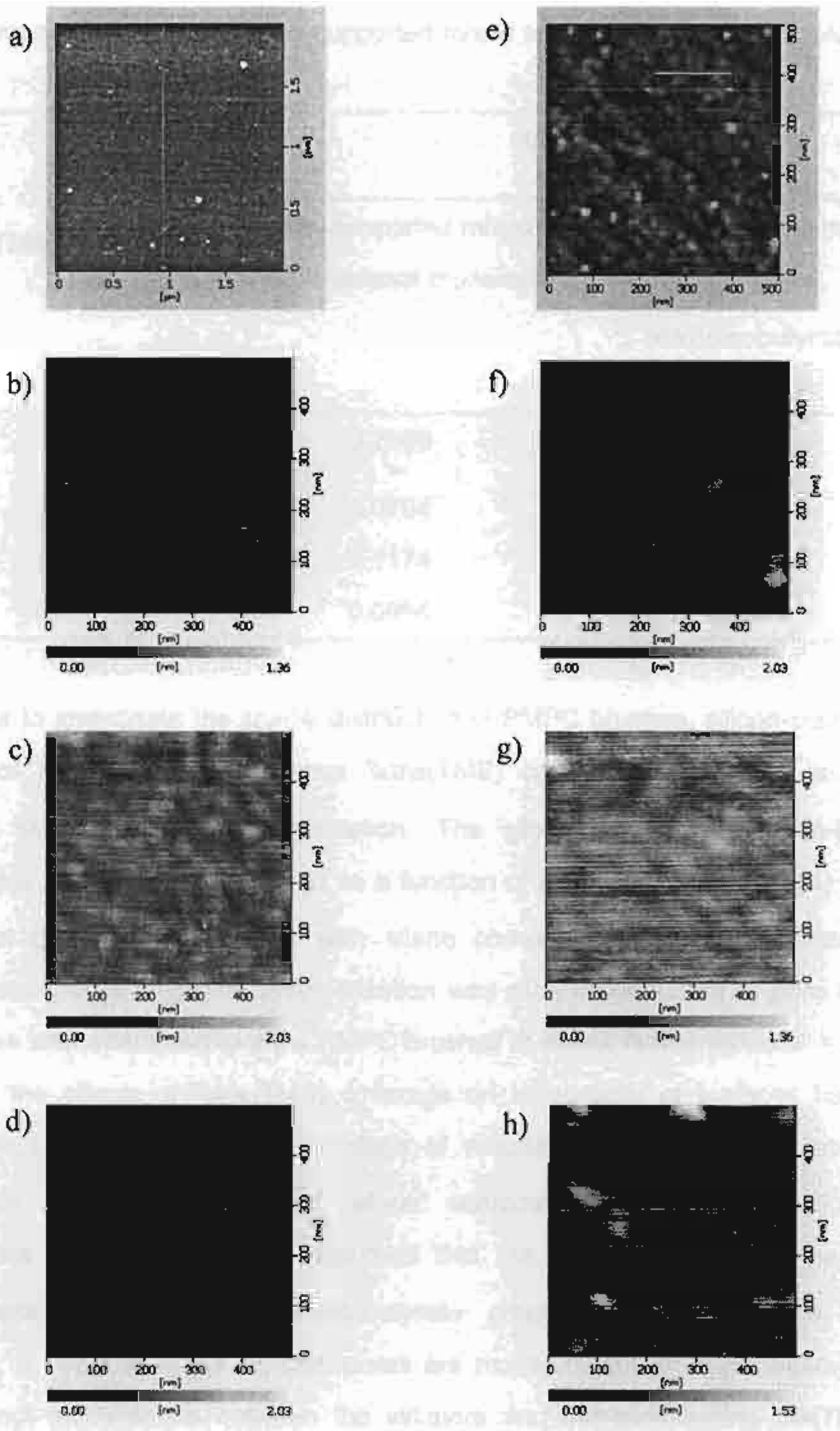


Figure 4.26 AFM images of silicon-supported mixed tris(TMS)/silanol monolayer having varied %tris(TMS) coverage: (a) 0%, (b) 51%, (c) 66%, and (d) 82% and silicon-supported mixed tris(TMS)/α-bromoisobutyrate monolayer having varied %tris(TMS) coverage: (e) 0%, (f) 51%, (g) 66%, and (h) 82%

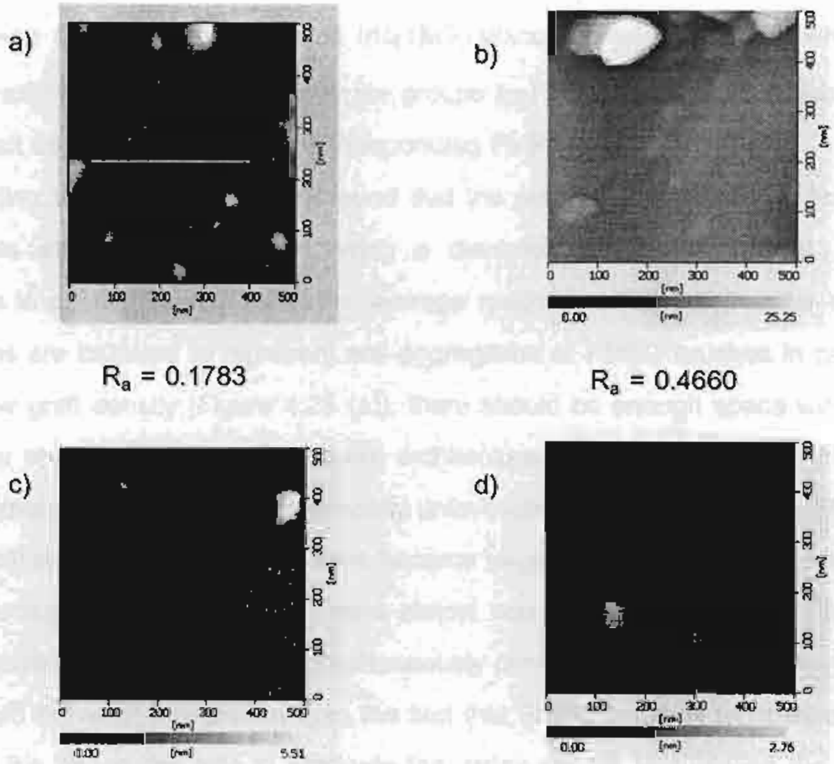
Table 4.6 Average roughness of silicon-supported mixed surfaces determined by AFM analysis

%tris(TMS) coverage	Ra (nm)	
	silicon-supported mixed tris(TMS)/silanol monolayer	silicon-supported mixed tris(TMS)/α-bromoisobutyrate monolayer
0	0.2006	0.1708
51	0.0794	0.1322
66	0.1174	0.1401
82	0.0954	0.1508

In order to investigate the spatial distribution of PMPC brushes, silicon-supported mixed tris(TMS)/silanol surfaces having various %tris(TMS) coverage were used as nano-scale templates for surface-initiated polymerization. The graft density of surface-tethered α-bromoisobutyrate groups was also varied as a function of reaction time (1-4 days) between the residual silanol groups in nanopores with silane compound having end-functionalized α-bromoisobutyrate groups (n_3). The polymerization was mostly conducted in pure methanol. In some cases, we also attempted to grow PMPC brushes in mixed methanol:water = 4:1 (v/v).

Firstly, the effects of %tris(TMS) coverage on topography of surfaces having grafted PMPC brushes were explored. Using 4 days of reaction between silicon-supported mixed tris(TMS)/silanol monolayers and the silane compound having end-functionalized α-bromoisobutyrate groups (n_3), it was assumed that the residual silanols in nanopores are completely replaced by the α-bromoisobutyrate groups that are capable of initiating polymerization. In another word, the nanopores are mostly filled with surface-tethered initiator and there is not much space between the initiators and the surrounding tris(TMS) groups. Originally, we envisioned that protrusions representing the aggregates of PMPC brushes in the nanopores should appear on the surfaces. And the higher the %tris(TMS) coverage, the smaller the size of protrusions. AFM images of silicon-supported mixed tris(TMS)/PMPC brushes having different %tris(TMS) coverage using polymerization time of 1h in methanol shown in Figure 4.27 suggested that it was not the case. All surfaces did not show any features that can be an evidence of distribution of polymer brushes grown from the nanopores. According to the data in Table 4.7, the surfaces become even smoother after a longer period of polymerization was used.

It was postulated that the densely grafted PMPC brushes within the limited space in nanopores may be forced by the surrounding tris(TMS) to stretch away from the surface when the polymer chain was relatively short and densely packed inside the nanopores. Under sufficiently long period of polymerization, the polymer chains became so long that they can no longer stretch out, thereby tend to fold over the tris(TMS) monolayer. For that reason, the surface presumes relatively smooth topography despite of its low overall graft density in the presence of tris(TMS). That is exactly what is observed in Figure 4.27. Nonetheless, the surfaces may become increasingly rough if the extensive growth of polymer brushes was allowed.



R_a = 0.1783

R_a = 0.4660

R_a = 0.2359

R_a = 0.1398

Figure 4.27 AFM images of silicon-supported mixed tris(TMS)/PMPC brushes prepared in methanol for 1 h having varied %tris(TMS) coverage : (a) 0%, (b) 51%, (c) 66% and (d) 82%

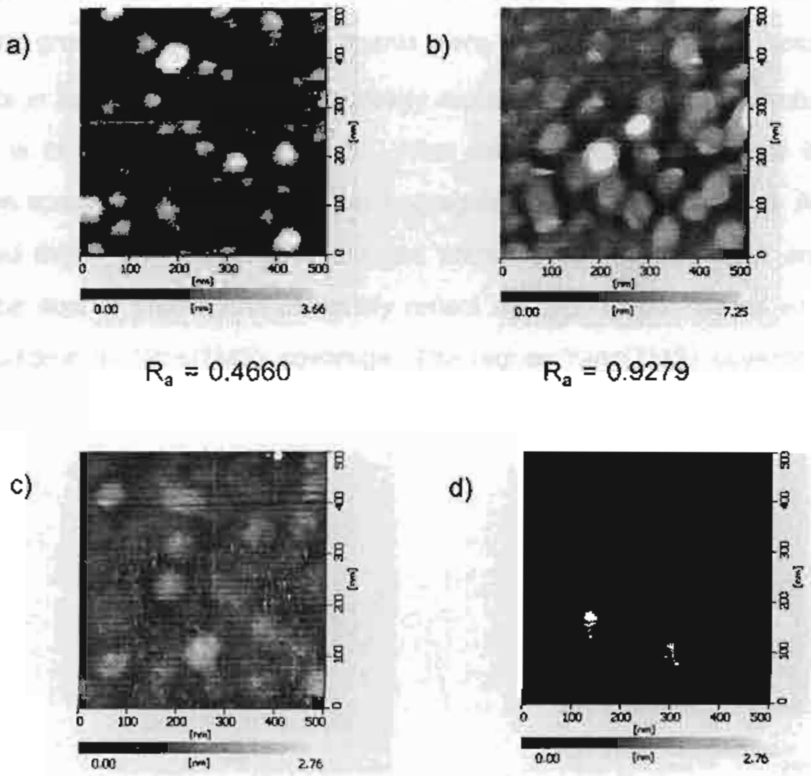
Table 4.7 Average roughness (R_a) of silicon-supported mixed tris(TMS)/PMPC brushes prepared in methanol determined by AFM analysis

% tris(TMS) coverage	R_a of silicon-supported mixed tris(TMS)/ PMPC brushes (nm)	
	Polymerization time of 1h	Polymerization time of 5h
0	0.1783	0.1322
51	0.4660	0.1598
66	0.2359	0.1727
82	0.1398	0.1404

In order to visualize the evolution of PMPC brushes grown from nanopores, two alternative routes were undertaken in order to regulate the graft density of polymer brushes. Firstly, by fixing %tris(TMS) coverage at 82%, the strategy exploited the kinetics control of the reaction between silicon-supported mixed tris(TMS)/silanol monolayers and silane compound having end-functionalized α-bromoisobutyrate groups (n_3) from 1 to 4 days which later yielded low to high graft density of initiator and corresponding PMPC brushes.

According to Figure 4.28, it was found that the surfaces having varied graft density of PMPC brushes exhibited protrusions having a diameter less than 100 nm. The size of protrusion was in good agreement with the average roughness (R_a) listed under each images. The protrusions are believed to represent self-aggregation of PMPC brushes in nanopores. In the case of low graft density (Figure 4.28 (a)), there should be enough space within the pores for the polymer chains to adopt more coil-like architecture or aggregated form instead of being in extended forms which are thermodynamically unfavorable. As the packing of PMPC brushes (Figure 4.28 (b)) was denser, the protrusions became larger and so did the roughness.

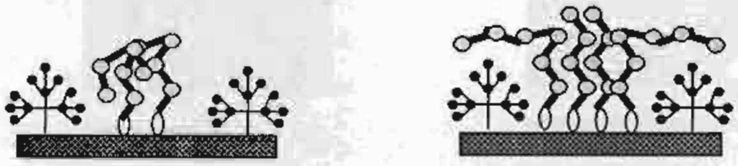
The surfaces whose nanopores were almost completely covered with PMPC brushes were quite smooth and the protrusions simultaneously diminished as can be observed in Figure 4.28 (c-d). Such behavior might stem from the fact that PMPC brushes were dense enough to be able to fill the nanopores and to eliminate the valley-and-hill features on the surface. The disappearance of the protrusions may be facilitated by two possible actions of polymer brushes previously explained. The first action involves the polymer chains being forced to stretch away from the surface and thus covering the nanopores while the other involves the folding of polymer chains over the tris(TMS) layer. This latter action should be favorable only when the polymer chains are sufficiently long. Behavior of polymer brushes can be schematically concluded in Figure 4.29.



R_a = 0.4660 R_a = 0.9279

R_a = 0.2487 R_a = 0.1398

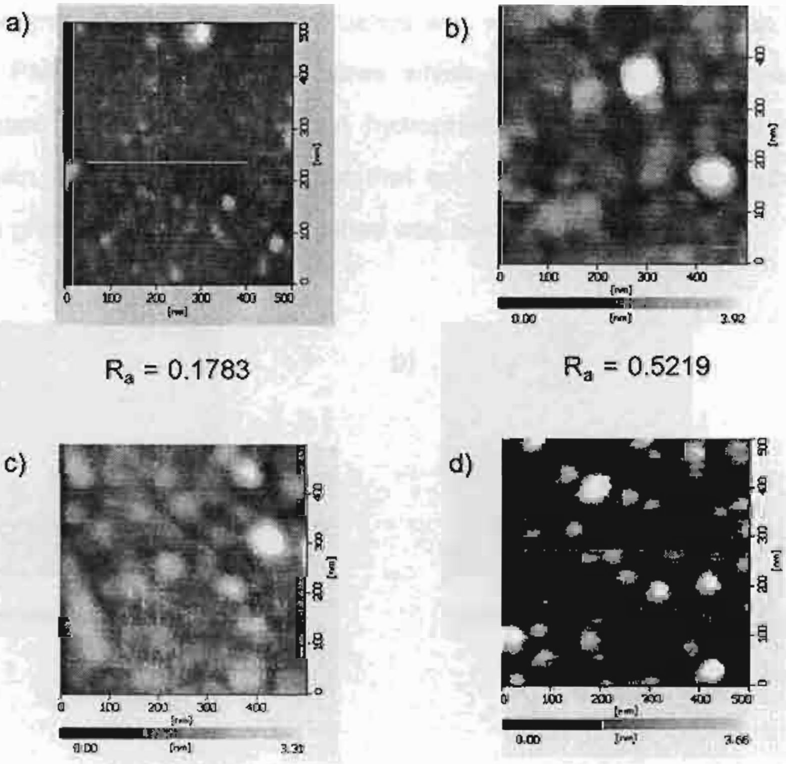
Figure 4.28 AFM images of silicon-supported mixed tris(TMS)/PMPC brushes having 82% tris(TMS) coverage prepared in methanol for 1 h by controlling grafting time of initiator: (a) 1 day, (b) 2 days, (c) 3 days and (d) 4 days



low graft density high graft density

Figure 4.29 Schematic representation of possible orientation of polymer brushes grown from nanopores having different graft density

Since the density of PMPC brushes in nanopores is the key to the ability to visualize the polymer distribution, the second alternative route was then done by varying %tris(TMS) coverage, but using a short reaction time (1 day) between silicon-supported mixed tris(TMS)/silanol monolayers and the silane compound having end-functionalized α-bromoisobutyrate groups (n_3). These experiments were designed in order to make sure that the residual silanols in nanopores are not completely replaced by the α-bromoisobutyrate groups that are capable of initiating polymerization. After the formation of polymer brushes, there should be some space for PMPC brushes to aggregate inside the nanopores. AFM images of silicon-supported mixed tris(TMS)/PMPC brushes prepared by this approach are illustrated in Figure 4.30. The size of protrusions obviously reflect the size of nanopores which should be inversely proportional to %tris(TMS) coverage. The higher %tris(TMS) coverage, the smaller the protrusions.



R_a = 0.1783

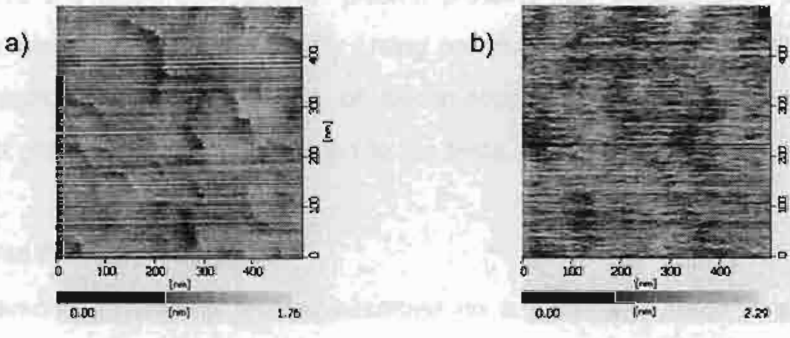
R_a = 0.5219

R_a = 0.3720

R_a = 0.4660

Figure 4.30 AFM images of silicon-supported mixed tris(TMS)/PMPC brushes prepared in methanol for 1 h using grafting time of initiator for 1 day and having varied % tris(TMS) coverage: (a) 0%, (b) 51%, (c) 66% and (d) 82%

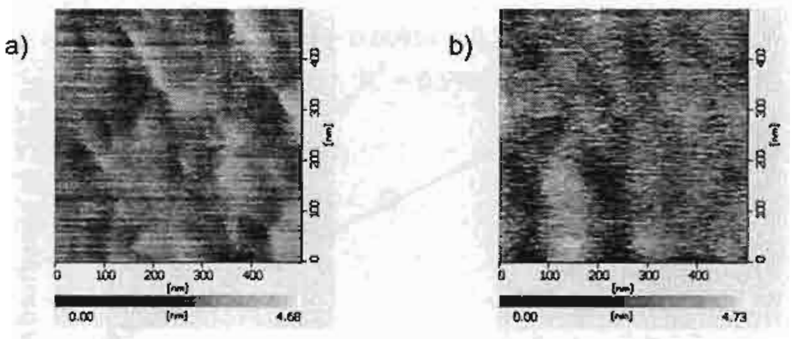
Comparative investigation was conducted on the system of poly(*t*-butyl methacrylate) (P*t*BMA) brushes. Figure 4.31 shows AFM images of silicon-supported mixed tris(TMS)/P*t*BMA brushes having 82% tris(TMS) coverage. P*t*BMA brushes was grown in toluene for 1 h from silicon-supported mixed tris(TMS)/α-bromoisobutyrate having different graft density of α-bromoisobutyrate groups. The reaction between silicon-supported mixed tris(TMS)/silanol monolayers and silane compound having end-functionalized α-bromoisobutyrate groups (n_3) for 1 day and 4 days yielded low and high graft density of α-bromoisobutyrate groups, respectively. Even though both surfaces have different graft density of P*t*BMA brushes, their surface topographies are indistinguishable and relatively smooth in texture. The surfaces became rougher when polymerization time was extended from 1h to 5h (Figure 4.32) without affecting surface topographies. Unlike PMPC brushes, P*t*BMA brushes are hydrophobic. The mixed tris(TMS)/P*t*BMA brushes system thereby doesn't exhibit nanoscopic phase separation although the surface graft density of P*t*BMA brushes was low. These results also implies that self-aggregation of PMPC brushes in nanopores which appears as protrusions is truly a consequence of phase incompatibility between hydrophilic PMPC brushes and hydrophobic tris(TMS). Once again, it should be highlighted that such nanoscopic phase separation was noticeable when the graft density of PMPC brushes was low.



R_a = 0.1993

R_a = 0.2596

Figure 4.31 AFM images of silicon-supported mixed tris(TMS)/PtBMA brushes having 82% tris(TMS) coverage prepared in toluene at 90°C for 1 h by controlling grafting time of initiator: (a) 1 day and (b) 4 days



R_a = 0.5559 R_a = 0.5630

Figure 4.32 AFM images of silicon-supported mixed tris(TMS)/PtBMA brushes having 82% tris(TMS) coverage prepared in toluene at 90 °C for 5 h by controlling grafting time of initiator: (a) 1 day and (b) 4 days

4.8 Biocompatibility of PMPC brushes

As a biocompatible polymer, PMPC is well recognized for its non-fouling characteristic, meaning it resists nonspecific interaction with plasma proteins and cells. One way to test the biocompatibility of PMPC brushes is to determine the interactions between surfaces bearing PMPC brushes and blood components: plasma proteins from platelet-poor plasma (PPP) and platelets from platelet-rich plasma (PRP). Using poly(methyl methacrylate) (PMMA) brushes as a positive control substrate, a series of silicon-supported PMPC brushes having different thickness and graft density were subjected to the tests.

4.8.1 Plasma Protein Adsorption

The amount of plasma protein adsorbed on a material surface is a primary factor in evaluating the blood compatibility of the material. When a blood-incompatible material is in contact with blood, a surface induces thrombosis which is initiated by the adsorption of plasma protein, followed by adhesion and activation of platelets. Here we determine the amount of plasma protein adsorption using BCA microassay. A calibration curve using albumin as a standard is displayed in Figure 4.33.

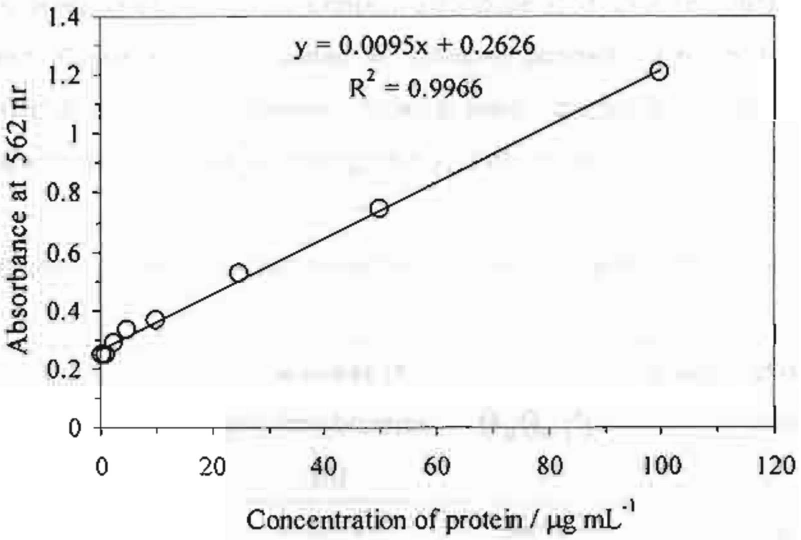


Figure 4.33 Calibration curve of the amount of albumin adsorbed and the absorbance obtained from BCA microassay

Protein adsorption data are concluded in Table 4.8. Due to the hydrophilic nature of silicon, the amount of protein adsorption was quite low. The amount of adsorbed protein slightly increased on a more hydrophobic silicon-supported α-bromoisobutyrate monolayer. Even though the amount of plasma protein adsorbed on the surfaces having PMPC brushes was lower than that on the surface grafted with α-bromoisobutyrate, the difference was not that significant considering the more hydrophilic nature of PMPC in comparison with the surface covered with α-bromoisobutyrate monolayer. In order to verify that the reduction of protein adsorption was really due to the presence of PMPC brushes, poly(methyl methacrylate) (PMMA), known as a blood incompatible polymer, was chosen as a positive control. PMMA brushes were grown from silicon-supported α-bromoisobutyrate monolayer, the same surface used for growing PMPC brushes. The fact that PMMA brushes adsorbed a larger amount of protein as opposed to PMPC brushes helped confirming the above statement.

The amount of protein adsorbed on the surface having PMPC brushes was almost independent of the thickness of polymer brushes implying that PMPC brushes being as thin as ~10 Å was sufficient to possess protein-resistant property. Apparently, the coverage of tris(TMS) played a significant role in controlling protein adsorption. The higher the content of tris(TMS) or the lower graft density of PMPC brushes on the surface, the higher the quantity of adsorbed protein. Responses to plasma proteins also depended upon the thickness of polymer brushes, however. Considering the series of silicon-supported mixed 66%tris(TMS)/PMPC brushes, it seemed that the longer polymer brushes were capable of repelling plasma protein despite their relatively low graft density or high %tris(TMS) coverage.

Table 4.8 The amount of plasma protein adsorbed on various silicon substrates

Surface	Thickness of polymer brushes (Å)	θ_A/θ_R (°)	Amount of protein adsorbed (mg/cm^2)
Silicon	-	34/15	0.32 ± 0.12
Silicon-supported α-bromoisobutyrate monolayer	-	71/62	0.65 ± 0.30
Silicon-supported PMPC brushes	10	23/0	0.45 ± 0.09
	26	22/0	0.40 ± 0.05
	64	20/0	0.34 ± 0.04
Silicon-supported PMMA brushes	18	66/54	1.47 ± 0.60
	30	67/58	2.40 ± 0.39
silicon-supported mixed 66%tris(TMS)/PMPC brushes	26	56/30	3.39 ± 0.33
	30	54/27	1.23 ± 0.10
	43	54/20	0.13 ± 0.05
silicon-supported mixed 75%tris(TMS)/PMPC brushes	23	63/31	5.42 ± 0.18
silicon-supported mixed 82%tris(TMS)/PMPC brushes	18	70/40	7.83 ± 0.54
silicon-supported mixed 88%tris(TMS)/PMPC brushes	6	77/52	24.04 ± 0.78

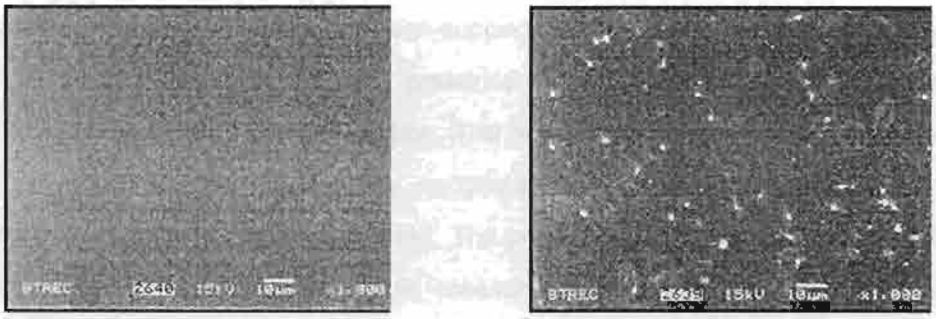
This outcome is in good agreement with the assumption previously proposed based on topographic evidence obtained from AFM analysis. It was postulated that the stretching of long polymer chains is thermodynamically unfavorable so the polymer chains tend to fold over the tris(TMS) monolayer. The lower receding contact angle as a function of polymer thickness can be an indication of PMPC brushes dominating at polymer/water interfaces or under hydrated condition. As a result, the coverage of PMPC brushes is no longer directly correlated with %tris(TMS). In another word, the protein resistance is not dictated by %tris(TMS) but by the thickness of PMPC brushes instead.

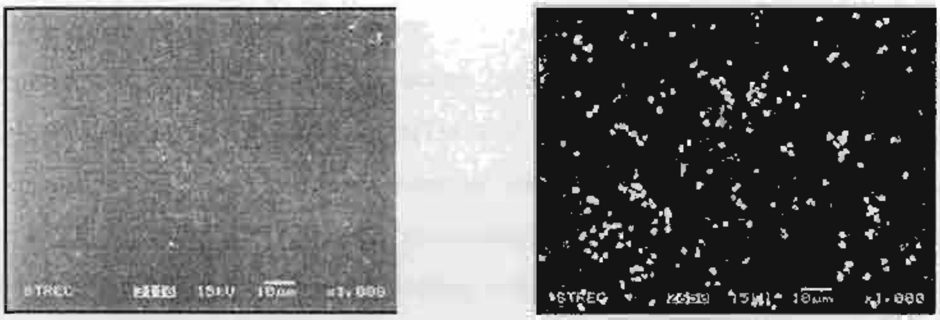
In the case of homogeneously and densely grafted PMPC brushes grown from silicon-supported α-bromoisobutyrate monolayer, the protein adsorption does not really depend on the thickness of polymer brushes as long as the surface is fully covered by the polymer brushes. The extremely thin layer of polymer brushes is certainly enough for generating non-fouling surface. On the other hand, the thickness and the graft density of polymer brushes become important parameters in controlling protein adsorption on heterogeneously and loosely grafted PMPC brushes grown from silicon-supported tris(TMS)α-bromoisobutyrate monolayer. When polymer brushes are quite short, the polymer chains are forced to stretch out or aggregated in the nanopores surrounded by tris(TMS), the protein adsorption is varied as a function of %tris(TMS) or the graft density of PMPC brushes. In contrast, when the polymer brushes are so long that they can fold and partly cover tris(TMS), the protein adsorption no longer relies on %tris(TMS), but is mainly influenced by the length or the thickness of the polymer brushes.

4.8.2 Human Platelet Adhesion

It is well known that platelets also contribute to the thrombus formation. In general, a foreign substrate induces adhesion and activation of platelets with the adsorbed protein layer serving as a controlling factor of the platelet response. Figure 4.34 shows SEM micrographs of silicon surface, silicon-supported α-bromoisobutyrate monolayer, silicon-supported PMMA brushes, and silicon-supported PMPC brushes after contacting with PRP. Many platelets adhered to the PMMA brushes. A few platelets were adhered on the surface grafted with α-bromoisobutyrate but did not adhere on the silicon surface. The thickness layer of PMPC brushes does not seem to influence the adherent platelets. PMPC brushes completely inhibited platelet adhesion. The data from platelet adhesion studies agree very well with the plasma protein adsorption and confirm the blood compatibility of PMPC brushes.

SEM micrographes of silicon-supported mixed tris(TMS)/PMPC brushes after exposure to PRP are not shown mainly due to the fact that there was no specific correlation between %tris(TMS) or the graft density of PMPC brushes and platelet adhesion. Some area was covered with small aggregates of platelets whereas other area was completely absent of platelets. These results indicate that platelets do not recognize the nanoscaled distribution PMPC brushes having dimension of less than 100 nm (0.1 μm) which is ~ 20 times smaller than a diameter of platelet (2-3 μm)


(a) silicon (b) α-bromoisobutyrate


(c) PMPC brushes (~14 Å) (d) PMMA brushes (~16 Å)

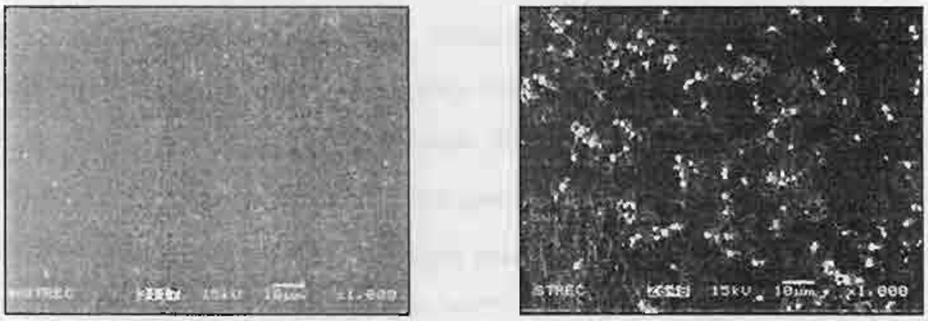

(e) PMPC brushes (~26 Å) (f) PMMA brushes (~30 Å)

Figure 4.34 SEM micrographs of various silicon surfaces after contacting with human PRP

CHAPTER V

EXECUTIVE SUMMARY

It has been demonstrated that hydrophilic polymer brushes of 2-methacryloyloxyethyl phosphorylcholine (MPC) can be successfully prepared by surface-initiated atom transfer radical polymerization (ATRP) from silicon-supported ∝-bromoisobutyrate monolayer using CuBr/bpy as a catalytic system in the presence of either propyl-2-bromoisobutyrate as a sacrificial initiator or $CuBr_2$ as a deactivator. The linear dependence of molecular weight and thickness on polymerization time clearly suggested that polymerization is living. The graft density of 0.3-0.5 chains/nm^2 was calculated. The presence of surface-tethered PMPC brushes was also verified by water contact angle measurements as well as XPS analysis. Mixed methanol:water = 4:1 (v/v) and pure methanol were found to be the suitable solvents for growing PMPC brushes in the living manner.

By controlling the kinetics of a reaction between silanol groups on the silicon surface with tris(trimethylsiloxy) chlorosilane (tris(TMSCl), silicon-supported mixed tris(TMS)/silanol surfaces having a range of tris(TMS) coverage were generated. The reaction progress was monitored by ellipsometry, contact angle measurements and XPS analysis. Nanoscaled holes (nanopores) in tris(TMS) monolayer containing unreacted silanol groups of silicon-supported mixed tris(TMS)/silanol monolayer further reacted with silane compounds having end-functionalized α-bromoisobutyrate and yielded silicon-supported mixed tris(TMS)/∝-bromoisobutyrate monolayer. These substrates were later used as nanoscaled templates for the synthesis of surface-tethered PMPC brushes. Due to the hydrophobicity of tris(TMS) groups surrounding ∝-bromoisobutyrate initiators, the growth of PMPC brushes from silicon-supported mixed tris(TMS)/∝-bromoisobutyrate monolayer was more favorable in a more surface-wettable solvent, methanol in this particular case, than water.

Surface topographies of polymer brushes were investigated by AFM analysis. Nanoscopic distribution of PMPC brushes which appeared as protrusions having a diameter of less than 100 nm was only visualized when the graft density of PMPC brushes in the nanopores was not too high. Under such circumstance, the size of protrusions depended upon both %tris(TMS) coverage as well as the graft density of PMPC brushes itself. Certain space in the nanopores was necessary for the polymer chains to adopt more coil-like architecture or aggregated form instead of being in extended forms which are thermodynamically unfavorable. In the case of high graft density of PMPC brushes in the nanopores, the surfaces became quite smooth due to two possible actions of polymer brushes. The first action involves the polymer chains being forced to stretch away from the surface and thus covering the nanopores while the other involves the folding of polymer chains over the tris(TMS) layer. This latter action should be favorable only when the polymer chains are sufficiently long. Comparative studies on the system of mixed tris(TMS)/P*t*BMA brushes confirmed that self-aggregation of PMPC brushes in the nanopores was truly a consequence of phase incompatibility between hydrophilic PMPC brushes and hydrophobic tris(TMS).

According to protein adsorption and platelet adhesion studies, homogeneously and densely grafted PMPC brushes are apparently blood compatible. It has also been demonstrated that surface grafting by extremely thin layer of PMPC brushes is an effective way for generating blood compatible surface. In the case of heterogeneously and loosely grafted PMPC brushes grown from silicon-supported mixed tris(TMS)α-bromoisobutyrate monolayer, the protein adsorption was varied as a function of %tris(TMS) or the graft density of PMPC brushes when polymer brushes were quite short. In contrast, when the polymer brushes were so long that they cannot stretch out, thereby tend to fold over the tris(TMS) monolayer, the protein adsorption no longer relied on %tris(TMS), but was mainly influenced by the length or the thickness of the polymer brushes. There was no specific correlation between %tris(TMS) or the graft density of PMPC brushes and platelet adhesion indicating that the distribution of PMPC brushes in the sub-micron scale (< 0.1 μm) was definitely too small for the micron-sized biomolecules like platelets to appreciate the heterogeneity of surface.

Knowledge gained through this research are very beneficial to the development of nanotechnology for a broad range of biomedical-related applications.

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OUTPUT

International Publication

1. Iwata, R.; Suk-in, P.; Hoven, V. P.; Takahara, A.; Akiyoshi, K.; Iwasaki, Y. "Control of Nano-biointerfaces Generated from Well-defined Biomimetic Polymer Brushes for Protein and Cell Manipulations" *Biomacromolecules*, **2004**, 5(6), 2308-2314.
2. Hoven, V. P.; Srinanthakul, M.; Kiatkamjornwong, S.; Iwata; R.; Iwasaki, Y. "Topographic Evidence of Nanoscaled Distribution of Polymer Brushes Generated by Surface-Initiated Polymerization" *In Preparation*
3. Hoven, V. P.; Suk-in, P.; Kiatkamjornwong, S.; Iwasaki, Y.; Watanabe, J.; Ishihara, K. "Ultrathin Coating by Surface-tethered Phosphorylcholine-containing Polymer Brushes for Blood-contacting Applications" *In Preparation*

International Presentation

1. Hoven, V. P.; Suk-In, P.; Srinanthakul, M.; Kiatkamjornwong, S.; Iwasaki, Y. "Preparation of Blood Compatible Surface by Surface-initiated Polymerization of 2-Methacryloyloxyethyl phosphorylcholine" *Abstracts of Papers of the American Chemical Society* 227: 029-POLY Part 1 Mar 2004.
2. Suk-In, P.; Kiatkamjornwong, S.; Iwasaki, Y.; Watanabe, J.; Ishihara, K.; Hoven, V. P. "Blood Compatible Polymer Brush of Poly(2-methacryloyloxyethyl phosphorylcholine)" *8th Pacific Polymer Conference,* November 24-27, 2003, Bangkok, Thailand.

National Presentation

1. Srinunthakul, M.; Kiatkamjornwong, S.; Iwasaki, Y.; Hoven, V. P. "Synthesis of Phophorylcholine-containing Polymer Brushes by Nanoporous Surface-initiated Polymerization" *30th Congress on Science and Technology of Thailand*, October 19-21, 2004, Bangkok, Thailand.

Appendix

International Publication #1

Control of Nanobiointerfaces Generated from Well-Defined Biomimetic Polymer Brushes for Protein and Cell Manipulations

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Control of Nanobiointerfaces Generated from Well-Defined Biomimetic Polymer Brushes for Protein and Cell Manipulations

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To better understand protein/material and cell/material interactions at the submolecular level, well-defined polymer brushes consisting of poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) on silicon wafers were prepared by atom transfer radical polymerization (ATRP). Silicon wafers were treated with 3-(2-bromoisobutyryl)propyl dimethylchlorosilane (BDCS) to form a monolayer that acts as initiators for ATRP. Silicon-supported BDCS monolayers were soaked in a methanol/water mixture solution containing Cu(I)Br, bipyridine, and a sacrificial initiator. After MPC was added to the solution, ATRP was carried out for 18 h. The molecular weight and thickness of the PMPC brush layer on the silicon surface increased with an increase in the polymerization time. The dense polymer brushes were obtained by the "grafting from" system. By selective decomposition of the BDCS monolayer by UV light-irradiation, the PMPC brush region and the sizes were well controlled, resulting in fabricating micropatterns of the PMPC brushes. When the thickness of the PMPC brush layer was greater than 5.5 ± 1.0 nm (3 h polymerization), serum protein adsorption and fibroblast adhesion were effectively reduced, i.e., proteins and cells could recognize such thin polymer brushes on the surface. In addition, the density of the adherent cells on the patterned PMPC brush surface could be controlled by changing the size of the pattern.

Introduction

There has been a considerable amount of theoretical and experimental interest in the micromanipulation of cell adhesion on solid surfaces, which makes use of the heterogeneous properties of the surface to control the cell adhesion.[1,2] Photolithographic and microcontact printing techniques have been used to prepare highly defined micropatterns, and their potential applicability to biosensors and drug screening arrays has been demonstrated.[3,4] In the presence of serum, adsorption of serum protein strongly influences cell adhesion. Control of serum protein adsorption is quite necessary to form a well-defined pattern of adherent cells. It is generally difficult to control protein adsorption on solid surfaces because nonspecific protein adsorption is the first phenomenon when the surface comes in contact with a physiological environment. The nonfouling properties of base materials may be important in the control of protein adsorption. To obtain a nonfouling polymer surface, we have been studying 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers synthesized as biomimetics in biomembrane structures.[5–8] The MPC polymers exhibit a property that resists nonspecific interaction with plasma proteins and cells.[9,10] Further, it has been shown that the activation and inflammatory response of cells in contact with MPC polymers are not induced.[11,12] While immobilization with MPC polymers to obtain nonfouling and biocompatible surfaces is successful, the uses of polymers to manipulate proteins and cells on surfaces are rarely reported. Furthermore, the effects of the surface structures of MPC polymers in a submolecular scale on biofouling have not yet been studied.

To produce well-defined polymers, controlled "living" radical polymerization has been explored.[13] Atom transfer radical polymerization (ATRP) is one of the best methods for this process because it can be applied to a wide variety of monomers.[14–17] An alternative process, pioneered by Huang and Wirth[18,19] and Tsujii and co-workers,[20,21] to prepare well-defined polymer brushes on solid surfaces with ATRP is considerably theoretical and deals with experimental interests in the control of surface properties. Surface-initiated graft polymerization is called a "grafting from" system and has the advantage of preparing dense polymer brushes as compared with the adsorption of functionalized polymers to solid/liquid interfaces (i.e., a "grafting to" system) due to steric hindrance of polymer.[20]

Numerous "grafting from" systems to improve wettability and nonfouling properties have been reported that use photoirradiation, plasma discharge, and corona discharge

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treatments.[22–25] While these processes were quite successful, their use precludes accurate control of the polymer structure. In contrast, ATRP is a robust method because of well-controlled molecular architecture.[13,24,27] A previous paper described the effectiveness of ATRP in preparing a well-defined graft polymer on a solid surface.[28–33] ATRP has also been applied for the fabrication of polymer brush micropatterns on solid surfaces.[34–37]

Control of surface properties is very important in the production of bio-related materials that can be used in biomedical and diagnostic applications. ATRP may be quite useful for this characteristic. However, only a few ATRP trials to optimize protein/material or cell/material interfaces, that is, biointerfaces, have been reported.[38,39]

Quite recently, Feng et al.[40] independently reported the preparation of PMPC brushes on silicon wafers. However, the effect of the surface properties of dense PMPC brushes with nanometer thickness on protein adsorption, cell adhesion, and their manipulations has not yet been studied.

Here, we report on the preparation of well-defined PMPC brushes to optimize the surface structures of bio-related materials and the effect of surface properties on protein adsorption and cell adhesion and their micromanipulation.

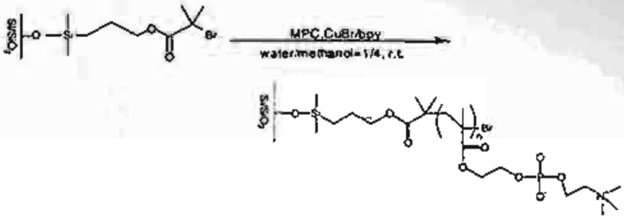
Materials and Methods

Materials. Silicon wafers (100 orientation, P/B doped) were purchased from Nakayama Semiconductor Co., Ltd., Tokyo, Japan. MPC was synthesized by previously reported methods.[41] 3-(2-Bromoisobutyryl)propyl dimethylchlorosilane (BDCS) was synthesized as previously described.[28,32] Purified water (reverse osmosis) was further purified on a Millipore Milli-Q system that involves reverse osmosis, ion exchange, and filtration steps (18.2 MΩ). Tetrahydrofuran was used after conventional distillation. Other chemicals were obtained from Aldrich and used without further purification.

Pretreatment of Silicon Substrate. Silicon wafers varied in thickness from 450 to 575 μm. Disks (100 mm) were cut into 1.2 cm × 1.2 cm pieces. The samples were held in a custom-designed holder and put in a freshly prepared mixture of 7 parts concentrated sulfuric acid containing dissolved sodium dichromate (~3−5 wt %) and 3 parts 30% hydrogen peroxide. Upon preparation, the solution turns from red-brown to green, warms to 80−90 °C, and foams extensively due to the formation of oxygen and ozone. Plates were submerged in the solution overnight, rinsed with 4 30-mL aliquots of water, and placed in a clean oven at 120 °C for 2 h. Silanization was carried out immediately after treatment of the plates in this manner.

Monolayers of BDCS on Silicon Wafer. To avoid a cross-linking reaction of organosilane and to prepare the homogeneous monolayer of the initiator on the silicone wafer,[42] monochlorosilane was synthesized. The BDCS monolayer on the silicon wafer was prepared by the method previously reported.[32] Briefly, cleaned silicon wafers (1.2 cm × 1.2 cm) were placed in a dry flask to which 30 mL of dry toluene and BDCS (33 μL, 0.15 mmol) were added under an argon gas atmosphere. The flask was allowed to stand for 18 h. The wafers were then removed from the solution, rinsed with toluene, acetone, and absolute ethanol, and dried in a nitrogen or argon stream.

Scheme 1. Synthetic Route of PMPC Brushes on Silicon Wafer via ATRP



Preparation of Well-Defined PMPC Brushes on Silicon-Supported BDCS Monolayer. A mixed solvent of 4 parts methanol and 1 part water was used as a solvent for ATRP of MPC. Argon gas was purged in these solvents to eliminate oxygen before the polymerization. Copper bromide (I) (29 mg, 0.20 mmol) and 2,2′-dipyridyl (63 mg, 0.40 mmol) were dissolved in 12 mL of methanol with stirring under argon at 0 °C, to which 6 mL of water was added. Then, ethyl 2-bromoisobutyrate (9 μL, 0.060 mmol) was added as a sacrificial initiator. After being stirred for 30 min under an argon gas atmosphere, the BDCS-immobilized silicon wafers were then submerged into the flask. MPC (3.6 g, 0.012 mol) was separately dissolved in 12 mL of methanol purged with argon for 1 h to eliminate oxygen. The MPC solution was added to the flask and polymerization occurred at room temperature with stirring under an argon gas atmosphere. The silicon wafers were periodically removed from the polymerization mixture and rinsed with methanol and water. Subsequently, they were extracted with a Soxhlet apparatus in methanol for 72 h and dried in an argon stream. The weight-averaged molecular weight of free polymer in solution was measured with a Tosoh GPC system with a refractive index detector and size-exclusion columns, Shodex, SB-804 HQ and SB-806 HQ with a poly(ethylene glycol) (PEG, Tosoh standard sample) standard in distilled water containing 10 mM LiBr. Scheme 1 shows the synthetic route of a PMPC polymer brush on a silicon surface.

Surface Patterning with UV Light Irradiation. A surface pattern of a BDCS monolayer on a silicon wafer was prepared with UV light (λ = 185 nm) (GL15ZH, Sankyo Denki Co., Ltd., 15 W) irradiation through a mesh for transmission electron microscopy (L, hole 450 μm, bar 50 μm; M, hole 120 μm, bar 50 μm; S, hole 45 μm, bar 40 μm; Okenshoji, Tokyo, Japan) for 3.5 h in an air atmosphere. The UV-irradiated silicon-supported BDCS monolayers were soaked in monomer solution and the ATRP of MPC was performed under conditions similar to those mentioned above.

Serum Protein Adsorption Test. Silicon wafers with surface patterning were exposed to 0.45 g/dL fluorescein isothiocyanate- (FITC-) labeled bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline solution (PBS) for 30 min and then rinsed with PBS and water. The sample was dried in an argon stream and observed with a fluorescent microscope (E-600, Nikon, Tokyo, Japan).

Fibronectin adsorption on the sample surface from 10% FBS/PBS was also determined by immunoassay. The bovine serum fibronectin adsorption onto the sample surface from the cell culture medium was detected by a method based on the antigen/antibody reaction with FITC-labeled immunoglobulin.[11] Briefly, the silicon wafers with surface patterning were placed in a 24-well tissue culture plate. Some of the cell culture medium was added to each well and allowed to remain for 60 min at 37 °C. The wafers were then rinsed with PBS. For the determination of fibronectin adsorption, the wafers were soaked in a BSA (Sigma Chemical Co., St. Louis, MO) solution (1 wt % BSA in PBS) to inhibit any undesirable reactions and nonspecific adsorption with the following antibody. They were incubated with one of the primary antibodies (anti-bovine fibronectin rabbit polyclonal antiserum; Yagai Co., Ltd., Yamagata, Japan) for specific proteins for 1 h at 25 °C. The primary antibody was reacted with the adsorbed proteins and then rinsed with PBS. The secondary antibody was applied to the wafers. FITC-conjugated immunoglobulin (anti-rabbit immunoglobulin G FITC conjugate, Sigma Chemical Co.) was used as the secondary antibody. After sufficient rinsing of the samples with PBS, the surface was observed in the fluorescence microscope (E-600, Nikon, Tokyo, Japan).

Cell Culture Experiment. Mouse fibroblasts (L-929 cells) were purchased from Riken Cell Bank. The L-929 cells were maintained in the culture medium [Eagle's modified essential medium (MEM); Nissui Pharmaceutical, Tokyo, Japan] containing 10% FBS at 37 °C in a humidified atmosphere f air containing 5% CO_2. The contents of the flasks for cell maintenance were detached by trypsin treatment. The concentration of the L-929 cells was adjusted to 5.0 × 10^4 cells ml/L. The L-929 cells were seeded on silicon wafers and cultured for 20 h in the CO_2 incubator with 95% humidity. After the medium was aspirated, the wafer was rinsed three times with PBS and in contact with 8 μM Nile Red (Sigma Chemical Co., St. Louis, MO)/PBS for few seconds. The wafers were then rinsed with PBS and placed in 2.5 vol % glutaraldehyde solution to fix the adherent cells on the wafer. The wafer was repeatedly rinsed with distilled water and observed with a scanning fluorescence microscope (E-600, Nikon, Tokyo, Japan).

Surface Analysis. The surface composition was measured by X-ray photoelectron spectroscopy (XPS) on a Scienta ESCA 200 spectrometer (Uppsala, Sweden) with Al Kα X-rays. All the XPS data were collected at takeoff angles of 5° and 90°. The dynamic contact angles for the sample plates were recorded by use of a probe fluid, purified water, an Erma G-1 contact angle goniometer (Tokyo, Japan), and l-mont syringes. The advancing (θ_A) and receding (θ_R) contact angles were measured with addition to and withdrawal from the drop (0−20 μL), respectively. The thickness of the polymer brush was measured on an imaging ellipsometer (NL-MIE, Nippon Laser & Electronics Lab., Nagoya, pan) operating with a 532-nm YAG laser at a 50° incident angle.

The surface morphology and viscoelasticity of the model sample were observed with an atomic force microscope (AFM; SPI-3800, Seiko-I, Tokyo). Surface viscoelastic functions were evaluated in microviscoelastic mode (VE-AFM).[43] Images were captured in a 100 μm × 100 μm area.

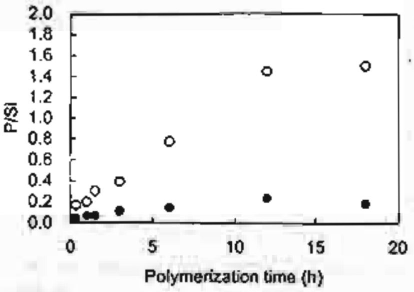


Figure 1. Time dependence of XPS elemental ratio (P/Si) of silicon-sputtered PMPC brush produced by ATRP: 15° (○) and 90° (●) takeoff angle data.

Results and Discussion

Controlled radical polymerization techniques, such as nitroxide-mediated radical polymerization, atom-transfer radical polymerization, and reversible addition/fragmentation chain transfer, allow good control of molecular weight with narrow distribution.[13] Well-defined polymer brushes on a solid surface are important to clarify protein/material and cell/material interactions at their interfaces. In this work, well-defined PMPC brushes on silicon wafers were prepared by surface-initiating ATRP, and surface properties were investigated physicochemically and biologically to optimize the biointerface.

Synthesis of PMPC Brushes on Silicon Surface via ATRP. Figure 1 shows the polymerization kinetics of MPC on silicon-supported monolayers. The phosphorus composition (P/Si ratio determined by XPS) increased with an increase in the polymerization periods of MPC and reached a plateau after 12 h. It can be confirmed that the monolayers of the PMPC brushes were prepared on the silicon wafer because the P/Si ratio at 90° of the takeoff angle was quite low (~0.25). ATRP of MPC in protic solvents was studied by Armes and co-workers.[44] They reported that MPC could be polymerized to high conversions in both water and methanol at ambient temperature. Slower polymerization and narrower polydispersities were always obtained in an alcoholic solution. The kinetics of polymerization could be well controlled by monomer concentration.[40] In this work and as mentioned by Armes and co-workers,[44] the rate of polymerization of MPC in aqueous solution was too fast (data not shown) to control the molecular weight of the PMPC brush. A mixed solvent of water and methanol was then used as a polymerization medium. We have demonstrated the adsorption of end-reactive PMPC on an organosilane monolayer.[45] The XPS phosphorus composition of the PMPC brush surface prepared by ATRP was much higher than that of the PMPC polymer-adsorbed surface. Figure 2 shows the water contact angle data for BDCS monolayer and the PMPC brushes. The water contact angles (θ_A/θ_R) of silicon-supported BDCS monolayer rapidly decreased with an increase in the polymerization periods of MPC and reached a plateau at 15−18/1° after 60 min. The surface wettability was not affected by the substrate after the reaction period. In contrast, equilibrated

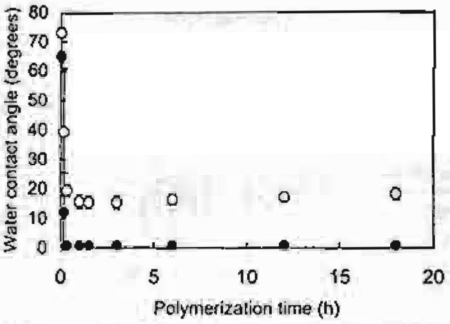


Figure 2. Water contact angle data for silicon-sputtered PMPC brush produced by ATRP: Advancing contact angle (○) and receding contact angle (●).

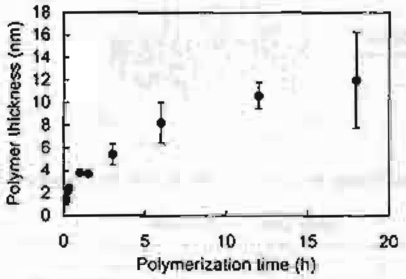


Figure 3. Thickness of silicon-sputtered PMPC brushes as a function of polymerization time.

contact angles of the PMPC brush surface prepared by a "grafting to" system were 60/5°.[45] As mentioned in a previous paper,[46] the surface density of the PMPC brushes prepared by a "grafting from" system would then be higher than that prepared by a "grafting to" system. Moreover, the hysteresis of the equilibrated contact angles ($\theta_A - \theta_R$) of the PMPC brush surface prepared by ATRP was <20°. This value is significantly lower than that of the PMPC brush surface prepared by the "grafting to" system or poly(MPC-co-*n*-butyl methacrylate) (PMB), which is mentioned in much of the literature as a nonfouling material.[5-12,41] From the water contact angle measurement, it has been shown that the mobility of the PMPC brushes prepared by ATRP is relatively low and the surface is homogeneous.

Figure 3 indicates the time dependence of the polymer thicknesses on the silicon wafer surface, which were determined by an ellipsometer. The polymer thickness increased with the polymerization time and was controlled from 0~15 nm.

The molecular weight of a PMPC brush on a silicon wafer was determined by measuring the molecular weight of a free polymer because reports have described that these molecular weights have similar values.[46] Figure 4 shows the change in the molecular weight of free PMPC with polymerization time. The profile of this graph is similar to that of polymer thickness. The molecular weight reached 6.5×10^4, at which it became a plateau. In this reactive condition, the ratio of MPC to the free initiator is 200/1. The molecular weight of MPC is 295.3, giving the theoretical molecular weight of the bulk polymer as 5.9×10^4, which coincides with the experimental value.

From the data in Figure 5, a cross-sectional area per chain, A_x, can be determined from the molecular weight of the chain, *M*, and the corresponding film thickness, *t*, by

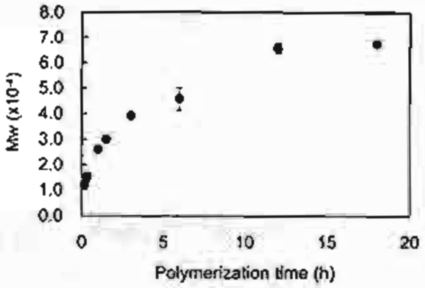


Figure 4. Molecular weight of free PMPC produced by the sacrificial initiator in solution.

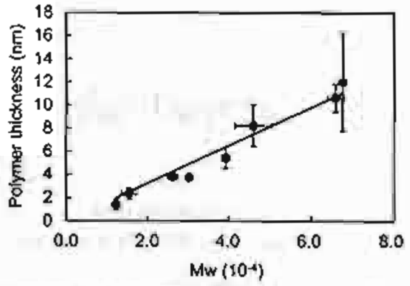


Figure 5. Correlation of the monolayer thickness of silicon-supported polymer brush with the molecular weight of free PMPC produced by the sacrificial initiator in solution.

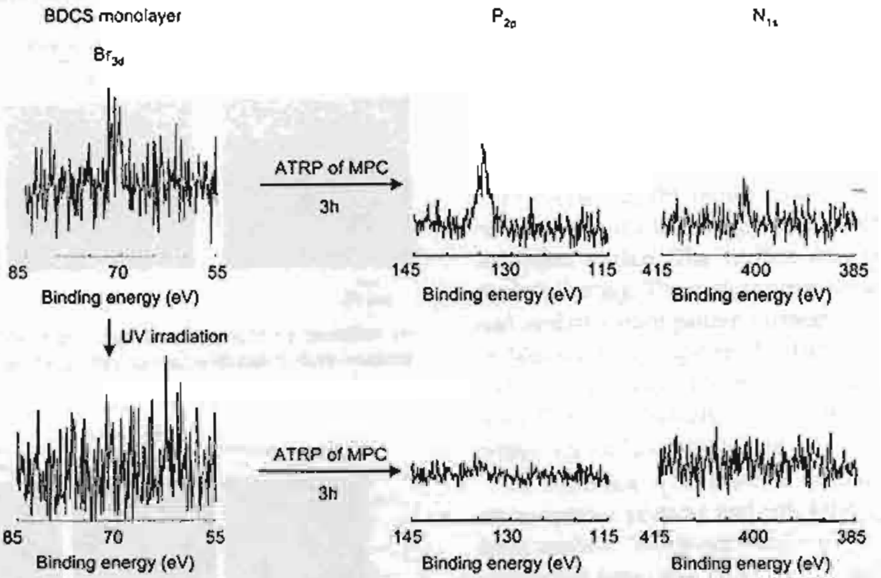
$$A_x = M/t\rho N_A$$

where ρ is the mass density determined with an oscillation U-tube (1.30 g/cm^3 for PMPC) and N_A is Avogadro's number. A_x was estimated at ca. 600 Å^2. The approximate cross-sectional area of PMPC determined with a CPK model is ca. 550 Å^2. It can be seen that the chains grown from the surface almost cover the surface.

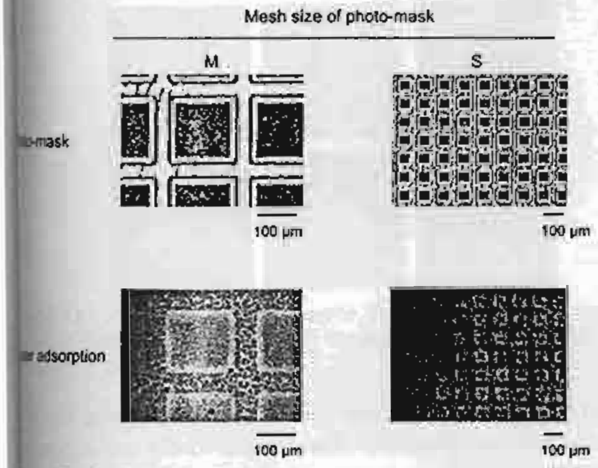
Fabrication of Patterning Polymer Surface. By UV irradiation, the water contact angles (θ_A/θ_R) of the BDCS monolayer changed from 72/62° to 54/43°. Figure 6 shows the XPS spectra of the BDCS monolayer surface that was irradiated with UV light and both surfaces on which ATRP of MPC was carried out. By UV irradiation, the bromine signal disappeared from the surface. On the BDCS monolayer surface, phosphorus and nitrogen signals are attributed to the phosphorylcholine group of the PMPC. In contrast, these signals were not observed on the UV-irradiated monolayer surface even after the same reactive condition. Physical adsorption of free PMPC was not observed.

Figure 7 shows the water absorption pattern on the patterned PMPC brush surface. The wettability of the PMPC brush region was improved by the hydrophilic polymer brushes. Although water absorption was observed on both the UV-treated and PMPC brush regions, the water drops adsorbed on the PMPC brush region spread wider and stuck longer than did those adsorbed on the UV-irradiated region.

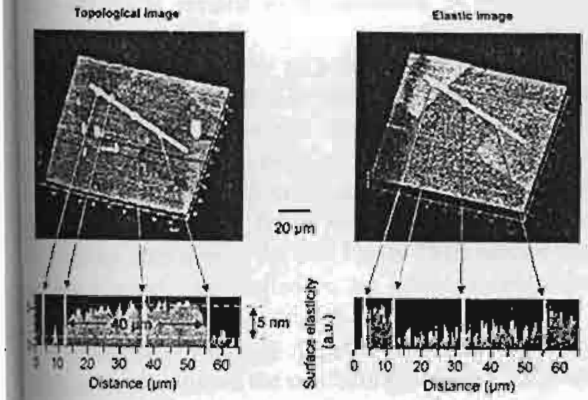
Figure 8 shows surface topological and elastic micrographs of the pattern surface produced by polymerization for 3 h. The thickness of the PMPC brush layer was about 5 nm, which was in good agreement with the data obtained by ellipsometry. The elastic image showed a pattern opposite that of a topological micrograph. This result indicates that the elasticity of the PMPC brush layer is lower than that of the UV-irradiated region.



ure 6. XPS spectra of BDCS monolayers on which were performed ATRP of MPC before and after UV light treatment.



ure 7. Optical micrographs of water adsorption pattern on erned PMPC brush surface.



ure 8. AFM image of patterned polymer brush produced on silicon ace by polymerization for 3 h: (a) topography AFM, (b) elastic M.

igure 9 is a schematic representation of microfabrication patterned graft polymer brushes by UV irradiation owed by graft polymerization.

ontrolled Protein Adsorption and Cell Adhesion. orption of FITC-labeled BSA was well controlled on the erned graft polymer surface, as shown in Figure 10. On the UV-irradiated region that has no polymer brush, the fluorescence intensity was significantly high, indicating that a large amount of BSA is adsorbed in this region. Conversely, BSA adsorption was remarkably reduced in the PMPC polymer brush layer. Figure 11 shows the fibronectin adsorption pattern on the pattern surface after contact with the cell culture medium for 60 min. The adsorption pattern was determined by immunoassay. On the polymer brush prepared by polymerization for 10 min, fluorescence caused by the adsorbed fibronectin was observed homogeneously. The difference in fibronectin adsorption on the patterning surface was clearly related to the polymerization time of the

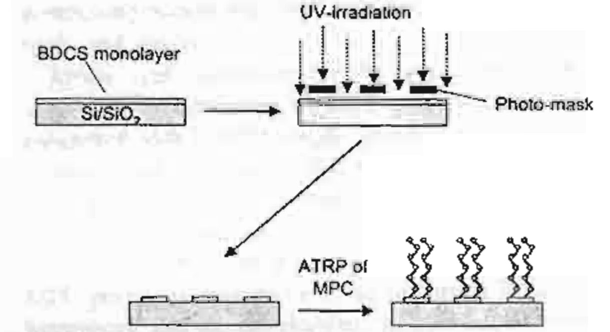


Figure 9. Schematic representation for fabrication of a patterned PMPC brush layer by UV light irradiation.

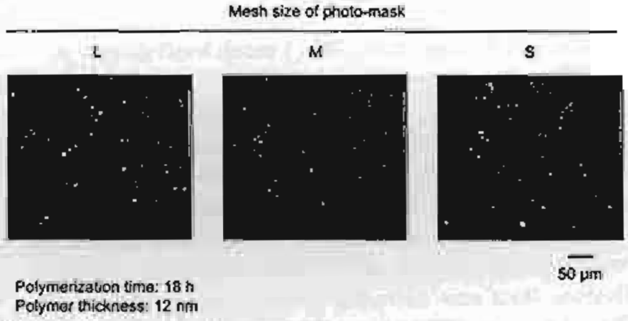
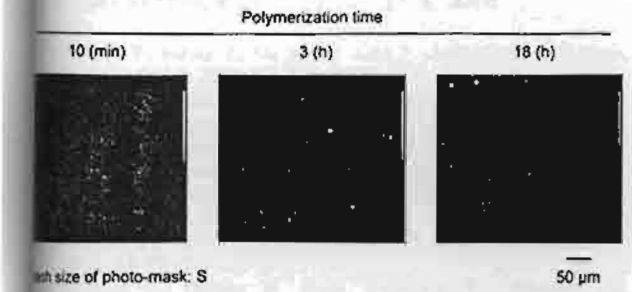
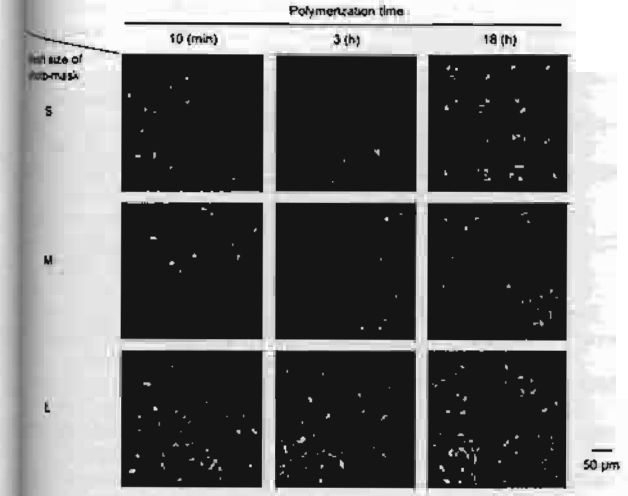


Figure 10. Fluorescence micrographs of FITC-albumin adsorption on patterned PMPC brush surface after contact with 0.45 g/dL FITC-albumin in PBS for 30 min.



gure 11. Fluorescence micrographs of fibronectin adsorption on ttemed PMPC brush surface after contact with cell culture medium r 60 min.



gure 12. Fluorescence micrographs of fibroblast adhesion on ttemed PMPC brush surface after incubation for 20 h. [Fibroblast] 5.0 × 10^4 cells/mL.

lymer brushes. On the PMPC brush surface, fibronectin sorption was effectively reduced. The reduction of plasma otein adsorption on MPC polymer surfaces has been ported by previous literature, and the mechanism is nsidered to be related to the structure of the polymer rface.[47]

The study to clarify the difference in surface structure etween a poly(MPC-*co*-BMA) (PMB) coating and the dense MPC brush prepared in this study on the reduction of otein adsorption is still ongoing. However, it was shown at PMPC brushes just 5 nm in thickness prepared by the rafting from" system could reduce protein adsorption. lthough the thickness of the cast film of PMB that we made s been normally controlled on the submicrometer scale, e PMPC brush thickness can be controlled on the scale of few nanometers. This is a great advantage for surface odification to improve the nonfouling properties of micro- nanodevices.

Figure 12 represents the fluorescence micrographs of roblasts that adhered on the pattern surface. The effect of rface grafting of PMPC by polymerization for 10 min on broblast adhesion was not observed and the cells adhered mogeneously to the surface. By adjusting the polymeri- tion time, cell adhesion was controlled and fibroblasts hered to UV-irradiated regions that have no PMPC ushes. This result is coincident with protein adsorption on e surface. Above a PMPC brush thickness of about 5 nm, otein adsorption and cell adhesion was remarkably reduced, i.e., they were able to recognize the thickness of the thin brush. The effect of grafting density of poly(ethylene glycol) on protein adsorption has been reported by Sofia et al.[48] The grafting density also may be important to control protein adsorption on the PMPC brush surface.

Furthermore, the number of adherent cells can also be controlled with a change in the surface area of the UV-irradiated region. The surface area of the pattern affected the cell density. The surface areas for adherent cells on small- and medium-sized pattern surfaces were 844 ± 185 and 1188 ± 240 μm^2/cell, respectively. The large-sized pattern surface had a cell density of 159 ± 34 μm^2/cell, which is significantly high. Cell communication depends on the area of the surface where the cells could adhere.

To fabricate a microscale pattern on a solid surface, microcontact printing and soft lithography techniques have been applied. Whitesides and co-workers[49−51] reported that microscale patterning provides a versatile method for creating novel adhesive substrates that are useful for spatially positioning mammalian cells and controlling their viability, form, and function.

Armes and co-workers[44,52−54] have reported that the molecular architectures of MPC polymers can be easily controlled with ATRP in protic solvents. As a result, a wide variety of polymer surface designs will be possible. Surface modification with well-defined MPC polymers would be considered as one of the robust methods to optimize biointerfaces on a molecular scale. Microfabrication with MPC polymers may prove to be important in separations, biosensors, and the development of biomedical materials.

Conclusion

A well-defined dense PMPC brush on a silicon wafer was prepared by the "grafting from" system with ATRP. The surface properties could be easily controlled even on a nanoscale. The dense brush layer could reduce serum protein adsorption and fibroblast adhesion with a polymer brush thickness of about 5 nm. Because a microscale pattern of the brush layer could be fabricated by decomposition of the initiating monolayer with UV irradiation, manipulation of protein adsorption and cell adhesion was well controlled. Surface modification with dense PMPC prepared by ATRP might be important in creating optimal biointerfaces because protein/material and cell/material interactions could be controlled with thin polymer brushes on a molecular scale. PMPC immobilization with ATRP is also a robust process for improving nonfouling properties in the micro- and nanofabrication of biomedical materials.

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ganizer: Dana Garcia
esiding: Matthew Laskoski

26 Oligomeric cyanate ester resins: A new class of thermosetting polymers
Matthew Laskoski, Teddy M. Keller

27 Preparation and characterization of poly(propylene oxide) dimethallyl and epoxy dimethallyl ethers
Nicolette Prevost, Alexander A. Lambert III, **Navzer Sachinvala**, Karol Maskos

28 Poly(arylene sulfide sulfone) copolymer composites for proton exchange membrane fuel cell systems: Extraction and conductivity
K. B. Wiles, C. M. de Diego, J. E. McGrath

29 Preparation of blood compatible surface by surface-initiated polymerization of 2-methacryloyloxyethyl phosphorylcholine
Vipavee P. Hoven, Piyawan Suk-In, Mayuree Srinanthakul, Suda Kiatkamjornwong, Yazuhiko Iwasaki

30 Enzymatic polymerizations for biomedical applications
Kamel Omrane, Erica Bryant, Anja Mueller

31 Incorporation DNA with diblock copolymers
Qingshan Mu, Jianren Lu, Andrew Lewis, Steven p. Armes

32 A-B-A Triblock copolymers derived from 5-methyl-5-benzyloxycarbonyl-1,3-dioxan-2-0ne (MBC) and L,L-lactide
Jamie M. Messman, Robson F. Storey, Chau N. Tang, Adam J. Pesek

33 Core-surface crosslinked micelles fabricated from PCL/PEG brush copolymers
Peisheng Xu, Maciej Radosz, **Youqing Shen**

34 Surface properties and morphologies of polyurethane via amphiphilic polyoxetane telechelics
Tomoko Fujiwara, Kenneth J. Wynne

35 Telechelic polyelectrolytes a novel class of associative polymers
Constantinos Tsitsilianis, Ilias Katsampas, Vasiliki Sfika, Nikoletta Stavrouli

36 Novel synthesis of 2, 2â€™-halogenated-4, 4â€™, 5, 5â€™-tetramethyl biphenyl and their derivative dianhydrides
Limin Sun, Dong Zhang, Huabin Wang, Z. D. Stephen Cheng, W. Frank Harris

37 Emulsion-based control of electrospun fiber morphology
Jay C Sy, I-Wei Chen, V. Prasad Shastri

General Papers -- Next Session

Symposium Grid -- Division of Polymer Chemistry -- Session Listing

The 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004

OLY 29

eparation of blood compatible surface by surface-initiated lymerization of 2-methacryloyloxyethyl phosphorylcholine

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preparation of biocompatible polymer brush was accomplished by surface-initiated atom transfer radical ymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) from surface-tethered -moester in the presence of CuBr/bpy. Polymerization from surface occured rapidly at room temperature in ueous media as opposed to alcoholic media. We found that the thickness of polymer brushes increased ost linearly with reaction time. The existence of phospholipid analogous groups on the surface was ealed by X-ray photoelectron spectroscopy (XPS). As demonstrated by water contact angle asurements, the surfaces having poly(MPC) brush showed improved hydrophilicity. The blood compatibility oly(MPC) brushes was evaluated by protein adsorption and platelet adhesion. The fact that the platelet esion is absent on poly(MPC) brushes as opposed to PMMA brushes supported the assumption that the ymer brushes are blood-compatible independent of the thickness in the range of 11-50 Å. Preparation of y(MPC) brushes from a nanoporous surface of mixed tris(trimethylsiloxy)silyl/silanol surface was empted.

General Papers

Division of Polymer Chemistry

The 227th ACS National Meeting, Anaheim, CA, March 28-April 1, 2004

PREPARATION OF BLOOD COMPATIBLE SURFACE BY SURFACE-INITIATED POLYMERIZATION OF -METHACRYLOYLOXYETHYL PHOSPHORYLCHOLINE

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uction

he control of surface properties is a key significance to several ercially important technologies ranging from biotechnology to advanced electronics. Recently an approach of "grafting-from" method or surface- ed polymerization has been introduced.[1] Since the polymer chain growth erally promoted from initiators which are attached to the surface, this ach can provide higher graft density and stable attachment.

here is growing interest in atom transfer radical polymerization (ATRP) it was discovered in 1995.[2] The living characteristic and the atibility with a variety of functional monomer render ATRP an attractive od for surface-initiated polymerization in producing well-defined ner brushes. The process allows a better control of target molecular t and molecular weight distribution. The success of ATRP in esizing hydrophilic polymers provides an additional advantage over the ional living ionic polymerization.[3]

As inspired by the biomembrane-like structure, a methacrylate monomer ng a polar phospholipid group, 2-methacryloyloxyethyl phorylcholine (MPC) was developed.[4] The excellent blood compatibility e MPC-containing materials is proven to originate from the ability to e self-assembly of natural phospholipids. Since hydrophilic MPC polymer possesses inferior mechanical properties, MPC is generally used form of copolymers, polymer coating and grafting. The issue related the ation of grafted poly(MPC) with well-defined structure, molecular weight raft density is a subject of considerable interest and yet to be explored. we propose a way to generate biocompatible polymer brushes by surface- ted polymerization of MPC using ATRP in both aqueous and alcoholic . Blood compatibility was addressed in terms of plasma protein ption and platelet adhesion. An attempt to prepare poly(MPC) brushes a nanoporous surface of mixed tris(trimethylsiloxy)silyl/silanol surface is eported.

erimental

Materials. Toluene anhydrous (99%), phosphate buffer saline (PBS), chonic assay kit (QuantiPro™ BCA assay) and protein standard (Bovine m Albumin: BSA) were obtained from Sigma-Aldrich. 2-Bromoisobutyryl ide (98%), 2,2'-bipyridyl (99%), copper(I) bromide (99.99%), dimethoxy e, sodium dodecyl sulfate (SDS) and sodium sulfate anhydrous were ased from Fluka. Dimethylethoxysilane and tris(trimethylsiloxy) osilane were obtained from Gelest, Japan. 2-Methacryloyloxyethyl phorylcholine (MPC) was synthesized by a method previously ibed.[4] Ultrapure distilled water was obtained from Mill-Q Lab system. on wafer was obtained from Siltron Inc. Korea. Platelet-poor plasma) and platelet-rich plasma (PRP) were obtained from Thai Red Cross ety.

Instrumentation. X-ray photoelectron spectra were collected using A-200, SCIENTA, Uppsala, Sweden. Contact angle meter model FACE, was used for the determination of water contact angles. The thickness of ner brush was characterized using L115C WAFER™ ELLIPSOMETER. ace morphology of polymer brushes were characterized by Atomic Force oscope (SPI-3800, Seiko I, Japan).

Preparation of Mixed Tris(trimethylsiloxy)silyl/silanol Surface. ned disks were covered with anhydrous toluene (20 mL) containing diisopropylamine (0.34 mL, 2.0 mmol) and tris(trimethylsiloxy) osilane (0.70 mL, 2.0 mmol). Reactions were carried out at 70°C under gen for 1-3 days. After the disks were isolated, they were rinsed with toluene (3x), isopropanol (2x), ethanol (3x), 1:1 ethanol:H_2O (2x), DI water (2x) and ethanol (2x), respectively. The disks were then dried in an oven at 120°C for 10 min.

Preparation of Surface Grafted ∝-Bromoester Initiators. Surface-tethered ∝-bromoester were prepared by soaking the cleaned disks or the disks bearing mixed tris(trimethylsiloxy)silyl/silanol in anhydrous toluene containing 2 mM of 3-(dimethylethoxysilyl)propyl-2-bromoisobutyrate[1] under nitrogen for a certain period of time at ambient temperature, followed by rinsing in toluene (3x), ethanol (3x), 1:1 ethanol:H_2O (2x), DI water (2x), ethanol (2x) and DI water (3x), respectively and dried under vacuum.

Preparation of Poly(MPC) Brushes. The substrates having surface grafted initiators were placed in a Schlenk flask containing CuBr (4.75 mg, 0.033 mmol), bpy (10.5 mg, 0.067 mmol), MPC (0.2 g, 0.67 mmol) in 30 mL degassed solvent was then added via cannular under nitrogen atmosphere. The polymerization was allowed to proceed for a set reaction time (30-180 min) at ambient temperature. The substrates were subjected to repetitive sonication in DI water and washed with isopropanol (IPA) and dried under vacuum.

Blood Compatibility Test. Plasma protein adsorption of the substrates bearing polymer brushes after exposure to platelet-poor plasma was determined using bicinchonic assay kit (QuantiPro™ BCA assay). Scanning electron microscopy (SEM Model JSM-5800L) was used to observe the morphology of surface-adherent platelets.

Results and Discussion

Formation of Poly(MPC) Brushes. The surface active ∝-bromoester initiating system (I) was prepared by a reaction of allyl alcohol with 2-bromoisobutyryl bromide in the presence of triethylamine followed by hydrosilylation of the resulting alkene terminated ester with dimethylethoxy silane.[2,8] The ∝-bromoester derivative (I), was attached to silanol groups on the silicon oxide surface (**Figure 1**). The surface reaction has reached its completion after 24h of reaction with a layer thickness of 10 Å and a water contact angle of $\theta_A/\theta_R = 71°/62°$.

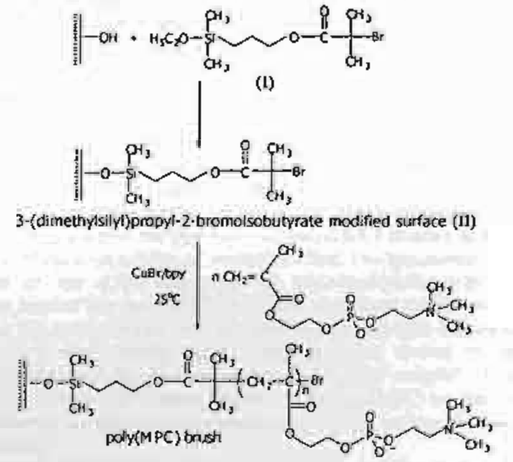
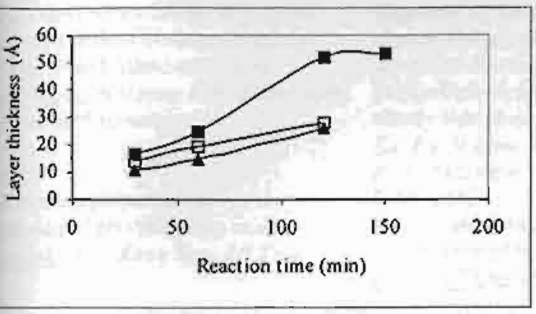


Figure 1. Reaction scheme for preparation of poly(MPC) brush.

Poly(MPC) brushes were grown from the surface bearing ∝-bromoester group (II) via ATRP mechanism in the presence of CuBr/bpy using H_2O or IPA as a reaction media. **Figure 2** shows the relationship between poly(MPC) brush thickness and reaction time. It was found that the layer thickness increased almost linearly with reaction time. The fact that polymer brushes grow more rapidly in a more polar aqueous media in comparison with a less polar alcoholic media is in good agreement with the data obtained in solution as described by Armes and coworkers.[5] The polar media, especially water facilitates the CuBr catalyst solubility and dissociation and thus accelerates the polymerization. The existence of phosphorylcholine groups of poly(MPC) brushes was revealed by the appearance of N_{1s} and P_{2p} as outlined in **Table 1**. The N/P ratios varying in the range of 0.7-1.8 reasonably agree with the stoichiometric ratio of MPC unit. Due to a partial X-ray damage of bromine during XPS analysis, the percentage of Br_{3d} is neither quantitative nor consistent with the percentages of N_{1s} and P_{2p}. The surfaces having polymer es (θ_A/θ_R ~ 55/30°) were more hydrophilic than the surface bearing α-ester groups (θ_A/θ_R ~ 71/62°) which is an indication of hydrophilic horylcholine moieties.



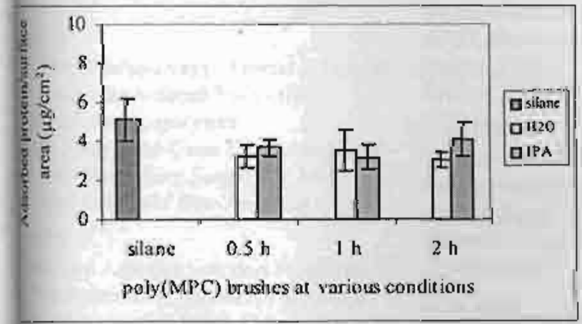
re 2. Ellipsometric thickness of poly(MPC) brushes prepared in H_2O (■), IPA/H_2O (□), and IPA (▲).

ble 1. Elemental Surface Composition (%) as Analyzed by XPS and Water Contact Angle Data.

les	C_{1s}	O_{1s}	N_{1s}	P_{2p}	θ_A/θ_R (°)
l surface	45.28	27.10	-	-	59/49
moester surface	41.03	26.90	-	-	71/62
MPC) brush (2 h, H_2O)	48.89	23.13	0.81	1.12	54/29
MPC) brush (2h, 50%IPA)	35.38	28.11	0.96	0.85	54/32
C brush (2 h, IPA)	20.54	38.22	0.86	0.47	57/33

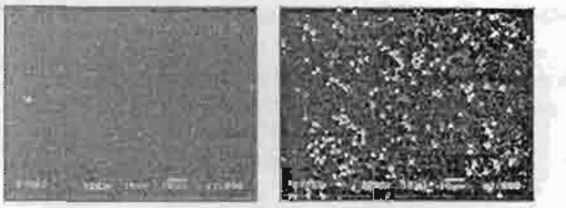
The independence of water contact angles on the poly(MPC) thickness ently implied that the growing of each polymer brush is simultaneous and g in character. According to AFM analysis, all poly(MPC) brushes having hickness in the range of 10-50 Å are relatively smooth (R_a ~ 0.2-0.3 nm).

Blood Compatibility of Poly(MPC) Brushes. The amount of plasma in adsorbed on material's surface is a primary factor in evaluating the d compatibility of material. When a blood-incompatible material is in ct with blood, a surface induced thrombosis is initiated by the adsorption asma protein, followed by adhesion and activation of platelets. **Figure 3** s the amount of plasma protein adsorbed on the surface bearing α-oester groups in comparison with the surfaces carrying poly(MPC) hes. As anticipated, the adsorption of plasma protein was suppressed as a equence of hydrophilic nature of phosphorylcholine groups of poly(MPC) hes. The complete absence of platelet adhesion of poly(MPC) brushes contacting with human PPP (**Figure 4**) confirmed the blood compatibility oly(MPC) brushes. On the other hand, there were a number of platelets ed on the positive controlled surface of poly(methyl methacrylate) MA) brushes prepared from the same α-bromoester surface.



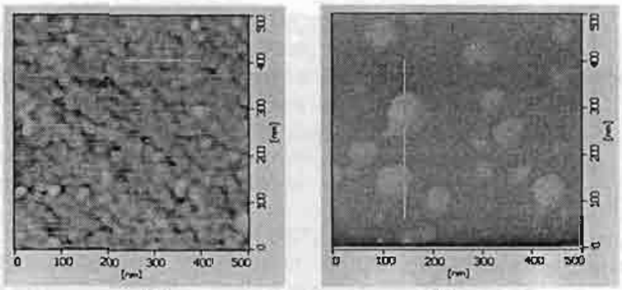
re 3. The total amount of protein adsorbed on substrates after contacting PPP for 3h.

Surface-initiated Polymerization of MPC from Mixed Tris ethylsiloxy)silyl/silanol surface. The extremely slow kinetic of a ion between silanol groups and tris(trimethylsiloxy)chlorosilane allows ficient control of the surface density of tris(trimethylsiloxy)silyl groups on the surface. It has been reported earlier by Fadeev and McCarthy that the nanopores containing silanol groups were capable of reacting with a number of smaller silane reagents.[6] Poly(MPC) and PMMA brushes were grown from mixed tris(trimethylsiloxy)silyl/α-bromoester surface prepared from mixed tris (trimethylsiloxy)silyl/silanol surface. The surface morphologies shown in Figure 5 suggested that the nano-scale poly(MPC) brushes can be formed from the mixed surface in contrast with PMMA brushes.



(a) (b)

Figure 4. SEM micrographes of substrates after contacting with PPP for 1h: (a) poly(MPC) brushes, (b) PMMA brushes.



(a) (b)

Figure 5. AFM micrographs of (a) poly(MPC) brushes, (b) PMMA brushes prepared from mixed tris(trimethylsiloxy)silyl/α-bromoester surface

Conclusions

It has been proven that surface-initiated polymerization of poly(2-methacryloyloxyethyl phosphorylcholine (poly(MPC)) by atom transfer radical process can be successfully used for generating blood compatible surface. The growth of poly(MPC) brushes can be efficiently controlled by reaction time, reaction media. The results of protein adsorption and platelet adhesion implied that the thin layer of poly(MPC) brushes is sufficient to improve blood compatibility of material surface. The appearance of nanoscale domain on the AFM micrograph of tris(trimethylsiloxy)silyl/poly(MPC) brushes implied that poly(MPC) brush was distributed nanoscopically on the surface. We anticipate that MPC polymer brushes with well-defined molecular weight and graft density would allow a better control of the blood compatibility at microscopic or nanoscopic scale of material's surface. The correlation between the density of immobilized poly(MPC) brushes with blood compatibility is a subject of ongoing studies and will be later discussed during the meeting.

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International Presentation #2

Poster Session Tuesday, 25th November 2003- 16.30-18.30

lymer Engineering, Processing and Characterization *(continued)* Abstracts p. 132-136

5 [361]
rospun non-woven fabrics of poly(ε-
olactone) and their biodegradation by
cultures of soil filamentous fungi
*aku Ohkawa, Hakyong Kim, Keunhyung
and Hiroyuki Yamamoto*

5 [362]
ct of solution properties on
rospinning of poly(ethylene oxide)
*Ho Park, Won Keun Son, Mi Yeon
k*

P-25 [373]
Rigid-rod polyesters with flexible side chains- Phase behavior including nematic, layered and hexagonal columnar phases in poly(*p*-biphenylene terephthalate) with alkoxy side chains.
Kai Fu, Masato Sone, Masatoshi Tokita and Junji Watanabe

P-25 [374]
Helical undulation in cholesteric planar texture formed by polypeptide: PBDoLG
Jirakorn Thisayukta and Junji Watanabe

P-25 [378]
UV/ozone surface modification process for continuous polymer composite manufacturing: Reactor design
Paisan Khanchaitit and Duangdao Aht-Ong

P-25 [450]
Elastic Modulus and Molecular Structure of Interconnecting Amorphous Section in an Oriented Crystalline Polymer
Ulmas Gafurov

P-25 [451]
Internal Energy Contribution of Polymers during Elongation
Xiaozhen Yang

lloids, Surfaces and Interfaces Abstracts p. 35-40

5 [030]
e fitting of the drug release from layered
ts containing ethylcellulose after acetone
r treatment
watchai Phaechamud

5 [080]
ace modification of Nafion® membranes
ugh plasma treatment
*gchan Bae, Heung Yong Ha, In–Hwan
nd Dukjoon Kim*

5 [082]
lation of enzymatic activity by
pholipid polymer conjugated with
rate
*Takei, Junji Watanabe, Madoka
i, and Kazuhiko Ishihara*

5 [102]
ace ordering of a fan-shaped molecule
fluorinated chain
*Ki Yoon, Su Rim Lee, Sang-Hyun Park,
Sun Min and Hee-Tae Jung*

5 [123]
ication of nano-arrays of metal dots with
-high density induced from self-
mbly of block copolymer
*ung Kwon, Dae-Geun Choi, Jong-Ryul
g, Dae-Hwan Jung,Sung-Chul Shin,
g-Man Yang, and Hee-Tae Jung*

[177]
esis and Aqueous Solution Properties of
Thermosensitive Polymer, Poly(ethyl
dyl ether), and its Block Copolymer with
ethylene oxide)
*hiro Ogura, Aya Koide, Shin-ichiro
yashi and Masayoshi Watanabe*

P-26 [181]
Surface Modification of carbon nanotube and carbon nanofiber by grafting of polymers
Norio Tsubokawa, Manabu Mikuni, Jinhua Chen, Wei Gang, Kazuhiro Fujiki

P-26 [240]
Specific selectivity for ortho-isomers and cyclodextrin by poly(vinyl pyridine)-grafted silica
Dong Wei, Takamasa Mimaki, Hamid Reza Anarian, Mahanaz Derakhshan, Toshihiko Sakurai, Makoto Takafuji, Shoji Nagaoka and Hirotaka Ihara

P-26 [241]
Preparation of poly(1-vinylimidazole)-grafted magnetic nano particles and its application to metal ion adsorbent
Shunichi Ide, Makoto Takafuji, Kenji Kato, Zhenghe Xu and Hirotaka Ihara

P-26 [242]
Enhanced molecular-shape selectivity and enantioselectivity through β-structural poly(L-alanine) on silica
Makoto Takafuji, Atsuomi Shundo, Toshihiko Sakurai, Shoji Nagaoka, Hirotaka Ihara

P-26 [287]
Surface modification of natural rubber by hydroxylation reaction
Porntip Rojruthai, Wipawee Pongsuwan, Yasuyuki Tanaka, and Jitladda Sakdapipanich

P-26 [288]
Adhesion between natural rubber and hydrogenated nitrile rubber
Anothai Saelee, Krisda Suchiva, Jacques Schultz and Marie-France Vallat

P-26 [349]
Aggregation structure and molecular motion at surface of isotactic polypropylene films
Atsushi Sakai, Keiji Tanaka, Toshihiko Nagamura, Atsushi Takahara, and Tisato Kajiyama

P-26 [357]
An effect of chain confinement on segmental motion at polystyrene surfaces
Kei-ichi Akabori, Keiji Tanaka, Toshihiko Nagamura, Atsushi Takahara and Tisato Kajiyama

P-26 [412]
Blood-compatible polymer brush of poly(2-methacryl-oyloxyethyl) phosphorylcholine)
Piyawan Suk-In, Suda Kiatkamjornwong, Yasuhiko Iwasaki, Junji Watanabe, Kazuhiko Ishihara, and Vipavee P. Hoven

P-26 [434]
Study on Admicellar Polymerization of Styrene on Silica from Rice Husk
Thanawat Ou-udomying, Ratana Rujiravanit, Manit Nithitanakul, and Richard M. Laine

P-26 [435]
Characterization of Polystyrene Formed via Admicellar Polymerization: Effect of Initiator Concentration
Nattharika Aumsuwan, Nang Sam Hom, Manit Nithitanakul, Boonyarach Kitiyanan, and John H. O'Haver

P-26 [439]
Acid-containing Tyrosine-derived Polycarbonates: Wettability and Surface Reactivity
Adisorn Poopattanapong; Joachim Kohn; Vipavee P. Hoven

Blood-Compatible Polymer Brush of Poly(2-methacryloyloxyethyl phosphorylcholine)

Piyawan Suk-In[1], Suda Kiatkamjornwong[2], Yasuhiko Iwasaki[3], Junji Watanabe[4], Kazuhiko Ishihara[4], Vipavee P. Hoven[5*],

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Abstract

The preparation of biocompatible polymer brush was accomplished by surface-initiated atom transfer radical polymerization (ATRP) of 2-methacryloyloxyethyl phosphorylcholine (MPC) from surface-tethered ∝-bromoester in the presence of CuBr/bpy. Polymerization from surface occured rapidly at room temperature in aqueous media as opposed to alcoholic media. We found that the thickness of polymer brushes increased almost linearly with reaction time. The existence of phospholipid analogous groups on the surface was revealed by X-ray photoelectron spectroscopy (XPS). As demonstrated by water contact angle measurements, the surfaces having PMPC brush showed improved hydrophilicity. The blood compatibility of PMPC brushes was evaluated by protein adsorption and platelet adhesion. The fact that there is such a small amount of protein adsorption and the platelet adhesion is absent on PMPC brushes supported the assumption that the polymer brushes are blood-compatible independent of the thickness in the range of 11-50 Å.

Keywords: polymer brush, atom transfer radical polymerization (ATRP), phospholipid polymer, blood compatibility, surface-initiated polymerization

Introduction

The control of surface properties is a key significance to several commercially important technologies ranging from biotechnology to advanced microelectronics. One of the novel surface modification methods is to use polymer brushes. Conventionally, polymer brushes are prepared by adsorption of block copolymers or by covalent grafting of end-functionalized polymer.[1] The former method suffers from its noncovalent nature leading to the unstable attachment. While the latter is not effective due to the fact that grafted polymer chains can sterically shield the remaining active sites on the surface, resulting in limited graft density and thickness of polymer brushes. Until recently an alternative approach of "grafting-from" method or surface-initiated polymerization has been introduced.[2-3] Since the polymer chain growth is generally promoted from initiators which are attached to the surface, this approach can provide higher graft density and stable attachment.

There is growing interest in atom transfer radical polymerization (ATRP) since it was discovered in 1995.[4] The living characteristic and the compatibility with a variety of functional monomer render ATRP an attractive method for surface-initiated polymerization in producing well-defined polymer brushes. The process allows a better control of target molecular weight and molecular weight distribution. The success of ATRP in synthesizing hydrophilic polymers provides an additional advantage over the traditional living ionic polymerization. Research work conducted by Armes and coworkers has demonstrated that the polymerization of some hydrophilic monomers such as 2-hydroxyethyl methacrylate (HEMA), methoxy-capped oligo (ethylene glycol)methacrylate (OEGMA), 2-methacryloyloxyethyl phosphorylcholine (MPC) can even be performed at ambient temperature in aqueous or alcoholic media.[5-6]

As inspired by the biomembrane-like structure, a methacrylate monomer bearing a polar phospholipid group, 2-methacryloyl oxyethyl phosphorylcholine (MPC) was developed.[7] The excellent blood compatibility of the MPC-containing materials is proven to originate from the capability to induce self-assembly of natural phospholipids. Since hydrophilic MPC homopolymer possesses inferior mechanical properties, MPC is generally used in the form of copolymers, polymer coating and grafting. The issue related the formation of grafted PMPC with well-defined structure, molecular weight and graft density is a subject of considerable interest and yet to be explored. Here we propose a way to generate biocompatible polymer brushes by surface-initiated polymerization of MPC using ATRP in both aqueous and alcoholic media. Blood compatibility was addressed in terms of plasma protein adsorption and platelet adhesion. We anticipate that MPC polymer brushes with well-defined molecular weight and graft density would allow a better control of the blood compatibility at microscopic or nanoscopic scale of material's surface.

Experimental

Materials and Methods

Toluene anhydrous (99%), phosphate buffer saline (PBS), bicinchonic assay kit (QuantiPro™ BCA assay) and protein standard (Bovine Serum Albumin: BSA) were obtained from Sigma-Aldrich. *2*-Bromoisobutyryl bromide (98%), *2,2'*-bipyridyl (99%), copper(I) bromide (99.99%), dimethoxy ethane, sodium dodecyl sulfate (SDS) and sodium sulfate anhydrous were purchased from Fluka. Dimethylethoxysilane was obtained from Gelest, Japan. *2*-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized by a method previously described.[7] Ultrapure distilled water was obtained from Mill-Q Lab system. Silicon wafer was obtained from Siltron Inc. Korea. Platelet-poor plasma (PPP) and platelet-rich plasma (PRP) were obtained from Thai Red Cross Society. X-ray photoelectron spectra were collected using ESCA-200, SCIENTA, Uppsala, Sweden. Contact angle meter model FACE, Japan was used for the determination of water contact angles. The thickness of polymer brush was characterized using L115C WAFER™ ELLIPSOMETER.

Preparation of Surface Grafted ∝-bromoester Initiators

Surface-tethered ∝-bromoester were prepared by soaking the cleaned disks in anhydrous toluene containing 2 mM of 3-(dimethylethoxysilyl)propyl-2-bromoisobutyrate[2-3] under nitrogen for a certain period of time at ambient temperature, followed by rinsing in toluene (3x), ethanol (3x), 1:1 ethanol:H_2O (2x), DI water (2x), ethanol (2x) and DI water (3x), respectively and dried under vacuum.

Preparation of Poly(MPC) Brushes

The substrates having surface grafted initiators were placed in a Schlenk flask containing CuBr (4.75 mg, 0.033 mmol), bpy (10.5 mg, 0.067 mmol), MPC (0.2 g, 0.67 mmol) in 30 mL degassed solvent was then added via cannular under nitrogen atmosphere. The polymerization was allowed to proceed for a set reaction time (30-180 min) at ambient temperature. The substrates were subjected to repetitive sonication in DI water and washed with IPA and dried under vacuum.

Blood Compatibility Test

Plasma protein adsorption of the substrates bearing polymer brushes after exposure to platelet-poor plasma (Thai Red Cross Society) was determined using bicinchonic assay kit (QuantiPro™ BCA assay). Scanning electron microscopy (SEM Model JSM-5800L) was used to observe the morphology of surface-adherent platelets.

Results and Discussion

Formation of PMPC Brushes

The surface active ∝-bromoester initiating system (I) was prepared by a reaction of allyl alcohol with *2*-bromoisobutyryl bromide in the presence of triethylamine followed by hydrosilylation of the resulting alkene terminated ester with dimethylethoxy silane.[2,8] The ∝-bromoester derivative (I), was attached to silanol groups on the silicon oxide surface (Fig. 1). The surface reaction has reached its completion after 24h of reaction with a layer thickness of 10 Å and a water contact angle of θ_A/θ_R = 71°/62°.

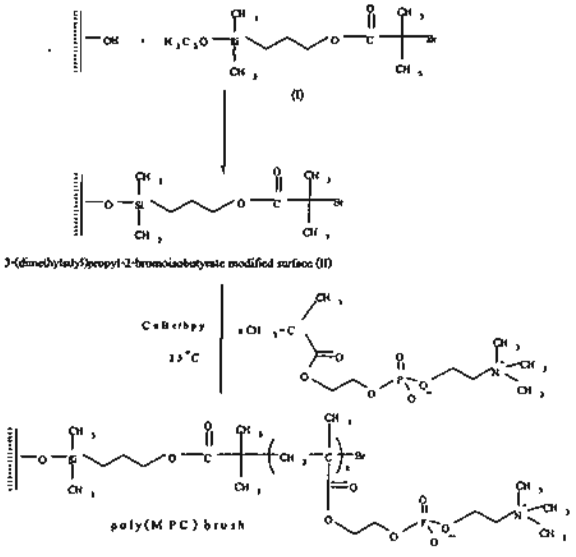

Fig. 1 Reaction scheme for preparation of PMPC brush.

PMPC brushes were grown from the surface bearing ∝-bromoester group (II) via ATRP mechanism in the presence of CuBr/bpy using H_2O or IPA as a reaction media (Fig. 1). Fig. 2 shows the relationship between PMPC brush thickness and reaction time. It was found that the layer thickness increased almost linearly with reaction time. The fact that polymer brushes grow more rapidly in more polar aqueous media in comparison with less polar alcoholic media is in good agreement with the data obtained in solution as described by Armes and coworkers.[6] The polar media, especially water facilitates the CuBr catalyst solubility and dissociation and thus accelerates the polymerization. Such a media, however, deteriorates the livingness of reaction and may cause premature termination as can be evidenced from the thickness of PMPC reaching its maximum of ~50 Å after 3h. The existence of phosphorylcholine groups of PMPC brushes was revealed by the appearance of N_{1s} and P_{2p} as outlined in Table 1. The N/P ratios varying in the range of 0.7-1.8 reasonably agree with the stoichiometric ratio of MPC unit. Due to a partial X-ray damage of bromine during XPS analysis, the percentage of Br_{3d} is neither quantitative nor consistent with the percentages of N_{1s} and P_{2p}. The surfaces having polymer brushes (θ_A/θ_R ~ 55/30°) were more hydrophilic than the surface bearing ∝-bromoester groups (θ_A/θ_R ~ 71/62°) which is an indication of hydrophilic phosphorylcholine moieties.

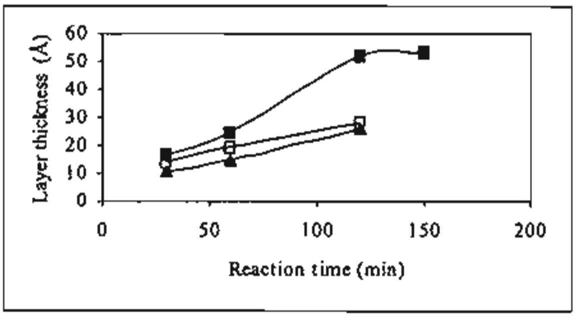

Fig. 2 Ellipsometric thickness of PMPC brushes prepared in H_2O (■), 50%IPA/H_2O (□), IPA (▲).

Table 1. Elemental surface composition (%) as analyzed by XPS.

Samples	C_{1s}	O_{1s}	N_{1s}	P_{2p}	θ_A/θ_R (°)
Silanol surface	45.28	27.10	-	-	59/49
α-bromoester surface	41.03	26.90	-	-	71/62
PMPC brush (2 h, H_2O)	48.89	23.13	0.81	1.12	54/29
PMPC brush (2 h, 50%IPA)	35.38	28.11	0.96	0.85	54/32
PMPC brush (2 h, IPA)	20.54	38.22	0.86	0.47	57/33

The growth of PMPC brush can also be promoted by an introduction of high MPC concentration. The PMPC thickness increased with the monomer concentration : PMPC thicknesses were 16 and 26 Å when [MPC] = 0.04 M. and 0.12 M, respectively. The independence of water contact angles on the PMPC thickness evidently implied that the growing of each polymer brush is simultaneous and living in character.

Blood Compatibility of PMPC Brushes

The amount of plasma protein adsorbed on material's surface is a primary factor in evaluating the blood compatibility of material. When a blood-incompatible material is in contact with blood, a surface induced thrombosis is initiated by the adsorption of plasma protein, followed by adhesion and activation of platelets. Fig. 3 shows the amount of plasma protein adsorbed on the surface bearing ∝-bromoester groups in comparison with the surfaces carrying PMPC brushes. As anticipated, the adsorption of plasma protein was suppressed as a consequence of hydrophilic nature of phosphorylcholine groups of PMPC brushes. The complete absence of platelet adhesion of PMPC brushes after contacting with human PRP (Fig. 4) confirmed the blood compatibity of PMPC brushes. On the other hand, there was few platelets sparingly adhered on the controlled α-bromoester surface.

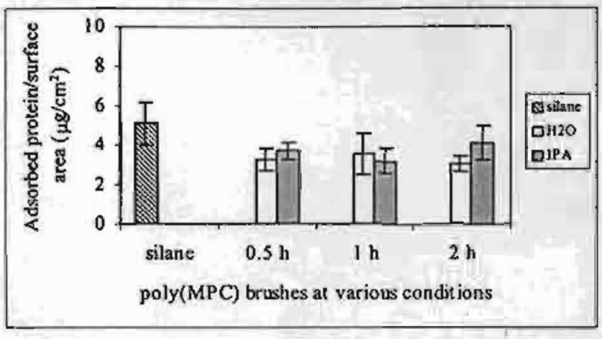

Fig. 3 The total amount of protein adsorbed on substrates after contacting with PPP for 3h.

α-bromoester surface PMPC brush (1h, H_2O)

PMPC brush (2h, H_2O) PMPC brush (2h, IPA)

Fig. 4 SEM micrographes of substrates after contacting with PRP for 1h.

Conclusion

It has been proven that surface-initiated polymerization by atom transfer radical process can be successfully used for generating blood compatible polymer brushes of poly(2-methacryloyloxyethyl phosphorylcholine (PMPC). The growth of PMPC brushes can be efficiently controlled by reaction time, reaction media as well as monomer concentration. The results of protein adsorption and platelet adhesion implied that the extremely thin layer of PMPC brushes is sufficient to improve blood compatibility of material surface.

Acknowledgement

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บทคัดย่อ
ABSTRACTS

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สมาคมวิทยาศาสตร์แห่งประเทศไทยในพระบรมราชูปถัมภ์
THE SCIENCE SOCIETY OF THAILAND
UNDER THE PATRONAGE OF HIS MAJESTY THE KING

คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
FACULTY OF SCIENCE
SRINAKARINWIROT UNIVERSITY

Abstract: A chelating ion-exchange membrane containing the hydroxamic acid functional group was synthesized from acrylamide-grafted-polyethylene (PE-g-AAm). The grafted polymer was obtained by 2-stage method. The polymer surface was first functionalized by decomposition of $K_2S_2O_8$ and subsequently grafted with acrylamide monomer by ceric ion technique. The chemical composition at the surface was analyzed by Attenuated Total Reflection Fourier Transforms Infrared Spectroscopy (ATR-FTIR) and ninhydrin method. Surface morphology of the grafted sample was characterized by Atomic Force Microscopy (AFM). The grafted polymer was then converted to poly(hydroxamic acid) by reacting with hydroxylamine hydrochloride under optimum condition. The existence of hydroxamic acid functional group was confirmed by ATR-FTIR. When the membranes were immersed in V^{5+}, Fe^{3+} and Cu^{2+} solutions, it was found that the membranes turned dark purple, deep brown and blue-green, respectively. The sorption behavior of the chelating membrane toward metal ions was also studied with UV-Visible spectroscopy. This behavior can not be observed in original PE and PE-g-AAm.

E0065-SYNTHESIS OF PHOPHORYLCHOLINE-CONTAINING POLYMER BRUSHES BY NANOPOROUS SURFACE-INITIATED POLYMERIZATION

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Abstract: Chemically grafted tris(trimethylsiloxy)silyl (tris(TMS)) monolayer on the silicon oxide substrate was used as a nanometer-scale template for controlling the graft density of phosphorylcholine-containing polymer brushes. Polymer brushes were synthesized by surface-initiated polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) from ∝-bromoester groups tethered to the residual silanol groups on the surface after creating a range of tris(TMS) coverage via atom transfer radical polymerization (ATRP) using CuBr/bpy as a catalytic system. The solvent, percentage of tris(TMS) coverage and polymerization time significantly influenced the thickness and morphology of PMPC brushes. Protrusions observed from AFM images of PMPC brushes evidently suggested that PMPC brushes distributed nanoscopically on the substrate. The size of protrusion and surface roughness corresponded quite well with the graft density of PMPC brushes.

E0067-Effects of Silane Coupling Agent on Natural Rubber Reinforced by Silica Generated *in situ* from Sol-Gel Process of Tetraethoxysilane in Latex

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Abstract: Sol-gel process of tetraethoxysilane (TEOS) was used to generate silica particles in natural rubber for preparing reinforced rubber by adding TEOS directly into concentrated latex. A sulfur-containing coupling agent, bis(3-triethoxysilylpropyl)tetrasulfide (TESPT), was added together with TEOS before the sol-gel step. The influences of the amount of TEOS, TESPT and ammonia on the physical properties of the composite were investigated by a statistical analysis method, namely 'two-level factorial design'. It was found that tensile modulus, tear strength, and hardness were the most significantly affected by TEOS content. The amount of TESPT affected the physical properties somewhat less. But ammonia content showed a negative effect. In other words, the NR composite with high mechanical properties can be obtained when adding a large amount of TEOS and TESPT without adding more ammonia to the latex mixture. In addition, the use of TESPT could reduce sulfur-cure time (t_{90}), compared to the composite without the coupling agent.

E0068-IMMOBILIZATION OF RGD PEPTIDES ON THE SURFACE OF TYROSINE-DERIVED POLYCARBONATE TO IMPROVE CELLULAR RESPONSE

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การสังเคราะห์พอลิเมอร์บรัชที่มีหมู่ฟอสโฟริลโคลีนโดยพอลิเมอไรเซชันริเริ่มบนพื้นผิวที่มีรูขนาดนาโน

SYNTHESIS OF PHOPHORYLCHOLINE-CONTAINING POLYMER BRUSHES BY NANOPOROUS SURFACE-INITIATED POLYMERIZATION

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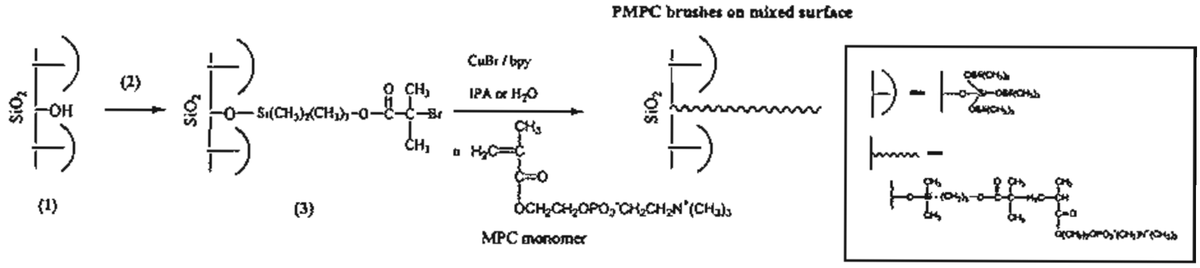
บทคัดย่อ: ซับสเตรตซิลิกอนออกไซด์ที่กราฟท์ด้วยโมเลกุลชั้นเดียวของตริสไตรเมทิลไซลอกซีไซลิล (ตริสทีเอ็มเอส) ถูกใช้เป็นแม่แบบที่มีสเกลในระดับนาโนเมตรในการควบคุมความหนาแน่นการกราฟท์ของพอลิเมอร์บรัชที่มีหมู่ฟอสโฟริลโคลีน สังเคราะห์พอลิเมอร์บรัชได้โดยการริเริ่มปฏิกิริยาพอลิเมอไรเซชันของ 2-เมทอะคริโลอิลออกซีเอทิลฟอสโฟริลโคลีน (เอ็มพีซี) จากหมู่แอลฟาโบรโมเอสเทอร์ซึ่งติดกับหมู่ไซลานอลที่เหลืออยู่บนพื้นผิวหลังจากถูกปกคลุมด้วยตริสทีเอ็มเอสเป็นปริมาณต่างๆด้วยอะตอมทรานส์เฟอร์แรดิคัลพอลิเมอไรเซชัน โดยใช้คอปเปอร์โบรไมด์/ไบไพริดีนเป็นระบบเร่งปฏิกิริยา ตัวทำละลาย เปอร์เซนต์การปกคลุมของตริสทีเอ็มเอส และเวลาในการทำปฏิกิริยาพอลิเมอไรเซชันส่งผลกระทบอย่างมีนัยสำคัญต่อความหนาและสัณฐานวิทยาของพีเอ็มพีซีบรัช ส่วนที่ยื่นนูนซึ่งสังเกตเห็นได้จากภาพเอเอฟเอ็มของพีเอ็มพีซีบรัชเป็นหลักฐานที่แสดงให้เห็นว่าพีเอ็มพีซีบรัชมีการกระจายตัวในระดับนาโนเมตรบนซับสเตรต ขนาดของส่วนที่ยื่นนูนและความขรุขระของพื้นผิวมีลักษณะสอดคล้องเป็นอย่างดีกับความหนาแน่นการกราฟต์ของพีเอ็มพีซีบรัช

Abstract: Chemically grafted tris(trimethylsiloxy)silyl (tris(TMS)) monolayer on the silicon oxide substrate was used as a nanometer-scale template for controlling the graft density of phosphorylcholine-containing polymer brushes. Polymer brushes were synthesized by surface-initiated polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) from ∝-bromoester groups tethered to the residual silanol groups on the surface after creating a range of tris(TMS) coverage via atom transfer radical polymerization (ATRP) using CuBr/bpy as a catalytic system. The solvent, percentage of tris(TMS) coverage and polymerization time significantly influenced the thickness and morphology of PMPC brushes. Protrusions observed from AFM images of PMPC brushes evidently suggested that PMPC brushes distributed nanoscopically on the substrate. The size of protrusion and surface roughness corresponded quite well with the graft density of PMPC brushes.

Methodology: Cleaned silicon oxide disks (1.5x1.5 cm^2) were covered with anhydrous toluene containing ethyldiisopropylamine and tris(trimethylsiloxy)chlorosilane (tris(TMS)Cl)). Reactions were carried out at 70°C under nitrogen for 1-3 days. After the disks were isolated, they were rinsed with a series of solvents before dried in an oven at

120°C for 10 min. Surface-tethered ∝-bromoester were then prepared by soaking the disks bearing mixed tris(TMS)/silanol groups (1) in anhydrous toluene containing 3-(dimethylethoxysilyl)propyl-2-bromoisobutyrate (2) under nitrogen at ambient temperature, followed by rinsing in a series of solvents. The substrates having surface grafted initiators (3) were placed in a Schlenk flask containing CuBr/bpy (1:2 by mole) (dissolved in solvent and bubbled for 1 h under nitrogen before use), MPC in degassed solvent was then added via cannula. The polymerization was allowed to proceed for a set reaction time at ambient temperature under nitrogen atmosphere. The substrates were removed from the solution, subjected to repetitive sonication in DI water and methanol, and soxhlet extraction with methanol before dried under vacuum.



Scheme 1 Synthesis of PMPC brushes using tris(TMS) monolayer as a template

Results, Discussion and Conclusion: The extremely slow kinetics of a reaction between silanol groups and tris(TMS)Cl allows an efficient control of tris(TMS) coverage on the surface. According to Table 1, a higher yield of silanization as a function of reaction time was confirmed by a higher carbon content from XPS analysis. The thickness of tris(TMS) monolayer reached its maximum of ~ 20 Å after 4 days of reaction. Initially there was a rapid rise in water contact angle to the value of 60°/43° within 12 h, followed by a gradual increase over a period of 1-4 days, indicating that the surface became more hydrophobic as the tris-TMS coverage was increased. Assuming the contact angle of 108° for the surface having 100% tris(TMS) and 0° for the surface having 100% silanol groups, we can calculate the percentage of tris(TMS) coverage as shown in Table1.

Table 1 Surface properties of substrates covered with a range of tris(TMS) monolayer

Sample	XPS atomic concentration (%) (15° take-off angle)			tris(TMS) coverage (%)	θ_a / θ_r (degree)	Tris(TMS) Monolayer Thickness (Å)
	Si	O	C			
silicon oxide	40.51	29.75	29.75	0	29/17	-
tris(TMS) 24 h	33.66	23.26	43.08	66	73/64	12
tris(TMS) 48 h	32.96	21.25	45.79	75	81/71	15
tris(TMS) 72 h	31.29	21.39	47.32	82	86/78	19

As demonstrated in Figure 1, the thickness of PMPC brushes which corresponded with the density and length of polymer brushes decreased as the percentage of tris(TMS) coverage increased. Due to the hydrophobicity of tris(TMS) groups surrounding ∝-bromoester initiators, the growth of polymer brush is more favorable in a more surface-wettable solvent, isopropanol (IPA) in this particular case, than water. As a result, PMPC brushes prepared in IPA are relatively thicker than those obtained in water. The growth of PMPC brushes from nanopores can also be tuned by reaction time. Figure 2 shows the thickness and receding water contact angle of the surface having 82% tris(TMS)/ PMPC brushes as a function of polymerization time. The longer the reaction proceeds, the thicker the polymer brush and the more hydrophilic the surface becomes.

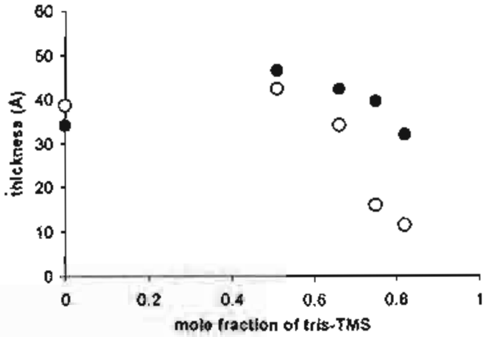


Figure 1 Thickness of PMPC brushes grown from the surface having a range of tris(TMS) coverage. (•) IPA and (○) water

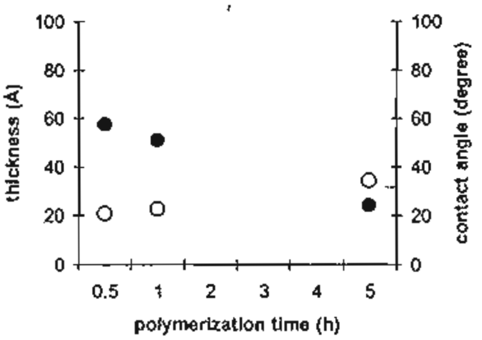
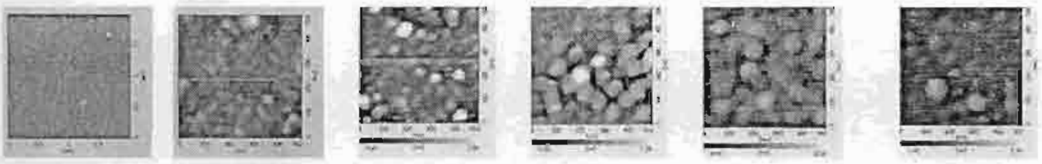


Figure 2 Thickness (○) and water receding contact angle (•) of the surface having 82% tris(TMS)/PMPC brushes as a function of reaction time

In order to investigate the spatial distribution of PMPC brushes, the mixed tris(TMS)/silanol surfaces having 82% tris(TMS) coverage were used as nano-scale templates for surface-initiated polymerization. The graft density of surface-tethered ∝-bromoester was varied as a function of reaction time (1-4 days) between the residual silanol groups in nanopores with (2). The polymerization was conducted in IPA for 1h. It was found that the cleaned silicon oxide substrate and PMPC brushes (without tris(TMS)) were almost featureless and relatively smooth. On the contrary, the surfaces having varied graft density of PMPC brushes exhibited protrusions having a diameter less than 100 nm. The size of protrusion was in good agreement with the average roughness (R_a). The protrusions are believed to represent self-aggregation of PMPC brushes in nanopores. Interestingly, the surfaces whose nanopores were almost completely covered with PMPC brushes (3-4 days) became quite smooth and the protrusions simultaneously diminished. Such behavior might stem from the fact that PMPC brushes were dense enough to be able to fill the nanopores and to eliminate the valley-and-hill features on the surface. This study has demonstrated that PMPC brushes having well-defined graft density can be generated using tris(TMS) monolayer as a nano-scale template. This would allow a better control of the property at microscopic or nanoscopic level of material's surface.



silicon oxide	PMPC	1 day	2 days	3 days	4 days
Ra = 0.2006	Ra = 0.1783	Ra = 0.4660	Ra = 0.9279	Ra = 0.2487	Ra = 0.1398

Figure 3 AFM images of PMPC brushes grown in IPA for 1h from the surface having 82% tris(TMS) coverage and a range of ∝-bromoester coverage which is varied as a function of reaction time: (1-4 days).

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Keyword: ATRP, polymer brush, nano-scale template, MPC, surface-initiated polymerization