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Controlled Release of Gentamicin Sulfate from Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) Wafers for the Treatment of Osteomyelitis

Gilson Khang*, Hak-Soo Choi, and John M. Rhee

Department of Polymer Science and Technology, Chonbuk National University, 664-14 Dukjin Dong 1 Ga, Dukjin Ku, Chonju 561-756, Korea

Sung Chul Yoon

Biomaterials Science Laboratory, Division of Life Science, Gyeongsang National University, Chinju 660-701, Korea

Jin Cheol Cho and Hai Bang Lee

Biomaterials Laboratory, Korea Research Institutes of Chemical Technology, P.O. Box 107, Yusung, Taejon 305-606, Korea

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Abstract : Biodegradable wafers were prepared with poly(hydroxybutyrate-*co*-hydroxyvalerate) (PHBV; 5, 10, and 15 mole% for 3-hydroxyvalerate) by simple heat pressing method for the sustained release of antibiotic agent, gentamicin sulfate (GS) to investigate the possibility of the treatment for osteomyelitis. The effects of hydroxyvalerate (HV) content, thickness of wafers, various types of additives such as sodium dodecyl sulfate (SDS), microcrystalline cellulose, polyvinylpyrrolidone, and hydroxypropylcellulose (HPC), and different initial drug loading ratio on the release profile have been investigated. *In vitro* release studies showed that different release patterns and rates could be achieved by simply modifying factors in the preparation conditions. PHBV wafers with 3 mm thickness, 10% of GS initial loading, 15% of HV content and addition of 5% of SDS and HPC were free from initial burst and a near-zero-order sustained release was observed for over 30 days. It might be suggested that the mechanisms of GS release may be more predominant simple dissolution and diffusion of GS than erosion of PHBV in our system.

Introduction

Gentamicin sulfate (GS) is one of the most potent antibiotics for bone infection such as osteomyelitis. Prolonged parenteral and oral antibiotic therapy for 4-6 weeks may be necessary for usual treatment.[1] Some disadvantages of prolonged parenteral therapy by intravenous or intramuscular antibiotic injections are the high cost of treatment, systemic toxicity and patient discomfort. Oral antibiotic delivery may also be associated with patient compliance problems. In addition, GS is also well known for causing rather severe secondary effects such as nausea, vomiting, headache, skin eruption, nephrotoxicity, and ototoxicity. Therefore, the localized delivery of an antibiotic to the infected site may be introduced to overcome the difficulties associated with parenteral and oral therapy. This method would deliver the drug at a continuous rate, and reduce the dose-dependent toxicity by minimizing the fluctuation in plasma concentration.[2] In the last three decades, localized antibiotic therapy has emerged as an important approach to treat orthopedic infections.

GS loaded poly(methyl methacrylate) (PMMA) beads have been employed clinically to prevent or treat osteomyelitis since the 1970s.[3-5] However, PMMA is a non-biodegradable material, so secondary surgery must be required to remove the beads after releasing of GS. Several biodegradable controlled antibiotic-release devices such as dideoxykanamycin loaded hydroxyapatite/poly(L-

*e-mail : gskhang@moak.chonbuk.ac.kr

lactide) (PLA) cylinders,[6] ampicillin loaded poly (D,L-lactide-*co*-glycolide) (PLGA), cefotiam loaded hydroxyapatite beads, and GS loaded PLA cylinders and microspheres have been developed.[7-14] However, the release properties of these devices were not found to be satisfactory primarily due to poor design and manufacturing. For example, in *in vitro* studies more than 40% of entrapped GS was released from PLA microcapsules in the first few hours. The release from uncoated PLA cylinders was barely longer than three weeks.[8]

Very recently, one of the most significant candidates for the study of biodegradable controlled release system is the family of polyhydroxyalkanoates (PHA) due to its relatively long biodegradability and relatively good biocompatibility with no acute inflammation, no abscess formation, and no tissue necrosis.[16] Also, these polymers are entirely natural and obtained from micro-organisms, *Alicaligen eutrophus* as gram-negative bacteria. Their use in drug release systems has been reported in recent years.[17-25] Poly(3-hydroxybutyrate) (PHB) and its copolymers with hydroxyvalerate (PHBV) with varying HV ratios are the most widely existing members of this biopolymer group. These biodegradable and biocompatible polyesters, because of their unique physicochemical properties, such as piezoelectricity, are claimed to induce bone reformation in load-bearing sites.[19, 20]

In this study, GS loaded PHBV wafers were prepared by simple compressing with heating method due to high stability of GS for high temperature, and the investigation of the effects of the copolymer ratio, initial drug loading, wafer thickness, and additive types on the release profile were carried out. Also, the *in vitro* release pattern of GS and the morphology of wafer have been investigated by means of high performance liquid chromatography (HPLC) and scanning electron microscope (SEM), respectively.

Experimental

Materials. GS was received as a gift from Dong Shin Pharmaceutics Co., Korea. PHBVs (D300G (pellet), D400G (pellet), and D600G (powder) ; 5, 10, and 15 mole% of 3-hydroxyvalerate, respectively) were received as a gift from Monsanto Chem. Co. Ltd., (Delaware, USA). Sodiumdodecylsulfate (SDS), hydroxypropylcellulose (HPC), polyvinylpyrrolidone (PVP), and microcrystalline cellulose (MCC) were purchased from Aldrich Chem. Co., USA. All other chemicals and solvents were a reagent grade.

Preparation of Wafer. GS loaded PHBV wafers were prepared by compressing the drug-polymer mixture using a manual tablet press (MH-50Y, Masada, Japan) with heating. Briefly, each of polymer pellets was pulverized to a homogeneous particle size and thoroughly mixed with drug by a freezer-mill (SPEX 6700, Metuchen, USA). The homogeneous mixture was applied to a stainless steel mold (20 mm diameter) with heating at 170°C, and the molten mixture was compressed under 20 kgf/cm^2 for 30 min (Figure 1). The resulting paste was cooled at room temperature for 30 min. The variables and conditions of wafer formulations are listed in Table I. The effects of the copolymer ratio, different thickness of wafers, different drug-loading ratio, and various additives on the release profile have been investigated.

***In Vitro* Release Investigation.** GS release patterns from the various wafer formulations were discussed by *in vitro* experiments. The wafers were extracted in 50 mL of phosphate buffered saline (PBS, pH 7.4). The vials were placed in a constant-temperature incubator kept at 37°C. The release medium (100 μL) was periodically taken out from the vials with pipette and same volume of the fresh medium was replaced. GS in

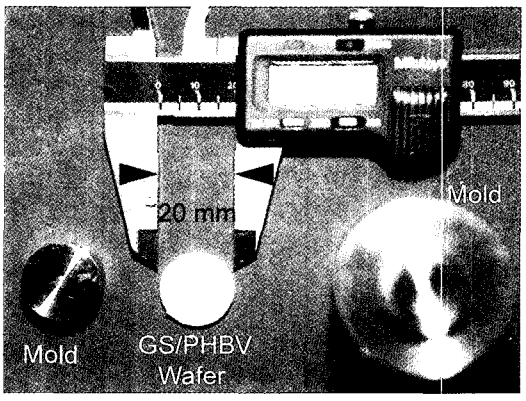

Figure 1. The photograph of waferring mold for GS loaded PHBV wafer composed of two punches with 20.0 mm diameter and the prepared wafer.

Table I. Preparation Conditions for PHBV Wafers with Gentamicin Sulfate

Batch	Polymer wt (g)	GS wt (g)	Initial Drug Ratio (%)	Additives (wt%)	Wafer Thickness (mm)
H1	0.09	0.01	10	-	0.3
H2	0.27	0.03	10	-	0.9
H3	0.45	0.05	10	-	1.5
H4	0.90	0.10	10	-	3.0
H5	1.35	0.15	10	-	4.5
H6	0.95	0.05	5	-	3.0
H7	0.80	0.20	20	-	3.0
H8	0.85	0.10	10	SDS (5)	3.0
H9	0.80	0.10	10	SDS (10)	3.0
H10	0.85	0.10	10	MCC (5)	3.0
H11	0.80	0.10	10	MCC (10)	3.0
H12	0.85	0.10	10	PVP (5)	3.0
H13	0.80	0.10	10	PVP (10)	3.0
H14	0.85	0.10	10	HPC (5)	3.0
H15	0.80	0.10	10	HPC (10)	3.0

the aqueous layer was analyzed by HPLC after the derivation with *o*-phthaldehyde (OPA).[9]

The HPLC system was consisted of solvent delivery pump and an UV detector (HP 1100 series, USA). The detection wavelength was set at 330 nm, the separation was achieved by using a reversed phase column (UG 120 5 μm packing, size 4.6× 150 mm, USA) and a flow rate of the mobile phase was 1.2 mL/min. The mobile phase consisted of methanol, distilled water, glacial acetic acid (70 : 25 : 5 by vol%), and 5 g of sodium 1-heptanesulfonate.[9] The HPLC assay method and OPA derivation for GS were based on the USP.

SEM Observation. Microphotographs of the wafers were observed by SEM (model S-2250N, Hitachi, Japan) operated at 15 kV in order to examine the surface and cross-sectional morphology. To obtain a cross-sectional morphology, the fractured samples were prepared by submerging the wafers into liquid nitrogen. Before observation, all samples were mounted on metal stubs with double-sided tape, and coated with a thin layer of platinum by means of a plasma sputtering apparatus (Emscope, Model SC 500K, UK) under argon atmosphere. The observations were studied before and after *in vitro* test.

Results and Discussion

In our previous study,[26] we developed PHBV microspheres for the sustained release of highly water soluble drug such as 5-fluorouracil. One of the most serious problem for the fabrication of PHBV durg carriers to get desirable release patterns of the drug was a poor solubility to organic solvent due to highly crystalline state of PHBV,[27] resulting in low loading efficiency into crystalline region. Originally, PHB and PHBV polymers are 100 % amorphous state inside bacteria. However, almost all amorphous regions were changing to crystalline state during the extraction process.[20] In this study, to overcome this problem such as homogeneous mixing with PHBV and GS, a simple compressing with heating method was developed because GS has been widely used a typical heat-resistant antibiotics.[1] Figure 2 shows the SEM microphotograph of the freezer-milled mixtures of PHBV (D600G) and 10% of GS in liquid nitrogen. We can observe the fine particles as well as homogeneous mixture below 20 μm to get homogeneous mixed wafer in figure.

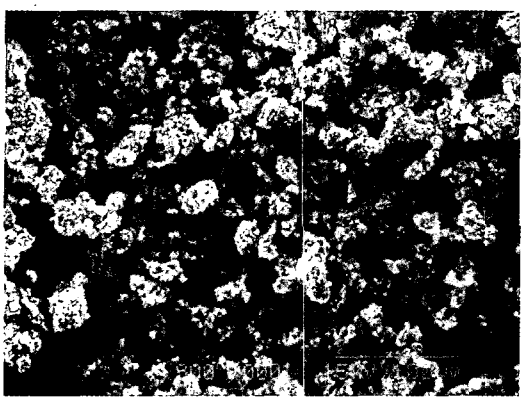

Figure 2. SEM microphotograph of the mixture of PHBV and GS (original maginification; × 300).

Effect of Thickness of Wafers on *In Vitro* Release. The influence of wafer thickness (0.3 (H1), 0.9 (H2), 1.5 (H3), 3.0 (H4), and 4.5 mm (H5) × 20 mm diameter) on the release profiles of GS from 10% GS-loaded PHBV (15% of 3-hydroxyvalerate) wafers is shown in Figure 3 (Formulation No. H1 to H5). It can be seen that the thickness of wafers has a significant effect on the release pattern. The release rate was in the order of 0.3, 0.9, 1.5, 3.0, and 4.5 mm for the initial loading and the duration of release was around 5, 12, 16, 20 and 30 days with near zero-order release, respectively. That is to say, the increase of wafer thickness significantly decreased initial burst and could be reached more near zero-order release pattern to 30 days. It can be explained that this wafer acts like simple "depot". The more thinner wafer, the faster the simple dissolution and diffusion due to small volume of depot of GS also could be happened. From these results, the desirable duration of GS release can be obtained by the controlling of the wafer thickness. The almost same release pattern from another PHBV with 5 and 10% of 3-hydroxyvalerate mole percent was observed (results were not shown).

Effect of Initial Loading Ratio on *In Vitro* Release. We observed that the higher drug loading ratio lead to increase little bit of initial burst in wafers in Figure 4. It can be seen that GS initial loading has a slight effect on release pattern between H4 (10% of GS), H6 (5% of GS), and H7 (15% of GS). The release rate of 5% initial loading with 3 mm thickness was lower than that of 20% initial loading. Generally speaking, however, in the considerably higher initial drug loading such as over 50% of GS, all the GS entrapped in the PHBV forms like a network which the majority of the crystals was in contact with each other, consequently, drug is released due to simple dissolution and diffusion.[28-30] In conclusion, very few initial burst of this system such as 5, 10 and 20% of initial loading was observed.

Effect of 3-Hydroxyvalerate Ratio on *In Vitro* Release. The effect of 3-hydroxyvalerate ratio of PHBV on the release pattern with 3 mm thickness is given in Figure 5. As can be seen in the Figure 5, the release rate reduced by the

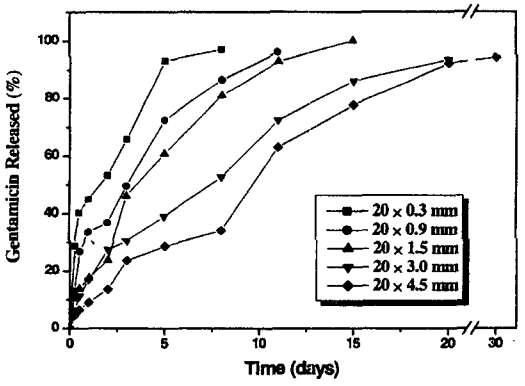

Figure 3. GS released behavior from different thickness of GS loaded PHBV wafer (10% GS/D600G). [■]; H1, [●]; H2, [▲]; H3, [▼]; H4, and [◆]; H5.

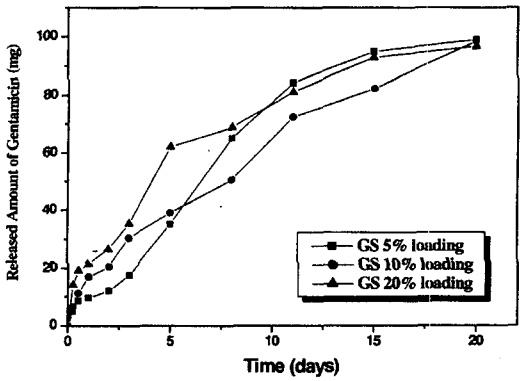

Figure 4. GS released behavior from GS loaded PHBV wafers from different loading ratio (1 gram PHBV base with 3 mm thickness; GS/D600G). [■]; H6, [●]; H4, and [▲]; H7.

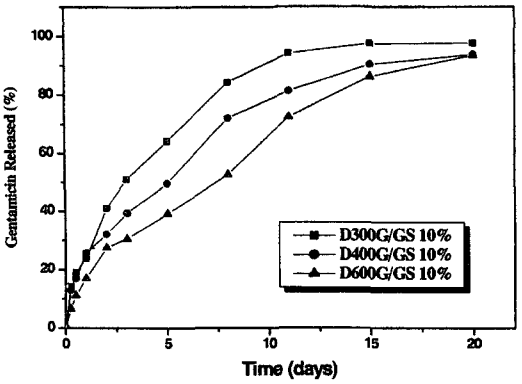

Figure 5. GS released behavior from PHBV wafers with different ratio of 3-hydroxyvalerate (1 gram PHBV base with 3 mm thickness). [■]; 5% of 3-hydroxyvalerate, [●]; 10% of 3-hydroxyvalerate, and [▲]; 20% of 3-hydroxyvalerate.

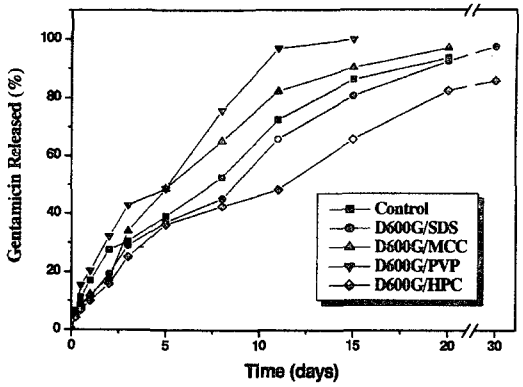

Figure 6. GS released behavior from PHVB wafers with various types of 5% additives (1 gram PHBV base with 3 mm thickness; 10% of GS initial loading; 15% of 3-hydroxyvalerate). [■]; control, H4, [●]; H8, [▲]; H10, [▼]; H12, and [◆]; H14.

higher content of 3-hydroxyvalerate as in the order of 5, 10 and 15% of 3-hydroxyvalerate. With use of D600G (15 mole% 3-hydroxyvalerate), a good linear relationship was observed and the initial percentage release decreased about 60 % compared with D300G (5 mole% of 3-hydroxyvalerate).[16] This result can be explained on the basis of the crystallinity of the PHBV decreased with increasing the content of 3-hydroxyvalerate,[19] that is to say, the increasing amorphous region in PHBV polymer with GS might be enhanced the entrapping of GS into PHBV wafer at same temperature, resulting in more homogenous mixing. Consequently, more desirable release duration could be reached. These results indicated that a desirable release pattern could be obtained by choosing an appropriate copolymer composition.

Effect of Additives on *In Vitro* Release. The influence of various additive types (as water soluble polymers) and different their amount with 5 and 10% was observed in Figures 6 and 7, respectively. It can be observed that the enhancement and the reduction of release rate of GS could be achieved by using MCC and PVP, and SDS and HPC, respectively. It has been widely recognized that the *in vitro* release of GS from biodegradable wafers without additive showed typical biphasic release kinetics, a slow diffusion release followed by a fast erosion-mediated

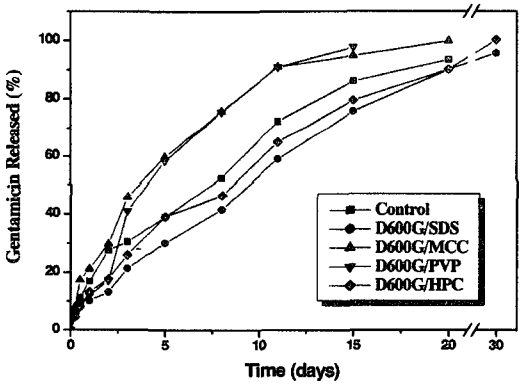

Figure 7. GS released behavior from PHVB wafers with various types of 10% additives (1 gram PHBV base with 3 mm thickness; 10% of GS initial loading; 15% of 3-hydroxyvalerate). [■]; control, H4, [●]; H9, [▲]; H11, [▼]; H13, and [◆]; H15.

release.[31] Figure 8 shows SEM microphotographs of the surface (a and c) and cross sectional (b and d) morphology before/after (30 days) *in vitro* release experiment for 10% GS loaded PHBV wafers with (a and b)/without (c and d) SDS. We can see the numbers of pores after GS releasing from physical dissolution. Also, the duration of biodegradation of PHBV is relatively longer compared that of the family of poly(α-hydroxyester).[9,26,28-30] For example, molecular weight of PHBV (19% of 3-hydroxyvalerate content) decreased from 292,300 to 248,000 g/mole at 1 month, whereas that of poly(L-lactide) decreased

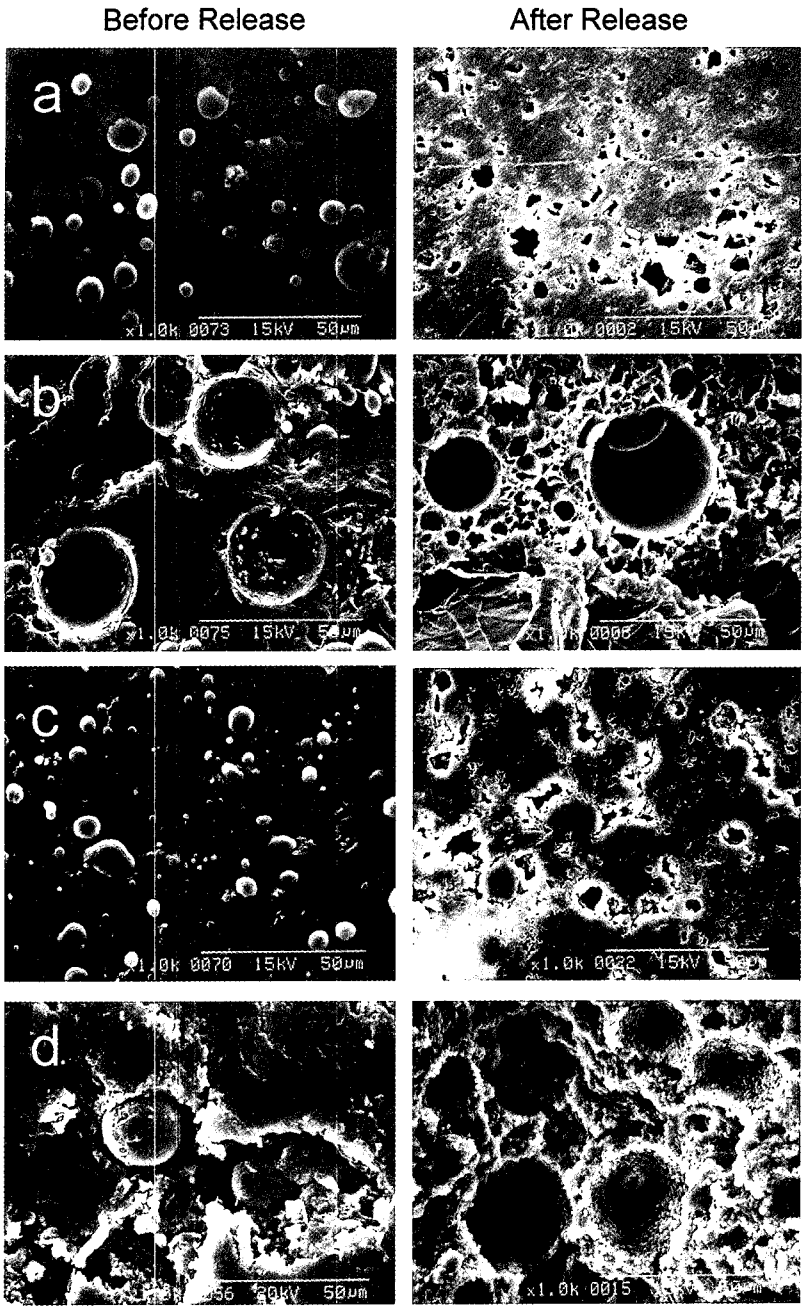

Figure 8. SEM microphotographs of surface (a and c) and cross section (b and d) of GS loaded PHBV wafers before/after (30 days) *in vitro* release experiments (original magnifications; × 1,000). (a and b): 10% GS loaded PHBV wafer with 3 mm thickness, and (c and d): 10% GS loaded PHBV (with 10% of SDS) wafer with 3 mm.

from 212,000 to 80,400 g/mole within same period,[16] i.e., the effect of erosion of PHBV on the GS release might be very low. From these results, it can be summarized that the mechanisms of GS release may be more predominant simple dissolution and diffusion of GS than erosion of PHBV

within 1 month.

Conclusions

Biodegradable controlled antibiotic release PHBV wafers have been prepared by means of simple compression with heating method and their *in vitro* release patterns have been investigated. The GS loaded PHBV wafers with 10% of GS initial loading, 3 mm thickness, higher 3-hydroxyvalerate content, and addition of water soluble polymers could be reached longer release period as over 30 days without any initial burst. This study has demonstrated that the release pattern of drug from wafer fabricated by simple method could be improved by optimizing the preparation conditions of the wafers. This direct compression method has the several merits; (1) it is applicable to unstable drug such as peptides, because no heating or contact with organic solvent, such as chlorinated solvent often used in the preparation of microspheres or nanospheres is required, (2) it can control the release rates even for low molecular weight with water soluble or water insoluble drugs, (3) the release rates can be controlled easily by appropriate selection of polymer species including highly crystalline polymers, and formulation.

The GS loaded PHBV wafers appears to be a promising antibiotic delivery device for the treatment of bone infection in orthopedic surgery without second operation for the removal of the implants after releasing of GS. Studies on the animal experiment are in progress.

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References

(1) K. Klemm, *Septopal a New way of Local Antibiotic Theraphy,* in *Local Antibiotic Treatment in Osteomyelitis and Soft Tissue Infections,* T. G. van Rens and F. H. Keyser, Eds., Excerpta Medica, Amsterdam, 1981, pp 24.
(2) J. Mauduit, N. Buhk, and M. Vert, *J. Control. Rel.,* **23**, 209 (1993).
(3) H. Wahlig, E. Dingeldein, R. Bergmann, and K. Reuss, *J. Bone Joint Surgery,* **60-B**, 270 (1978).
(4) D. H. Robinson and S. S. Sambath, *Drug Dev. Ind. Pharm.,* **15**, 2339 (1989).
(5) S. B. Trippel, *J. Bone Joint Surgery,* **68-A**, 1297 (1986).
(6) Y. Ikada, S. H. Hyon, K. Jamshidi, S. Higashi, T. Yamamuro, Y. Katutani, and T. Kitsugi, *J. Control. Rel.,* **2**, 179 (1985).
(7) K. Yamamura, H. Iwata, and T. Yotsuyanagi, *J. Biomed. Mater. Res.,* **26**, 1053 (1992).
(8) S. S. Sampath, K. Garvin, and D. H. Robinson, *J. Pharm.,* **78**, 165 (1992).
(9) J. C. Cho, G. Khang, J. M. Rhee, Y. S. Kim, J. S. Lee, and H. B. Lee, *Korea Polymer J.,* **7**, 79 (1999).
(10) J. A. Patterson, T. R. Tice, W. E. Meyers, J. W. Vincent, and G. C. Battisone, *Polym. Mater. Eng.,* **53**, 620 (1985).
(11) Y. Shinto, A. Uchida, F. Korkusuz, M. Araki, and K. Ono, *J. Bone Joint Surgery,* **74-B**, 600 (1992).
(12) D. Yu, J. Wong, Y. Matsuda, J. L. Fox, W. I. Higuchi, and M. Ostuka, *J. Pharm. Sci.,* **65**, 1563 (1992).
(13) A. A. Firsov, A. D. Nararov, and I. P. Fomia, *Drug Dev. Ind. Pharm.,* **13**, 1651 (1987).
(14) X. Zhang, P. Wyss, D. Pichora, and M. F. A. Goosen, *J. Macromol. Sci.-Macromol. Chem.,* **A30**, 933 (1993).
(15) G. Wei, Y. Katoura, M. Oka, T. Yamamuro, R. Wada, S. H. Hyon, and Y. Ikada, *J. Bone Joint Surgery,* **73-B**, 246 (1990).
(16) S. Gogolewski, M. Jovanoic, S. M. Perren, J. G. Dillon, and M. K. Hughes, *J. Biomed. Mater. Res.,* **27**, 1135 (1993).
(17) P. J. Hocking, *J.M.S.-Rev. Macromol. Chem. Phys.,* **C32**, 35 (1992).
(18) N. Gangrade and J. C. Price, *J. Microencapsulation,* **8**, 185 (1991).
(19) S. Akhtar and C. W. Pouton, *Drug News Perspectives,* **2**, 89 (1989).
(20) K. Juni and M. Nakano, *CRC Crit. Rev. Therap. Drug Carrier Syst.,* **3**, 209 (1987).
(21) T. W. Atkins and S. J. Peacock, *J. Microencapsulation,* **13**, 709 (1996).
(22) I. Gursel and V. Hasirci, *J. Microencapsulation,* **12**, 185 (1995).
(23) B. R. Conway, J. E. Eyles, and H. O. Alpar, *J. Control. Rel.,* **49**, 1 (1997).
(24) F. Koosa, R. H. Muller, S. S. Davis, and M. C. Davies, *J. Control. Rel.,* **9**, 149 (1989).
(25) K. Juni, M. Nakano, and M. Kubota, *J. Control. Rel.,* **4**, 25 (1986).
(26) G. Khang, S. W. Kim, J. C. Cho, J. M. Rhee, S. C. Yoon, and H. B. Lee, *Bio-Med. Mater. Eng., accepted* (2000).
(27) S. Akhtar, C. W. Pouton, L. J. Notarianni, and P. L.

Gould, *J. Pharm. Pharmacol.*, **40**, 118 (1988).
(28) G. Khang, J. C. Cho, J. W. Lee, J. M. Rhee, and H. B. Lee, *Bio-Med. Mater. Eng.*, **9**, 49 (1999).
(29) G. Khang, J. C. Cho, J. M. Rhee, J. S. Lee, and H. B. Lee, *Polymer Preprints*, **40**, 288 (1999).
(30) G. Khang, J. H. Lee, J. W. Lee, J. C. Cho, and H B. Lee, *Korea Polymer J.*, **8**, 80 (2000).
(31) H. Okada and H. Toguchi, *CRC Crit. Rev. Therap Drug Carrier Syst.*, **12**, 1 (1995).