Transactions of the Materials Research Society of Japan 32[2] 567-570 (2007)

Surface nanopatterning of phosphorylcholine unit by well-defined block copolymer containing 2-methacryloyloxyethyl phosphorylcholine

Ji Hun Seo, Ryosuke Matsuno, Tomohiro Konno, Madoka Takai, Kazuhiko Ishihara*
Department of Material Engineering, School of Engineering and Center for NanoBio Integration,
The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-8656, Japan
Fax: 81-3-5841-8647, e-mail: *ishihara@mpc.t.u-tokyo.ac.jp

Ordered surface patterning of biocompatible microdomain is potentially needed in many applications such as high sensitive biochips, biosensors, and nanobio devices for cell culturing. One of the efficient ways in the fabrication of patterned surface is using the microphase separation of well-defined block copolymers by controlling molecular weight and film formation process. Thus we synthesize several compositions of block copolymers containing 2-methacryloyloxyethyl phosphorylcholine (MPC) which is hydrophilic and shows excellent biocompatibility, and polydimethylsiloxane (PDMS) which has hydrophobic nature and usually used as soft lithography materials. All of the copolymers were synthesized by the atom transfer radical polymerization (ATRP) method at room temperature. The kinetics plot and molecular weight plot shows that the polymerizations of block copolymers were well controlled, thus the compositions of block copolymers were controllable. Two kinds of synthesized copolymers: the longest MPC segment and the shortest one, were cast on Si(100) substrate by spin casting or solvent casting. By using the IR-multichannel viewer and atom force microscope, we could conclude that the film formed by the longest MPC segment could not generate the well defined surface structure due to its long chain aggregation. By using the copolymer containing the shortest MPC segment, we could generate a nanoscale ordered surface patterning of phorsphorylcholine unit.

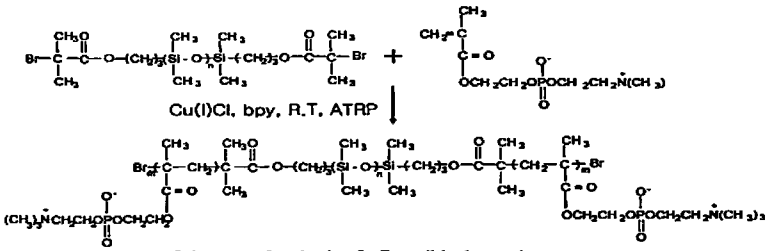
Key words: triblock copolymer, MPC, ATRP, phase separation

1. INTRODUCTION

To generate a well defined biocompatible domain structure is one of the challenging fields in the nanobio technology because of its potential utility in high sensitive biochips, biosensors, and research tools for cell movement [1-3]. One of the effective methods to construct the nanoscale surface patterning is introducing self organized system of the block copolymer.

Block copolymers consist of two or more chemically different homopolymers covalently bonded together to form a larger macromolecules. Since each blocks have their own distinctive properties, block copolymers have been suggested for many applications such as polymer micelles for nano template [4], nanocomposite for filler [5], and template for synthesize of nanowire [6]. One of the interesting and challenging fields of block copolymer research is about its self assembly nature. If the constituent blocks are immiscible, phase separation is directly induced on a various scale related to the chain length, volumetric composition and film formation process. A number of researches have been carried out to understand this phenomenon theoretically and experimentally [7-10]. The most of these researches have focused their final goals on the construction of long range ordered and defect free surfaces of block copolymer films. As a result, several outstanding methodologies to prepare the well defined domain structures have been produced [11-14]. The general way of generating surface nanostructures by using block copolymer can be classified into two main methods: using nonselective solvent and selective solvent. When block copolymer solutions are made by using a nonselective solvent, i.e. both of the blocks are dissolved well, filming conditions such as annealing temperature, annealing time, film thickness, substrate condition, etc. play an important role in controlling domain structures [15]. Compared with this, when block copolymer solutions are made by using a selective solvent, i.e. only one of the polymer blocks dissolved well, block copolymer micelles are previously formed in solution. This micellar structures are of great utility as nanoscale template and have a potential to be a main tool of a nanotechnology because of its simplified process. In this selective solvent system, nanopatterns can be simply tuned by adjusting solution concentration and block copolymer composition [16].

Here we address a simple direct way of surface patterning of biocompatible domain structure by using micellar structure of triblock copolymer. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was used as a building unit of A block because of its excellent biocompatible nature and ability to inhibit nonspecific protein absorption [17-20]. Poly(dimethyl siloxane) (PDMS) which is usually used as soft lithography and imprinting materials was used as B block unit because of its high affinity with proteins caused by hydrophobic interaction in solution [21]. In order to precisely control the block composition, atom transfer radical polymerization (ATRP) method was used because of its broad utility to synthesize block copolymer by using very different kind of two or more species like MPC and PDMS [22, 23]. The final goal of this study is to construct the well organized and stable phase separated phosphorylcholine patterning that the biomolecules can be selectively absorbed.



Scheme 1. Synthesis of ABA triblock copolymer

2. EXPERIMENTAL

2. 1. Materials

Poly(dimethylsiloxane) (Mn=1628) was kindly received from Dow Corning (Japan) and n=14 was purchased form Gelest (USA). 2, 2'-bipyridyl was obtained from Kanto Chemical (Japan) and solvent used in this study was purchased from Wako Chemical (Japan). Ally 2-bromoisobutyrate, Cu(I)Cl, 2-methyl- 1, 4 - naphthoquinon, and Karsted's catalyst were purchased from Sigma Aldrich (MO., USA).

2. 2. Synthesis of ABA block copolymer

PDMS macroinitiator was synthesized as reference [23]. A typical polymerization is as follows: 0.332g (0.232mmol) of synthesized PDMS macroinitiator was placed into a 20ml flask with 2.5g MPC (8.5mmol) and 5ml methanol. The solution was bubbled with Ar gas for 10 min. Then the mixture of 0.046g (0.46mmol) of Cu(I)Cl and 0.145g(0.928mmol) of 2,2'-bipyidyl was put into the flask and sealed with the rubber septum. Syringe capped Ar balloon was placed at the septum and the mixture was stirred at room temperature until homogeneous maroon solution was formed. Periodically 0.1ml aliquots of reaction mixture were removed for kinetic and molecular weight analysis. After the reaction, 10ml of the methanol was pored into the mixture then filtered through an alumina column to remove the transition metal complex. Clear colorless reaction mixture was then recrystallized in the large amount of ether and chloroform (7:3) mixed solvent followed by a dialysis process for a day. After freeze dried, white block copolymer was gained. Four different compositions of polymers were synthesized for the SEC (size exclusion chromatography) analysis and the polymer with the shortest MPC segment used in this report was synthesized same as above except that the amount of monomer was 1/5 scale.

2. 3. Preparation of polymer film

Block copolymer was dissolved in hexafluoro isopropanol (HFIP) (0.5wt%) and then cast on the Piranha solution (H_2O_2+H_2SO_4) treated SiO_2/Si(100) wafer. Solvent casting was carried out as follows: 5μl of the solution was cast on the Si substrate followed by vacuum treatment to remove the solvent.

2. 4. ATRP characterization

Monomer conversion was calculated by comparing NMR peak integrals due to the group in MPC monomer at δ=5.5 and δ=6.0 to those of the α-methyl group in the polymer chain at δ=0.5-1.1. SEC (size exclusion chromatography) measurement was conducted using a Jasco RI-1530 detector with a poly(methyl methacrylate) standard in HFIP (flow rate 0.2ml / min.).

2. 5. Surface characterization

Surface characterization of cast film was carried out by using 2 dimensional IR-multi channel viewer (JASCO IMV-4000, Japan), Nanoscope IIIa multimode Atomic Force Microscope (Nippon Veeco Co., Tokyo, Japan) and Transmission Electron Microscope (Hitachi, H-800, acceleration voltage : 100 kV)

3. RESULT AND DISCUSSION

3. 1. Synthesis of ABA block copolymer

The overall reaction scheme is illustrated in shceme1. A number of ATRP reactions initiated from various types of PDMS macroinitiator have been studied in the last decade [23-26]. Since 2-bromoisobutyrates are efficient initiators for polymerization of methacrylates [27], we synthesized PDMS macroinitiator containing this species by reference [23]. Polymerization of MPC initiated by this PDMS macroinitiator was carried out at room temperature.

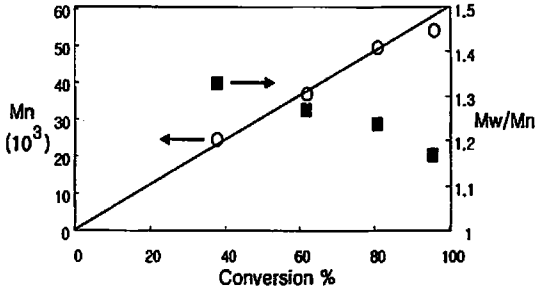


Fig. 1. Changes in the molecular weight of block copolymer during ATRP

In Fig. 1, the molecular weight (Mn), and polydispersity (Mw/Mn) were plotted against the monomer conversion. As indicated in the equation (1), a linear increase of Mn versus monomer conversion was observed. Although the kinetics data was not shown in here, semi-logarithmic monomer conversion with reaction time and linear kinetics plot could make us confirm that the overall ATRP condition was well controlled and monomer conversion could be over 90% at room temperature. Based on the relationship between polymerization time and monomer conversion, we could synthesize several compositions of well defined block copolymers. (The block copolymer with the shortest MPC segment was synthesized same as above except that the amount of monomer was 1/5 scale because it was hard to control the short reaction time i.e. control the monomer conversion). Table 1 showed the representative 2 kinds of block copolymers (the shortest

and the longest MPC segment).

$$Mn_{theory} = Mn_{macroinitiator} + \frac{[Mn]_{monomer}}{[Mn]_{macroinitiator}} Mn_{monomer} \times Conversion \quad (1)$$

Fig. 2 shows the SEC trace of the ATRP and it demonstrates how synthesized block copolymers are well defined. Although some shoulder was appeared at conversion 96% peak, the overall growing triblock copolymer peak remained monodal (Mw/Mn=1.16) throughout the reaction, and the polymer peak is continuously shifted to the higher molecular weight with monomer conversion. It indicates that all of the radical species in reacting mixtures are homogeneously participating in polymerization.

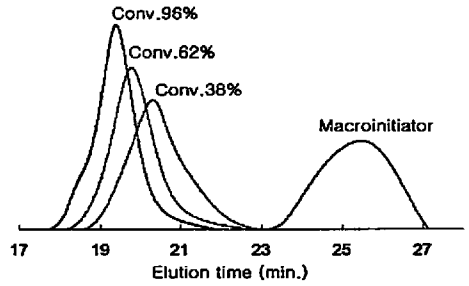


Fig. 2. SEC chart of PDMS macroinitiator and block copolymer obtained after ATRP
PDMS macroinitiator Mw/Mn=1.48

Furthermore the polymer peak becomes sharpened as it shifts to the high molecular weight region. As indicated in equation (2) [27], the theoretical polydispersity in ATRP must be decreased with monomer conversion (C). Polydispersities of each triblock copolymers are also plotted on Fig. 1 with molecular weight.

$$Mw/Mn = 1 + \left[\frac{[RX]_0 K_p}{K_{deact}[D]}\right]\left[\frac{2}{C} - 1\right] \quad (2)$$

$[RX]_0$: concentration of initiator
[D] : concentration of deactivator
K_p : radical propagate rate constant
K_{deact} : deactivation rate constant
C : monomer conversion

Based on these results, we could conclude that overall ARTP was well controlled, thus well defined triblock copolymers composed of PMPC and PDMS were synthesized even though two units have extremely different solubility to the methanol.

Table 1. Representative synthesized block copolymer

symbol	*A:B molar ratio (NMR)	Mn (SEC)	Mn (NMR)	Mw/Mn
M12P14	1:0.58	**	8.55k	**
M82P22	1:0.13	68.9k	50.3k	1.16

*: A indicates MPC unit and B is repeating unit of PDMS respectively
**: SEC measurement was impossible because of its limited amount and solubility

3. 2. Solvent casting of M82P22

Solvent casting method was used to induce the phase separation of block copolymer film (M82P22). Fig. 3 shows the optical microscopes of the casting film and 2-dimensional IR mapping (carbonyl functional group1720cm^{-1}) equivalent to that.

Two different kinds of morphologies were detected in the same film: MPC centered and PDMS centered aggregations. As the carbonyl functional groups are concentrated at the center of the aggregation, it could be concluded that Figs. 3 (a) and (b) show the MPC centered aggregation. Contrary to this, Figs. 3 (c) and (d) show reverse tendency i.e. PDMS centered aggregation. Further studying is now in progress to clearly understand this result. Although more researches must to be done to clarify this morphological phenomenon, we could conclude that triblock copolymers containing the longest MPC segment have a problem to make self assembled phosphorylcholine patterning due to its aggregation.

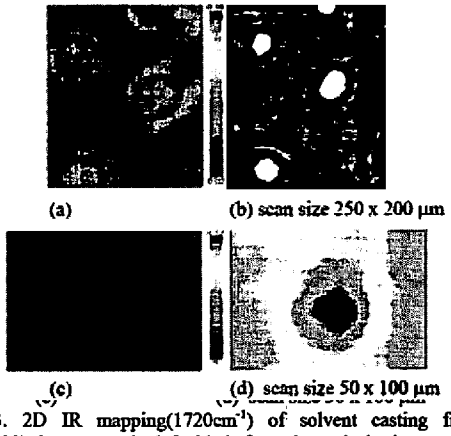


Fig. 3. 2D IR mapping(1720cm^{-1}) of solvent casting film (M82P22), images on the left side is from the optical microscopes and the right side is 2D IR mapping(1720cm^{-1}). The much of carbonyl group is showed as bright color in the right image according to the degree bar in the center. Carbonyl mapping was strongly detected in the center of the aggregation in (a) and (b). Contrary to this corona have the strong carbonyl peak in (c) and (d) (the different site on the same film).

3. 3. Solvent casting of M12P14

Solvent casting was carried out by using the triblock copolymer containing the shortest MPC segment among the synthesized polymers. Fig. 4 shows the TEM (bright field image) and AFM height / phase images of cast morphology with 0.3 wt. % concentration. Since cast film was oxidized with OsO_4 before observation, PMPC phase might be showed as dark region in TEM observation.

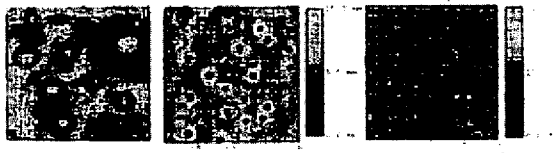


Fig. 4. TEM and AFM image (height and phase) of cast film (M12P14 0.3 wt. % scan size 1μm x 1μm, height scale bar : 0-10nm, phase scale bar : 10°-30°)

Several researches have been carried out to explain the morphologies of polymer surfaces by using height and phase mode of AFM.[28, 29] In most of the cases, it was difficult to know which part of the images were stiff or lower in friction by tapping mode AFM. Contrary to this, they could easily predict which phase is more hydrophilic by comparing height and phase images of AFM. Hydrophilic phase generally higher than hydrophobic phase about 1-2nm in height mode AFM and phase image was equivalent to height image. [28] Since we could confirmed that the outer PMPC phase by bright field TEM image, we could also confirmed that the phase images was

well fit with height mode i.e. higher hydrophilic part was higher degree of phase images. Fig. 4 explained the phase separated micellar structure which has PMPC as an inner phase and PDMS as an outer phase. Since dark side of TEM image was PMPC and it is very well known hydrophilic material, our height mode AFM data was well matched as expected because PMPC was about 2nm higher than PDMS phase. Moreover phase image was well matched with height mode image i.e. not inverse image with height image.

Although we could construct the phase separated micellar structure, the overall network, i.e. continuous phase separated morphology was not yet constructed by using 0.3 wt. % polymer solution. Ordered patterns of micellar structures by using selective solvent were previously reported by various research teams [30-32]. Such achievements suggested that the polymer compositions and the concentration of polymer solution play a dominant role in controlling the surface structure. Thus we cast the film by using the 0.5 wt. % polymer solution. Fig. 5 shows the height and phase mode AFM images of the casting film with 0.5 wt. % concentration.

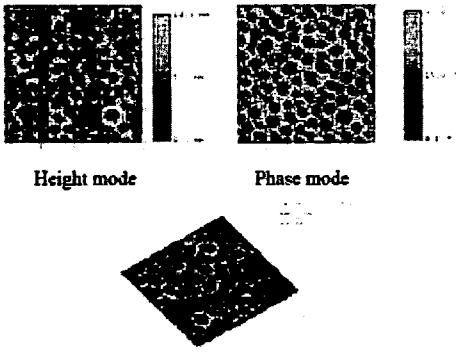


Fig. 5. Height and phase mode AFM image of solvent casting film (M12P14 0.5 wt. % scan size 1μm x 1μm, height scale bar : 0-10nm, phase scale bar : 10°-30°)

Since we confirmed that the dark region indicates the PDMS phase in both of the height and phase images comparing to the bright region by TEM observation, it could be thought that the PDMS centered micellar aggregation was formed.

This image indicates that the casting condition with 0.5 wt. % polymer solution was proper and minimum concentration for generating connected network of each micellar aggregations. We are now studying the best casting condition to generate the long range ordered and close packed stable surface structure.

4. CONCLUSIONS

ABA triblock copolymers composed of PMPC and PDMS with various compositions were synthesized by well controlled ATRP method. Each block copolymers have low polydispersity (<1.3) thus well defined block copolymers are used to cast film. Two types of synthesized block copolymers were used to make a film of self assembled micellar structure: the longest MPC chain length and the shortest one. Casting film formed by block copolymer containing longer chain makes it hard to form a self organized surface structure due to its long chain aggregation. Phosphorylcholine patterning, induced by self organized micellar structure, was successfully conducted by using the triblock copolymer containing shortest MPC segment with 0.5 wt. % concentration. Further researches are in progress to generate the long range ordered and stable surface structure. The final object of this study is to apply this thin film as a nano template for selective absorption of biomolecules.

REFERENCES

[1] Y. Cai and B. M.Ocko, *Langmuir*, **21**, 9274 (2005)
[2] H. Agheli, J. Malmstrom, E. M. Larsson, M. Textor and D. S. Sutherland, *Nanoletters*, **6**, 1165 (2006)
[3] H. Kaji, T. Kawashima, M. Nishizawa, *Langmuir*, **22**, 10784 (2006)
[4] Samson A. Jenekhe and X. Linda Chen, *Science* **283**, 372 (1999)
[5] K. Ishizu and K. Ochi, *Macromolecules*, **39**, 3238 (2006)
[6] J. Q. Lu, S. S. Yi, *Langmuir*, **22**, 3951 (2006)
[7] M. Larranaga, N. Gabilondo, G. Kortaberria, E. Serrano, P. Remiro, C. C. Riccardi, I. Mondragon, *Polymer*, **46**, 7082 (2005)
[8] G. Krausch and R. Magerle, *Adv. Mater.* **14**, 1579 (2002)
[9] A. Knoll, A. Horvat, K. S. Lyakhova, G. Krausch, G. J. A. Sevink, A. V. Zvelindovsky, and R. Magerle, *Phys.Rev.Lett*, **89**, 035501 (2002)
[10] D. Simon Connell, S. Collins, J. Fundin, Z. Yang, and I. W. Hamley, *Langmuir*, **19**, 10449 (2003)
[11] C. Park, Y. Joy, J. M. Fasolka, Anne M. Mayes, C.A. Ross, and Edwin L. Thomas, *Appl. Phys. Lett.* **79**, 6 (2001)
[12] S. O. Kim, H. H. Solak, M. P. Stoykovich, N. J. Ferrier, J. J. de Pablo and P. F. Nealey, *Nature*, **424**,24 (2003)
[13] M. Kimura, M. J. Misner, T. Xu, S. H. Kim and T. P. Russell, *Langmuir*, **19**, 9910 (2003)
[14] C. De Rosa, C. Park, E.L. Thomas, and B. Lotz, *Nature*, **405**, 25 (2000)
[15] S. Krishnamoorthy, C. Hinderling, and H. Heinzelmann, *MaterialsToday*, **9**, 40 (2006)
[16] T. Xu, H. C. Kim, J. DeRouchey, C. Seney, C. Levesque, P. Martin, C. M. Stafford and T. P. Russell, *Polymer*, **42**, 9091 (2001)
[17] K. Ishihara, N. Nakabayashi, K. Fukumoto, J. Aoki, *Biomaterials*, **13**, 145 (1992)
[18] Y. Iwasaki, N. Nakabayashi, K. Ishihara, *J. Biomed. Mater. Res.*, **57**, 72 (2001)
[19] T. Furuzono, K. Ishihara, N. Nakabayashi, Y. Tamada, *J. Polym. Sci.*, **73**, 2541 (1999)
[20] K. Ishihara, Y. Iwasaki, S. Ebihara, Y. Shindo, N. Nakabayashi, *Col. and Surf. B: Biointer.* **18**, 325 (2000)
[21] J. P. Renault, A. Bernard, A. Bietsch, B. Michel, H. R. Bosshard, and, E. Delamarche, *J.Phys.Chem.* B, **107**, 703 (2003)
[22] Y. Ma, Y. Tang, N. C. Billingham, and S. P. Armes *Macromolecules*, **36**, 3475 (2003)
[23] P. J. Miller and K. Matyjaszewski *Macromolecules*, **32**, 8760(1999)
[24] Y. Nakagawa, P. J. Miller and K. Matyjaszewski, *Polymer*, **39**, 5163 (1998)
[25] D. A. Brown, G. J. Price, *Polymer*, **42**, 4767 (2001)
[26] J. Pyun, S. Jia, T. Kowalewski and K. Matyjaszewski *Macromol.Chem.Phys.* **205**, 411 (2004)
[27] K. Matyjaszewski and J. Xia, *Chem.Rev.* **101**, 2921 (2001)
[28] Bryan B. Sauer, R. Scott McLean, and Richard R. Thomas, *Langmuir*. **14**, 3045 (1998)
[29] K. Hong Tan, Min Guo, Rongni Du, Xingyi Xie, Jiehua Li Yinping Zhong, Qiang Fu, *Polymer*. **45**, 1647 (2004)
[30]S. A. Jenekhe and X. L. Chen, *Science*, **283**, 372 (1999)
[31] J. P. Spatz, T. Herzog, S. Mobmer, P. Ziemann, and M. Moller *Adv. Mater.* **11**, 149 (1999)
[32] Y. Chen, Z. Wang, Y. Gong, H. Huang, and T. He, *J.Phys.Chem.B*, **110**, 1647 (2006)

(Received December 9, 2006;Accepted April 2, 2007)