Controlled Release of Bovine Serum Albumin using MPEG–PCL Diblock Copolymers as Implantable Protein Carriers

Moon Suk Kim,[1] Kwang Su Seo,[1,2] Hoon Hyun,[1,2] Gilson Khang,[2] Sun Hang Cho,[1] Hai Bang Lee[1]

[1]*Nanobiomaterials Laboratory, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong, Daejeon 305-600, Korea*
[2]*Department of Polymer/Nano Science and Technology, Chonbuk National University, 664-14, Duckjin, Jeonju 561-756, Korea*

Received 12 January 2005; accepted 12 July 2005
DOI 10.1002/app.23528
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: MPEG–PCL diblock copolymers consisting of methoxy polyethylene glycol (MPEG) and poly(ε-caprolactone) (PCL) as drug carriers were synthesized by ring-opening polymerization. It is possible to control the balance between hydrophilic and hydrophobic by changing the MPEG and the ratio of ε-CL to MPEG. Implantable wafers were easily fabricated by the direct compression method after physical mixing of diblock copolymers and bovine serum albumin–fluorescein isothiocyanate (BSA-FITC) as a model protein drug. The BSA release from wafers prepared by MPEG–PCL diblock copolymers were higher than that from PCL with the physical blending of MPEG. The wafers prepared by a variety of MPEG–PCL diblock copolymers exhibited the controlled BSA release profiles with a dependence on MPEG–PCL diblock copolymer compositions. In addition, the changing of MPEG and PCL molecular weights within MPEG–PCL diblock copolymer controlled the initial burst of BSA. We confirmed that the diblock copolymers could be served as protein delivery carrier in implantable wafer form. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 1561–1567, 2006

Key words: implantable wafer; drug carrier; MPEG–PCL; bovine serum albumin

INTRODUCTION

In general, peptide or protein drugs that require prolonged administration have been delivered mainly by an oral route even though they have poor oral bioavailability.[1,2] To improve bioavailability of the drugs, much effort has been made to control and maintain the release for a long period by other administration methods as well as an oral route.[3–5] The sustained peptide and protein delivery should release the loading drug as a continuous rate for a long period. It has become important to target research to develop the sustained and controlled delivery systems for peptides or proteins.

Meanwhile, various natural and synthetic polymers have been explored as drug delivery carriers.[6] Among them, the biodegradable synthetic polymers can have a larger potential as a carrier for a drug delivery.[7–10] Many systemic administration forms such as microsphere, film, wafer, tablet, and scaffold were examined by using a variety of biodegradable synthetic polymers as a drug carrier. Particularly, an implantable wafer for a drug delivery using the biodegradable synthetic polymers has been widely investigated over the past several years because it has been considered as one of the most convenient methods to capture drug inside administration forms.[11–15] In addition, it does not need to be removed by surgery after complete releasing of a drug because drug delivery carriers degrade *in vivo*.

In the view of drug carriers, aliphatic polyesters are one of the most attractive biodegradable polymers because their backbones easily cleave by hydrolysis, and thereafter; the nontoxic cleaved products are absorbed in and/or eliminated from tissues or cells.[16,17] Specially, polyesters like poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), or their copolyesters (PLGA) probably were one of the most biodegradable polymers that could be commonly used as drug carriers.[18]

Meanwhile, poly(ε-caprolactone) (PCL) has potential in biomedical applications as drug carriers due to good compatibility with a number of polymers and biodegradability, although they exhibit a much slower degradation rate than the PLGA series.[19,20] PCL has a high crystalline domain that may induce both positive

Correspondence to: M. S. Kim (hblee@krict.re.kr).
Contact grant sponsor: Sol-Gel Innovation Project of Korea; contract grant number: MOCIE, 10006921.

and negative effects in *in vivo* application. A high crystalline part in PCL may retard the diffusion of biologic fluid into the polymer segment, and therefore may be applied at a long-term drug delivery, but is able to cause harmful effects for tissue once it is use for a long period *in vivo*. Thus, to endow biocompatibility for tissue for a long period, various attempts have been examined by the modification of other polymer segments in the PCL segment.

Polyethyleneglycol (PEG), which was already approved by the FDA, is widely used in biomedical research and applications. PEG may be considered as one of the most promising polymers due to prevention of protein absorption and improvement of biocompatibility for the blood contact compound.[21,22] Therefore, some group introduced a PEG segment into the PCL segment to improve the biocompatibility.[23–25] In addition, the PEG segment as the hydrophilic part can change the physicochemical properties of hydrophobic and biodegradable PCL.

Based on the properties of PCL and PEG, we recently synthesized methoxy poly(ethyleneglycol)-*block*-poly(ε-caprolactone) (MPEG–PCL), a diblock copolymer, by living ring-opening polymerization of ε-caprolactone (ε-CL) via activated monomer cationic polymerization, which can suppress unfavorable reactions such as back-biting and diproportionation.[26,28] The polymerization provided MPEG–PCL diblock copolymers with a well-defined structure.

The aim of our research was to develop various drug administration forms fabricated by various drug delivery carriers. In this work, we chose the MPEG–PCL diblock copolymers as the drug delivery carrier and implantable wafer as the drug administration form to deliver bovine serum albumin (BSA) as the model protein drug. We here describe the first approach to evaluate the release behaviors of BSA from the implantable wafers prepared by MPEG–PCL diblock copolymers with different compositions.

MATERIALS AND METHODS

Materials

Methoxy poly(ethyleneglycol) (MPEG) (Aldrich, M_n 2000 and 5000), 2-(2-ethoxyethoxy)ethanol (carbitol, TCI), and HCl (Aldrich; 1.0*M* solution in diethyl ether) were used as received. ε-CL was distilled over CaH_2 under reduced pressure. CH_2Cl_2 was distilled sequentially from $CaCl_2$ and CaH_2 under nitrogen before use. Bovine Serum Albumin-FITC (Sigma) was handled under condition without light.

Characterization

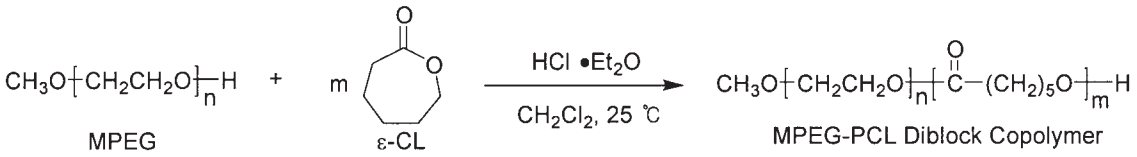
^{1}H-NMR spectra were measured using a Bruker 300 and 500 MHz instrument with $CDCl_3$ in the presence of TMS as the internal standard. IR spectra were measured with a Magna-IR™ spectrometer 550 Nicolet. Molecular weights and molecular weight distributions of MPEG and MPEG–PCL diblock copolymers were measured by a Futects At-3000 GPC system (Shodex RI-71 detector) using two columns (Shodex K-802 and Shodex Asahipak GF-510). $CHCl_3$ was used as the eluent at a flow rate of 0.6 mL/min. A scanning electron microscope (SEM, S-2250N, Hitachi, Japan) was used to examine the morphologic change of the wafers before and after *in vitro* release of BSA. The wafers were mounted on metal stubs and coated with a thin layer of platinum using a plasma-sputtering apparatus (Emitech, K575, Japan) under argon atmosphere.

Synthesis of poly(ethyleneglycol-*block*-ε-caprolactone) diblock copolymers (MPEG–PCL)

All glasses were dried by heating in vacuum and handled under a dry nitrogen stream. The typical process for the polymerization to give MPEG–PCL with a PCL molecular weight (6000, E2C6) is as follows. MPEG (M_n = 2000) (0.81 g, 0.4 mmol) and toluene (30 mL) were introduced into a flask. The MPEG solution was distillated by azeotropic distillation to remove water. Toluene was then distilled off completely. CH_2Cl_2 (2.5 mL) was added to MPEG, followed by the addition of ε-CL (2.41 g, 21.1 mmol) using a syringe. The polymerization was initiated by the addition of 1.0*M* solution of HCl in diethyl ether (0.8 mL, 0.8 mmol) at 25°C. After 24 h, the reaction mixture was poured into *n*-hexane to precipitate a polymer, which was separated from the supernatant by decantation. The obtained polymer was redissolved in CH_2Cl_2 and then filtered. The polymer solution was concentrated by rotary evaporator and dried in vacuum to give a colorless polymer of quantitative yield. The ε-CL monomer conversion was determined by ^{1}H-NMR spectroscopy before precipitation with *n*-hexane. The molecular weight of the PCL segment in the diblock copolymer was determined by the intensity of terminal methoxy proton signal of MPEG at δ = 3.38 ppm and methylene proton signal of PCL at δ = 2.31 ppm in ^{1}H-NMR spectroscopy. The hydrophilic and hydrophobic balance (HLB) values were calculated by a previously reported method (Becher and Schick, 1987).

Preparation of BSA-FITC loaded wafers

Freeze milling of a mixture of MPEG–PCL (99 mg) and BSA-FITC (1 mg) performed to disperse uniformly BSA-FITC into diblock copolymers. Ten milligrams of the mixed powder was compressed by a molder of a 3-mm diameter using Carver Press (MH-50Y CAP 50 tons, Japan) at 20 Kgf/cm^2 for 5 s at room tempera-



Scheme 1

ture. The wafers were 3 × 1-mm in size with a flat surface and stored at 0°C without light until use.

In vitro release of BSA-FITC

BSA-FITC loaded wafers were individually placed in a vial with 10 mL of PBS. The vial was constantly shaken at 100 rpm and 37°C. At a set time, 1 mL of solution was taken out from the vial and then 1 mL of PBS added to the vial. The solution taken immediately was measured by fluorescence spectroscopy (F-4500, Hitachi, Tokyo, Japan). The amount of cumulatively released BSA was calculated by the standard calibration curves predetermined with BSA-FITC. The release experiments were individually performed for three wafers and then calculated as an average value.

Water uptake ability

The wafers in PBS solution of pH 7.4 were taken out at day 1, followed by the removal of water from the wafer using soft KIM wipes. The obtained wafers were weighed to determine the water uptake of the wafer during release test. The CC8/MPEG blend used wafer was dried by the freeze dryer for 3 days and weighed to determine the mass loss from original wafer. The dried wafer was also measured by ^{1}H-NMR.

RESULTS AND DISCUSSION

Synthesis of MPEG–PCL diblock copolymers

To synthesize MPEG–PCL diblock copolymers as drug carriers, the polymerization of ε-CL by terminal alcohol of carbitol (M_n, 134) or MPEG (M_n, 2000 and 5000) as an initiator was performed with various feed ratios of ε-CL with regard to the initiator in the presence of HCl · Et_2O as the monomer activator (Scheme 1). The obtained MPEG–PCL diblock copolymers were summarized in Table I. MPEG–PCL diblock copolymers were obtained in almost a quantitative yield. The M_n values of the obtained MPEG–PCL diblock copolymers showed good agreement with those calculated from the feed ratio of the ε-CL to an initiator. Moreover, polydispersities of MPEG–PCL diblock copolymers have maintained the comparable narrow those (1.19–1.39) when compared with MPEG (1.12–1.17) as an initiator. As shown in Figure 1, carbitol–PCL and MPEG–PCL exhibited characteristic peaks of PCL as well as those of carbitol or MPEG. The polymerization gave MPEG–PCL diblock copolymers with the different ratios in MPEG and PCL. It was possible to control the hydrophilic and hydrophobic balance (HLB) value by changing the MPEG and the ratio of ε-CL to MPEG. IR spectroscopy of MPEG–PCL diblock copolymers exhibited carbonyl peaks of PCL around 1720 cm^{-1}.

BSA releasing from wafer

We chose a BSA as the model protein drug and an implantable wafer as the systemic administration form because it was able to be conveniently prepared. Through a simple direct compression method after physical mixing of diblock copolymers and BSA using a freeze mill, BSA was easily introduced inside implantable wafers.

In general, water-soluble drug releasing from the wafer could depend on the penetratrable ability of

TABLE I
Synthesis of MPEG-PCL Diblock Copolymers

No.	MW of initiator[a]	$[\varepsilon\text{-CL}]_o/[I]_o$	Yield[b] (%)	M_n calcd	M_n NMR[c]	M_w/M_n[d]	HLB[e]
CC8	PEG 134 (Carbitol)	69	93	134–7900	134–8400	1.24	0.3
E2C11	PEG 2000	98	98	2000–11,200	2000–11,400	1.39	3.0
E2C6	PEG 2000	53	95	2000–6000	2000–6000	1.33	5
E2C3	PEG 2000	24	99	2000–2800	2000–3200	1.29	7.7
E5C3	PEG 5000	26	94	5000–3000	5000–2900	1.19	12.7

Conditions: [HCl]/[Initiator] = 2, $[\varepsilon\text{-CL}]/[CH_2Cl_2]$ = 0.5 *M*, room temperature, 24 h.
[a] MPEG = 2000 (M_w/M_n = 1.17), 5000 (M_w/M_n = 1.12)
[b] *n*-Hexane insoluble Part.
[c] Determined ^{1}H-NMR.
[d] Measured by gel permeation chromatography (based on standard polystyrene).
[e] HLB = 20× (molecular weight of MPEG/total molecular weight).

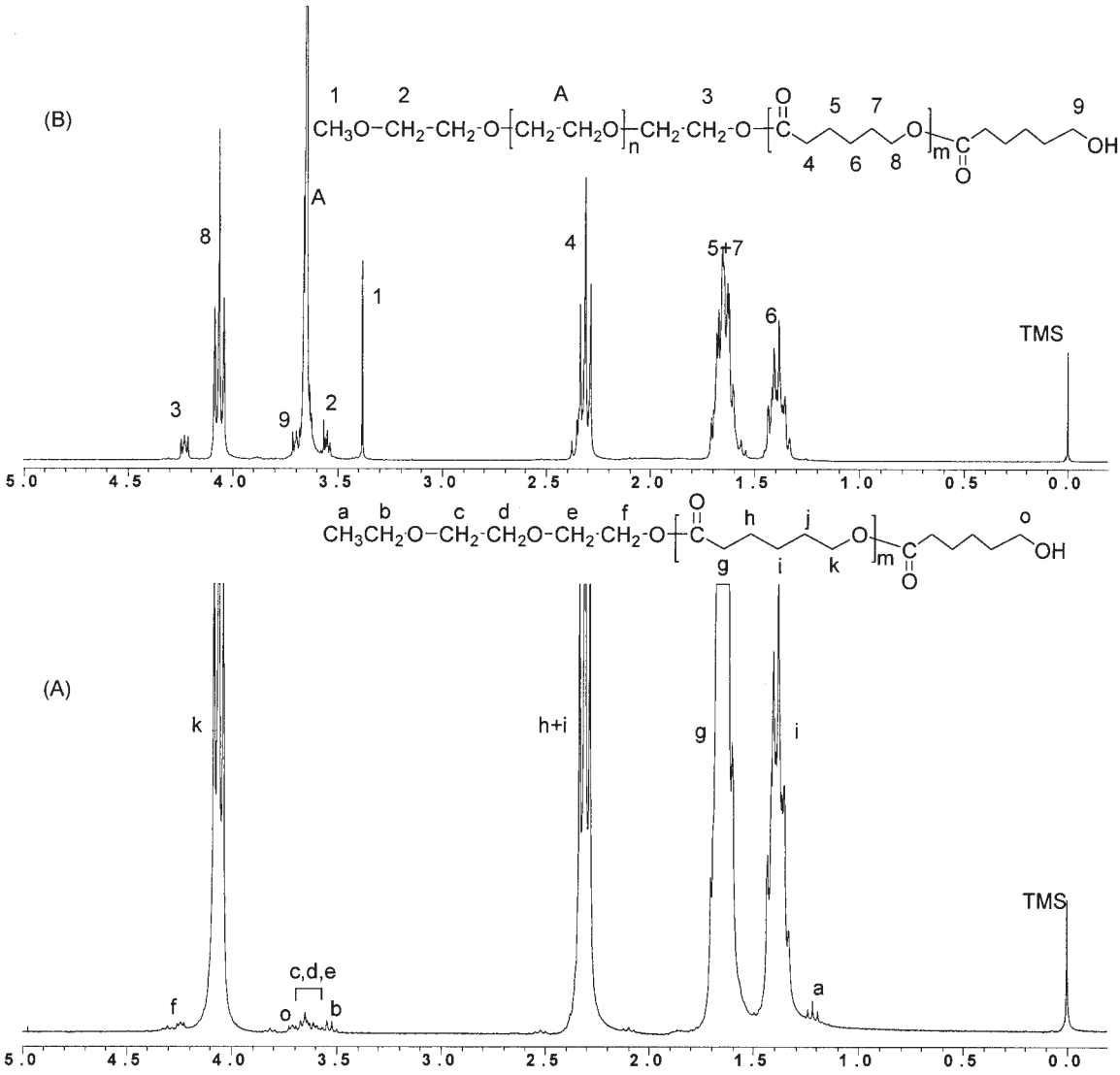


Figure 1 ^{1}H-NMR spectra of (A) Carbitol-PCL (CC8) and (B) MPEG-PCL diblock copolymer (E2C3).

water or biologic fluid inside the wafer. Considering the water-penetration ability for PCL polymer used as a carrier for the wafer, the addition of the hydrophilic PEG segment in the hydorphobic PCL segment can change the water-penetrating ability. Thus, it is first necessary to compare drug release from the wafers prepared by the PEG segment added PCL polymers through either chemical (covalent bonding) or physical (simple blending) method. The wafers were prepared by using the CC8, CC8/MPEG blend (90/10, wt/wt), and E2C6 as carriers to examine the effect in the release of BSA. Bovine serum albumin–fluorescein isothiocyanate (BSA-FITC) was used to detect the amount of BSA released from the wafer. The experiment of BSA release from the wafers was performed at 37°C for 30 days under shaking. The release profiles are show in Figure 2. The CC8 used a wafer exhibited only at 7% release of BSA even for 30 days. A slight increase for the released amount of BSA was detected

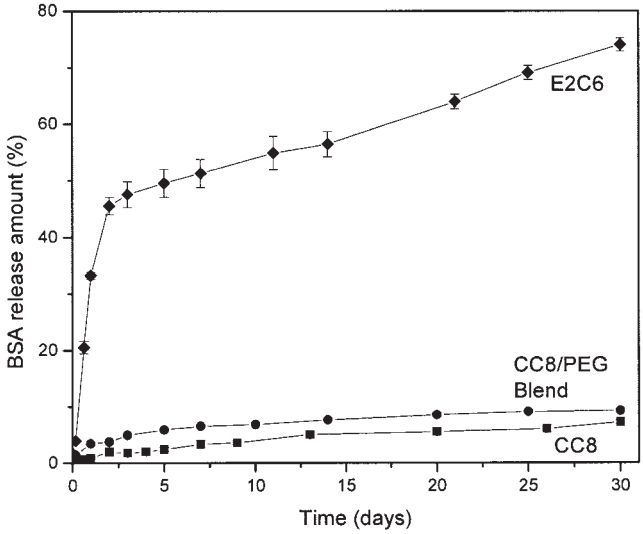
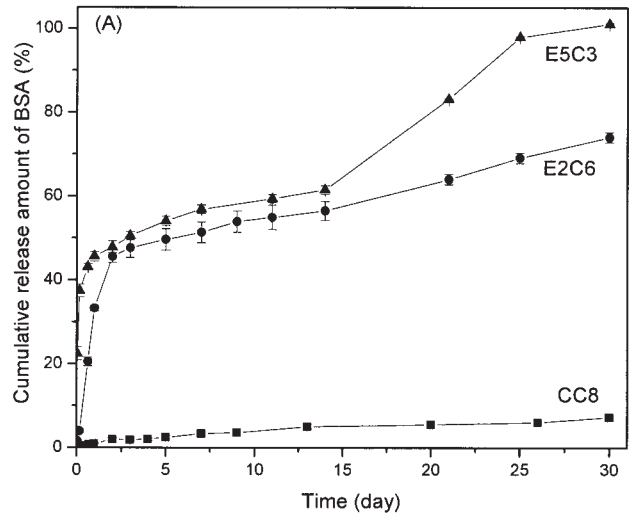


Figure 2 BSA amount released from wafers prepared by CC8, CC8/MPEG blend, and E2C6.

at the wafer prepared by using the CC8/MPEG blend and almost a similar BSA release was obtained at the wafer using the 70/30 and 90/10 (CC8/MPEG, wt/wt) polymer blend, whereas, surprisingly, E2C6 used a wafer showing the larger enhancement in the released amount of BSA when compared with those using the CC8 and CC8/MPEG blend.

Measurement of the water uptake amount for the wafers was performed to examine the changing of the BSA release amount with respect to the used polymers. Water uptake of the wafer using CC8 was below 5% at day 1, while E2C6 used a wafer that was approximately above 50%. The water absorption amount of E2C6 used a wafer that was larger than that using CC8, although the wafer using the CC8/MPEG blend decreased the weight slightly. After 1 day in PBS, the ^{1}H-NMR spectrum of the wafer prepared by the CC8/MPEG blend showed only CC8 peaks with the exception of MPEG, indicating that the MPEG completely dissolved out from the wafer. These results indicated that the BSA release could depend on the water uptake of the wafer, and the MPEG segment of the E2C6 diblock copolymer was an important effect on releasing BSA as the remaining state in the wafer under releasing condition.

Next, the wafers were prepared by using MPEG–PCL diblock copolymers to compare BSA release in the view of hydrophilic and hydrophobic balance. The wafers were prepared by using the diblock copolymers, CC8, E2C6, and E5C3, maintained as a total molecular weight of 8000 g/mol by changing the MPEG molecular weight (134, 2000, 5000 g/mol), and E2C3, E2C6, and E2C11, with changing of the PCL molecular weight in a constant PEG molecular weight (2000 g/mol). Figure 3 shows the releasing profiles of BSA from the wafers. As the relative MPEG segment in the diblock copolymers increased, the released amount of BSA increased, as shown in Figure 3(A). In the case of the PCL molecular weight change [Fig. 3(B)], the released amount of BSA increased as the relative PCL segment decreased. The wafers exhibited the controlled release profiles with a dependence on HLB value via changing of MPEG–PCL diblock copolymer compositions, even though they had an initial burst. Figure 4 shows the plot of the HLB value of diblock copolymers versus the released amount of BSA at day 1. The initial burst at day 1 increased when the relative MPEG amount in the diblock copolymers or the HLB value increased [Fig. 4(A)]. Water uptake of the wafers was examined at day 1. The water absorption amount of CC8, E2C11, and E2C6 was approximately 5, 15, and 50%, respectively. E5C3 and E2C3 exhibited a water absorption amount above 200% even at day 1, although the exact amount could not be determined due to a slight dissipation of the used diblock copolymers for the wafer. The water absorption amount increased as the relative MPEG



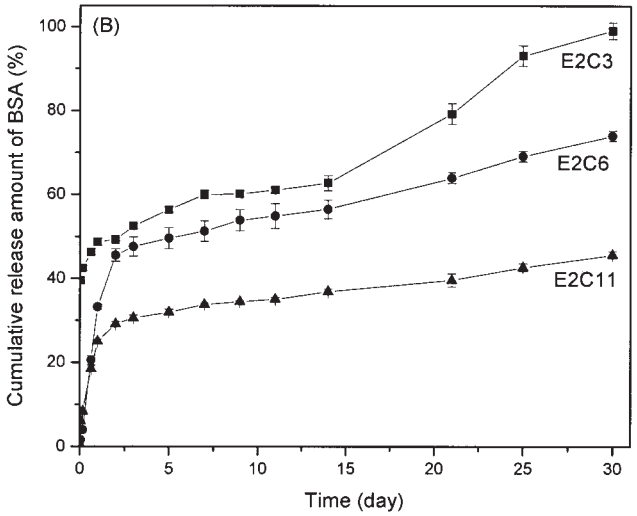


Figure 3 BSA amount released from wafers prepared by diblock copolymers: (A) CC8, E2C6, and E5C3 (total molecular weight is 8000 g/mol), and (B) E2C3, E2C6, and E2C11 with changing of PCL molecular weight in a constant PEG molecular weight (2000 g/mol).

segment or HLB value increased. The relative MPEG segment increased, resulting in enhancement of the water-penetrating ability of the wafer through an increase of the relative hydrophilicity of the diblock copolymers. Therefore, the larger water absorption inside the wafer in the initial stage probably induced the initial burst. After the initial burst, the wafers showed release profiles with a similar release rate, which was determined by the release slope until 14 days after initial burst. This means that the overall BSA release was attributed to initial burst amount. Figure 4(B) shows the plot of the HLB value of the diblock copolymers versus the time required to release 50% BSA from wafers prepared by diblock copoly-

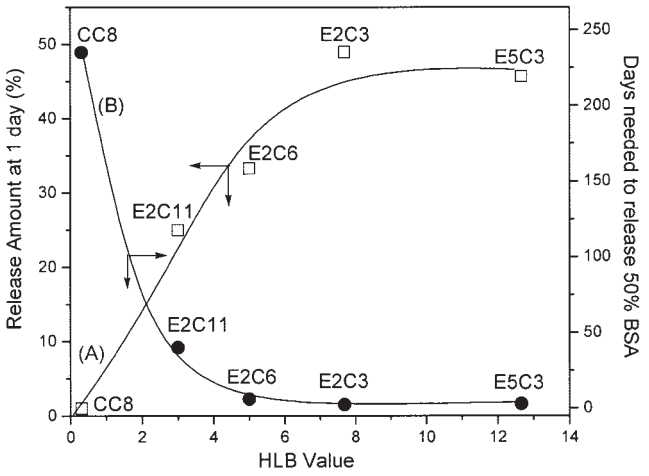


Figure 4 Plot of HLB value of diblock copolymers versus (A) release amount of BSA at 1 day, and (B) the days needed to release 50% BSA from the wafers. (The release days of CC8 or E2C11 diblock copolymer used wafer were calculated by conjecture through extrapolation of release profiles).

mers. As the MPEG/PCL ratio in the diblock copolymer or the HLB value increased, the shorter days needed to release 50% BSA from the wafers.

Figure 5 illustrated the wafers using diblock copolymers before and after BSA release for 30 days. A color of E5C3 used a wafer changed to white, indicating the almost release of BSA. Moreover, E5C3 used a wafer that did not completely maintained the shape, because E5C3 with high MPEG content disintegrated in water due to slow breaking stress under shaking in PBS. This induced an abrupt increase in the released amount of BSA after about 15 days, although E2C6 used a wafer that was a little yellowish and maintained the shape for 30 days. Before and after the BSA release test, the morphological changes of the wafer

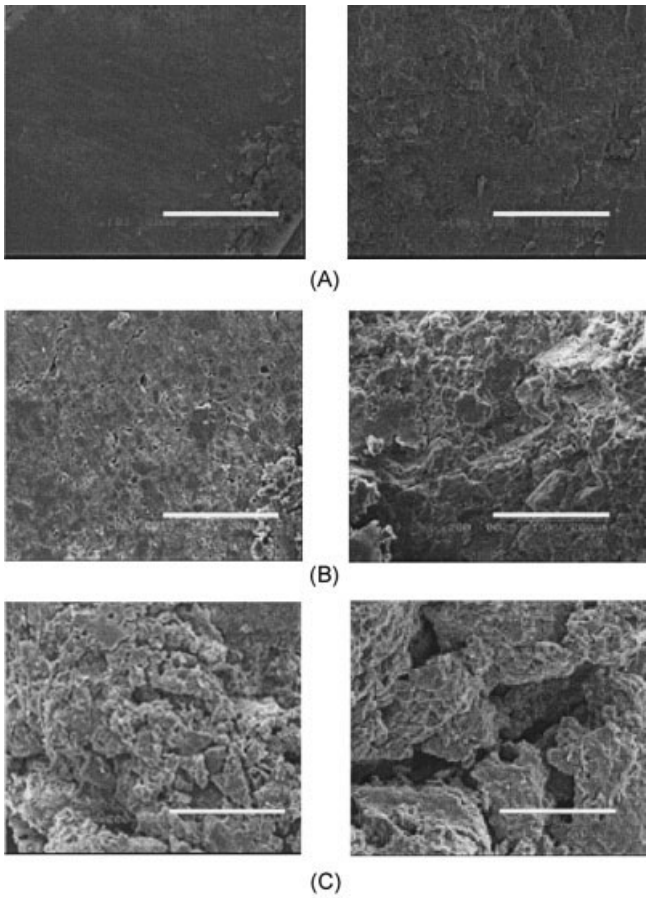


Figure 6 SEM microphotographs of BSA-loaded wafers: (A) before and (B) after releasing of BSA from E2C6 used wafer for 30 days, and (C) after releasing of BSA from E5C3 used wafer for 30 days. (Left: surface; right: cross-section, magnification is ×200, scale bar represents ×200 μm).

were also observed by SEM, as shown in Figure 6. The E5C3 used a wafer compared with E2C6, which used a wafer showing more structural metamorphosis of

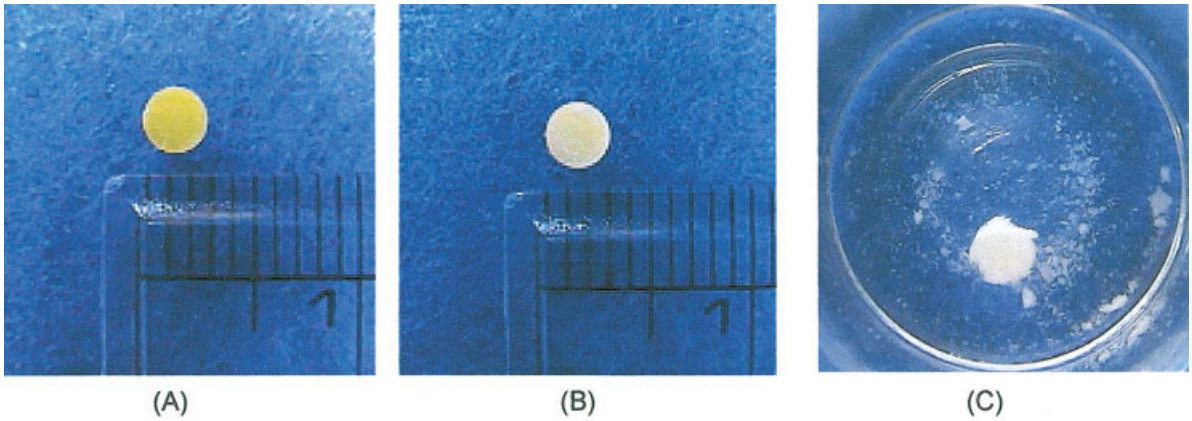


Figure 5 Pictures of wafers (A) before and (B) after releasing of BSA from E2C6 used wafer for 30 days, and (C) after releasing of BSA from E5C3 used wafer for 30 days. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the crack form, probably due to a higher water absorption of MPEG inside the wafer. The structure change of the wafer could also induce a faster BSA release.

CONCLUSION

We successfully prepared the MPEG–PCL diblock copolymers with various compositions. It is possible to control the balance between hydrophilic and hydrophobic properties by changing the MPEG and the ratio of ε-CL to MPEG. BSA-loading implantable wafers were easily prepared by direct the compression method after physical mixing of the diblock copolymers and BSA. The wafer prepared by the MPEG–PCL diblock copolymer exhibited higher BSA release than that of PCL with the physical blending of MPEG.

The prepared implantable wafers exhibited the controlled BSA release profiles with a dependence on MPEG–PCL diblock copolymer compositions, although the overall BSA release was attributed to an initial burst amount. In view of the results so far obtained, we confirmed the possibility of MPEG–PCL diblock copolymers as protein carriers for an implantable wafer possessing many advantages such as simple manufacture, long-term delivery, and controlled release. Further research on the biodegradability and the biocompatibility for tissue of diblock copolymers according to changing of MPEG and PCL compositions is now in progress.

References

1. Mahato, R. I.; Narang, A. S.; Thoma, L.; Miller, D. D. Crit Rev Ther Drug Carrier Syst 2003, 20, 153.
2. Fix, J. A. Pharm Res 1996, 13, 1760.
3. Bernkop-Schnurch, A.; Walker, G. Crit Rev Ther Drug Carrier Syst 2001, 18, 459.
4. Owens, D. R.; Zinman, B.; Bolli, G. Diabet Med 2003, 20, 886.
5. Torchilin, V. P.; Lukyanov, A. N. Drug Discov Today 2003, 8, 259.
6. Andrianov, A. K.; Payne, L. G. Adv Drug Deliv Rev 1998, 34, 155.
7. Prabhu, S.; Sullivan, J. L.; Betageri, G. V. Drug Deliv 2002. 9, 195.
8. Khang, G.; Rhee, J. M.; Jeong, J. K.; Lee, J. S.; Kim, M. S.; Cho, S. H.; Lee, H. B. Macromol Res 2003, 11, 207.
9. Yoo, J. Y.; Kim, J. M.; Khang, G.; Kim, M. S.; Cho, S. H.; Lee, H. B.; Kim, Y. S. Int J Pharm 2004, 276, 1.
10. Lee, J. S.; Chae, G. S.; Kim, M. S.; Cho, S. H.; Lee, H. B.; Khang, G. Biomed Mater Eng 2004, 14, 185.
11. Westphal, M.; Hilt, D.C.; Bortey, E.; Delavault, P.; Olivares, R.; Warnke, P. C.; Whittle, I. R.; Jaaskelainen, J.; Ram, Z. Neuro-Oncology 2003, 5, 79.
12. Seong, H.; An, T. K.; Khang, G.; Choi, S. U.; Lee, C. O.; Lee, H. B. Int J Pharm 2003, 251, 1.
13. Bromberg, L. E.; Buxton, D. K.; Friden, P. M. J Controlled Release 2001, 71, 251.
14. Lee, J. S.; Chae, G. S.; Khang, G.; Kim, M. S.; Cho, S. H.; Lee, H. B. Macromol Res 2003, 11, 352.
15. Seo, K. S.; Kim, M. S.; Kim, K.; Cho, S. H.; Lee, H. B.; Khang, G. Polymer (Korea) 2004, 28, 328.
16. Abou-Zeid, D. M.; Muller, R. J.; Deckwer, W. D. J Biotechnol 2001, 86, 113.
17. Steinbuchel, A.; Biopolymers, Doi, Y.; Steinbuchel, A., Eds. Polyester III Application and Commercial Products; Wiley-VCH: Berlin, 2001; Vol. 4.
18. Zhang, L.; Xiong, C.; Deng, X. J Appl Polym Sci 1995, 56, 103.
19. Allen, C.; Yu, Y.; Maysinger, D.; Eisenberg, A. Bioconjugate Chem 1998, 9, 564.
20. Pitt, C. Poly(ε-caprolactone) and its copolymer. In: Chasin, M.; Langer, R., Eds.; Biodegradable Polymers as a Drug Delivery System; Marcel Dekker: New York, 1990; p 71.
21. Burnham, N. L. Am J Hosp Pharm 1994, 51, 210.
22. Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. Adv Drug Deliv Rev 2003, 55, 217.
23. Ryu, J. G.; Jeong, Y. I.; Kim, I. S.; Lee, J. H.; Nah, J. W.; Kim, S. H. Int J Pharm 2000, 200, 231.
24. Hsu, S. H.; Tang, C. M.; Lin, C. C. Biomaterials 2004, 25, 5593.
25. Shuai, X.; He, Y.; Na, Y. H.; Inoue, Y. J Appl Polym Sci 2001, 80, 2600.
26. Kim, M. S.; Seo, K. S.; Khang, G.; Cho, S. H.; Lee, H. B. J Biomed Mater Res 2004, 70A, 154.
27. Kim, M. S.; Seo, K. S.; Khang, G.; Cho, S. H.; Lee, H. B. J Polym Sci Part A: Polym Chem 2004, 42, 5784.
28. Kim, M. S.; Seo, K. S.; Khang, G.; Lee, H. B. Macromol Rapid Commun 2005, 26, 643.