Effect of Glycolide Monomer on Release Behavior of Gentamicin Sulfate-Loaded PLGA Microparticles

Je Young Yoo,[1] Joon Hyun Shin,[1] Gilson Khang,[1] Hyung Sik Shin,[2] Soon Hong Yuk,[3] Yong Sik Kim,[4] Moon Suk Kim,[5] John M. Rhee,[1] Hai Bang Lee[5]

[1]*BK-21 Polymer BIN Fuston Res Team, Chonbuk National University, 664-14, Duckjin, Jeonju 561-756, Korea*
[2]*Department of Chemical Engineering, Chonbuk National University, 664-14, Duckjin, Jeonju 561-756, Korea*
[3]*Department of Polymer Science and Engineering, Hannam Uinversity, 133, Ojeongdong, Daedeog, Deajeon, 306-791, Korea*
[4]*Department of Orthopedic Surgery, Catholic Medical School, St. Mary's Hospital, Seocho, Seoul 137-040, Korea*
[5]*Nanobiomaterials Laboratory, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong, Daejeon 305-600, Korea*

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ABSTRACT: For the treatment of osteomyelitis and the prevention of infections after orthopedic surgery, topically implantable gentamicin sulfate (GS)-loaded poly(D,L-lactide-*co*-glycolide) microparticles (GSMP) containing glycolide monomer (GM), as a biodegradable and nontoxic material, were prepared by melt-extrusion method without organic solvent for controlled release. After preparation of polymer blend, the powders of different size (90–1200 μm) were obtained by means of freezer-mill. The influences of GM and particle size were investigated on the GS release patterns. GSMP containing GM (in case of 10% loaded) showed a near-zero order release from 2 to 7 days with the initial burst. GM affected to increase of GS release rate during the *in vitro* release test. The pH variations of the media were investigated to determine effect of GM on pH drop of media. The morphological evaluations, change of molecular weight, and thermal property of microparticles were characterized by scanning electron microscope, gel permeation chromatography, and differential scanning calorimeter, respectively. Bacterial inhibition zone test was established to identify antibiosis of GS. It showed antibiotic areas except control sample. From these results, the authors expected that GSMP containing GM would be a good dosage form as a topically implantable device that can get rid of lag period of GSMP. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1019–1025, 2007

Key words: poly(D,L-lactide-*co*-glycolide); gentamicin sulfate; glycolide monomer; microparticles; near-zero order release

INTRODUCTION

Gentamicin sulfate (GS) (Fig. 1) is one of the most potent antibiotics for the treatment of bone infections like osteomyelitis because of its good activity against gram-negative bacteria.[1–7] A serious infection like osteomyelitis can occur mainly by a pyrogenic microorganism, *Staphylococcus aureus*, after knee or hip replacement surgery.[1,4] Osteomyelitis can generally be medicated by administration of GS as a drug with systemic dosage forms such as injection and cream. However, with these dosage forms, it is difficult to exhibit a desirable treatment effect of the antibiotic because of the insufficient concentration of GS at the site of infection.[8] For instance, an injection of GS needs to be administered three times a day to achieve a sufficient treatment effect at the infection site because GS shows the short half-life in plasma.[6]

Moreover, GS is poorly absorbed by oral administration. This poor absorption of GS administrated orally shows low activity in the intestine and is easily eliminated through feces.[6] This can be attributed to a number of factors: no passage for the biological membrane, low absorbability in gastrointestinal tract, no metabolism, glomerular filtration reuptaked in proximal tubule-nephrotoxicity, and partial accumulation in inner ear-ototoxicity. These factors have limited the use of GS as an oral dosage form. Therefore, these systemic administrations of GS need excess administrations to maintain the effective level at the infection site. These excess administrations can also induce various side effects such as nausea,

Correspondence to: Prof. Gilson Khang (gskhang@chonbuk.ac.kr).

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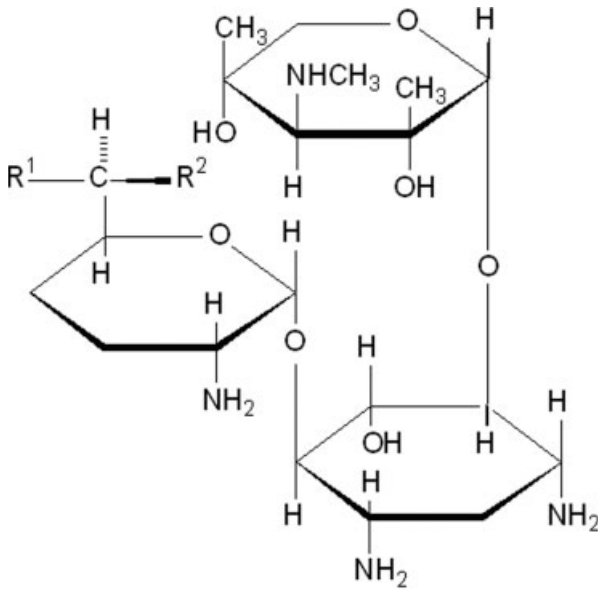

Figure 1 Structure of gentamicin sulfates.

vomiting, headache, skin eruption, ototoxicity, and nephrotoxicity.[6–8]

Much effort has been made to overcome these problems in systemic administration through the development of sustained drug delivery system.[1-6,8-11] The sustained drug delivery system would deliver the drug as a continuous rate and reduce the dose-dependant toxicity by minimizing the fluctuation in plasma concentration.[12] For example, GS-loaded poly (methyl methacrylate) (PMMA) beads have been reported as sustained drug delivery system to treat orthopedic infection. However, PMMA beads must need to be removed after the release of GS because of nonbiodegradability of PMMA.[13,14]

Therefore, much attention has been given to the development of sustained drug delivery system using poly(D,L-lactide-*co*-glycolide) (PLGA),[1,5,15-22] FDA approved material, since they are easily biodegraded to glycolic acid and lactic acid *in vitro* or *in vivo*. Consequently, it does not need to have additional operation to remove the residual materials after orthopedic surgery. Considering the advantage of PLGA, we have recently examined the GS-loaded PLGA devices as a sustained drug delivery system, for example, disc,[5] microspheres,[23] wafer,[24] double-layered cylindrical tablets,[11] and scaffold.[25] In our previous study, we used NaCl and PVP as additives to obtain the favorable drug release pattern in GS-loaded PLGA.[24] However, these additives did not show desirable drug release patterns. Hence, the present work focused on the glycolide monomer (GM), one of main composition of PLGA, used as a channeling agent for the controlled drug release and further pursued the possibility of applying GM blended with PLGA for the fabrication of microparticles prepared by melt-extrusion for topical administration of GS. GM was strongly recommended for desirable drug release without any lag periods from PLGA matrix because of its moderate solubility in water, which can continuously induce the hydrophilic drug release via formed pores from the hydrophobic PLGA matrix.

The aim of this study was to develop GS-loaded PLGA microparticles (GSMP) containing GM with various particle sizes for the development of desirable drug delivery system, which is able to maintain sustained-GS release over 1 week without lag periods, which occasionally might occur in PLGA devices. The release profiles of GS from GSMP according to the different formulations with various ratios of GM and the particle size were evaluated and optimized.

EXPERIMENTAL

Materials

GS was kindly obtained from Dong Shin Pharm. (Seoul, Korea). PLGA and GM (Resomer® RG 503 H, mole ratio of 50:50, molecular weight of 33 Kg/mol) were purchased from Boehringer Ingelheim (GmbH, Germany). *o*-Phthaldialdehyde was purchased from Sigma Chem. (St. Louis, MO). 1-Heptanesulfonate was purchased from Fluka Chem. (GmbH, Switzerland). All other solvents were used as extra pure grade.

Preparation of GSMP

The formulation of GSMP and GSMP containing GM (2, 5, and 10%) is shown in Table I. Briefly, the mixtures were melted at 120°C mold temperature using minimax molder (CS-183, MMX, USA) for 5 min and then solidified at room temperature. After fabrication of the polymer blends, they were grinded by freezer-mill (SPEX 6750, NJ) and divided into different particle size by sieve.

Measurement of drug content from fabricated GSMP

Some of the fabricated GSMP and GSMP containing GM samples were first put into methylene chloride to dissolve PLGA and then distilled water was added

TABLE I
Fabrication Formulations of GSMP With and Without GM

Sample no.	GS % (w/w)	GM % (w/w)	PLGA % (w/w)
Batch 1	10	0	90
Batch 2	10	2	88
Batch 3	10	5	85
Batch 4	10	10	80

in the solution to dissolve GS. The solutions were vigorously sonicated to dissolve each material. Supernatant solutions were centrifuged at 13,000 rpm. A portion of the solutions was mixed with isopropyl alcohol and *o*-phthaldialdehyde (1 : 1 : 0.4 as volume ratio) for derivation, and then the initial loading amount of GS was determined by high performance liquid chromatography (HPLC) method, based on our previous study.[4,5,24]

In vitro release test

The GSMP with and without GM were suspended in 5 mL of phosphate buffered saline (PBS, pH 7.4, Sigma Chem.) and incubated at 37°C. At the scheduled time, the media were taken out from the vials and same volume of the fresh PBS was replaced. The solutions with released GS were stored in refrigerator prior to analysis. All analyses were carried out with three solutions in separate vials. The cumulative drug concentration was plotted against time in days after determination of drug content.

Determination of pH variation of media during degradation test

The determination of the pH variation of the PBS with the microparticles was carried out with a pH meter (Corning 340, USA) equipped with a combined glass electrode. At the scheduled time, the pH of media was measured directly at room temperature.

Quantitative analysis of GS by HPLC

The amounts of released GS from the microparticles were determined by HPLC analysis. The HPLC system consisted of UV detector (UV-1000, Spectra System, USA), pump (Bischoff, Switzerland), and autosampler (AS-1000, Spectra System, USA). The separation of GS was achieved by using a reversed phase column (Capcellpak-C_{18}, Shishedo, Japan) at 37°C. The mobile phase consisted of methanol, distilled water, and glacial acetic acid (80 : 18 : 2) with 1-heptanesulfonate (5 g). It was degassed by vacuum filtration through a 0.45-μm filter prior to use. The eluent flow rate was 1 mL/min. The detection wavelength was set at 330 nm.

Morphological evaluation

The morphological changes of the GSMP with (in case of 10% loaded) and without GM during the *in vitro* release test were investigated to identify the degradation behaviors by scanning electron microscope (SEM, *S*-2250, Hithachi, Japan). Before SEM observation, all samples were mounted on metal stubs and coated with a thin layer of platinum by means of a plasma sputtering apparatus (Emitech, K 575, Japan) under argon atmosphere.

Molecular weight measurement of PLGA

GSMP with (in case of 10%-loaded) and without GM were determined to measure molecular weight change of PLGA during *in vitro* release test by size exclusion chromatography using a gel permeation chromatography (GPC) system that consisted of refractive index (Shodex, RI-71, Japan), pump (Futecs, NS 2001P, Korea), and columns (Shodex, GPC K-802, Asahipak GF-510 HQ, Asahipak GF-1G 7B, Japan). The eluent (chloroform) flow rate and temperature were 0.6 mL/min and 35°C, respectively.

T_g determination of microparticles

The thermal characteristics of selected samples of microparticles during *in vitro* release test were determined by differential scanning calorimeter (DSC, Q 1000, Universal V 3.7S, TA instruments, USA). The instrument was equilibrated at 10°C and purged with pure liquid nitrogen at a flow rate of 50 cc/min. The samples were heated from 10 to 100°C at a heating rate of 10°C/min under nitrogen atmosphere. The T_g of the samples was obtained by taking the midpoint of the slope.

Bacterial inhibition zone test

Cultivation and transfer of *Staphylococcus aureus* were carried out to identify the antibiosis of GS.[5] Prepared liquid medium, which had *Staphylococcus aureus* (1 mL) with soft medium (3 mL), was poured into

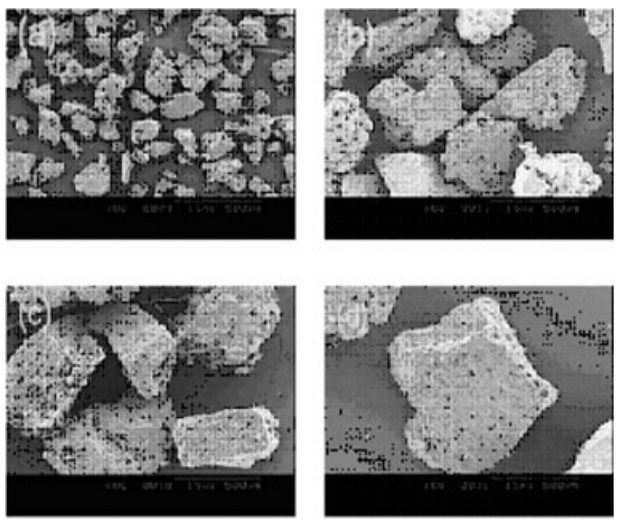

Figure 2 SEM of microparticles separated by sieve after melt-extrusion. (a) 90–180 μm, (b) 260–350 μm, (c) 430–600 μm, and (d) 710–1200 μm (original magnification ×80).

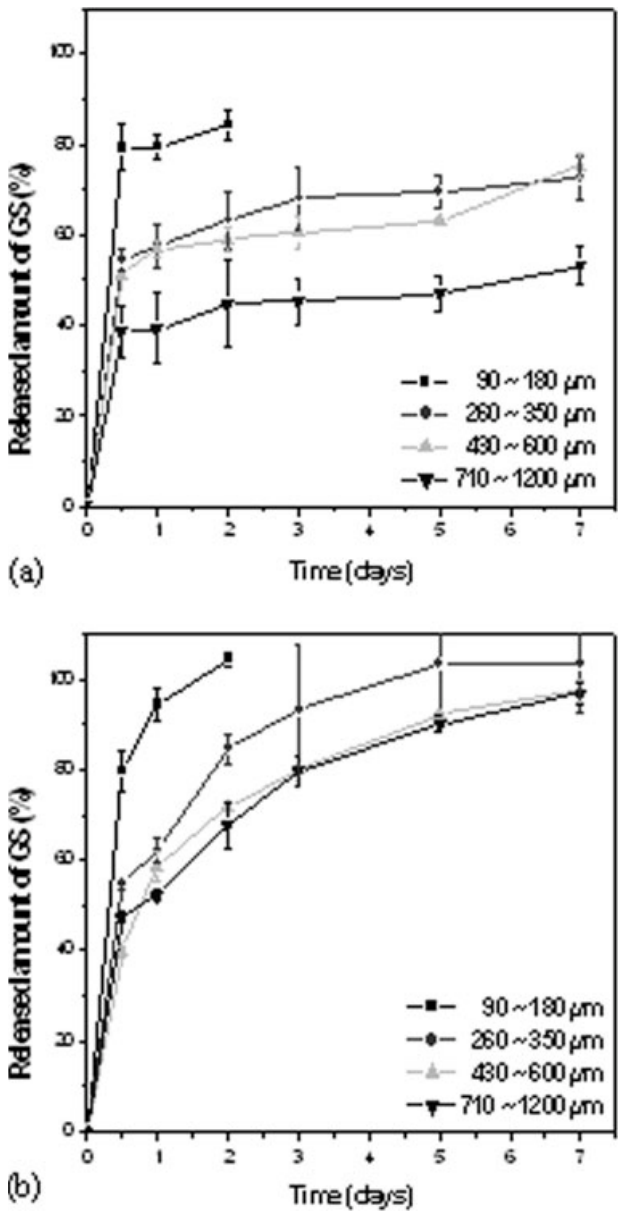

Figure 3 Cumulative release profiles of GS. (a) GSMP and (b) GSMP containing GM (10%).

solid medium (25 mL). After 10 min, cloning disks (I.D. 3 mm) were loaded on agar dish under condition of asepsis. After 0.5, 1, and 7 days, antibiotic areas formed by GS solutions taken from the *in vitro* test vials were observed and pictured.

RESULTS AND DISCUSSION

Characterization of GS-loaded PLGA microparticles

The GSMP with and without GM was prepared by melt-extrusion. All GSMP were grinded by freezer-mill and separated to obtain different sizes of microparticles by sieve. The drug contents of prepared GSMP was 98–100% considering the initial loading amount. The surface morphology of the obtained GSMP is shown in Figure 2. The GSMP showed some pores and irregular structures.

Cumulative release of GS from microparticles during *in vitro* release test

The GS release profiles from the all GSMP were investigated at 37°C for 7 days as shown in Figure 3. Figure 3(a) shows the release of GS from the GSMP with variable particle sizes. The initial burst from the GSMP was very quickly ranged around 40–80% in proportion to the decrease of particle size. Gener-

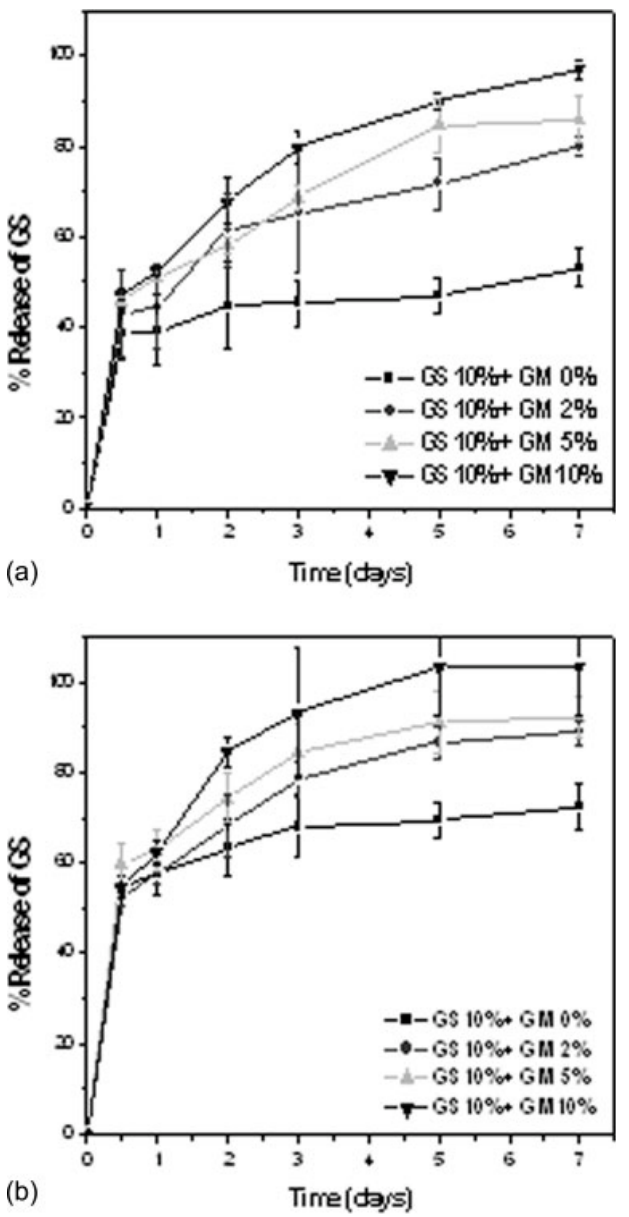

Figure 4 Cumulative release profiles of GS. (a) 710–1200 μm and (b) 260–350 μm.

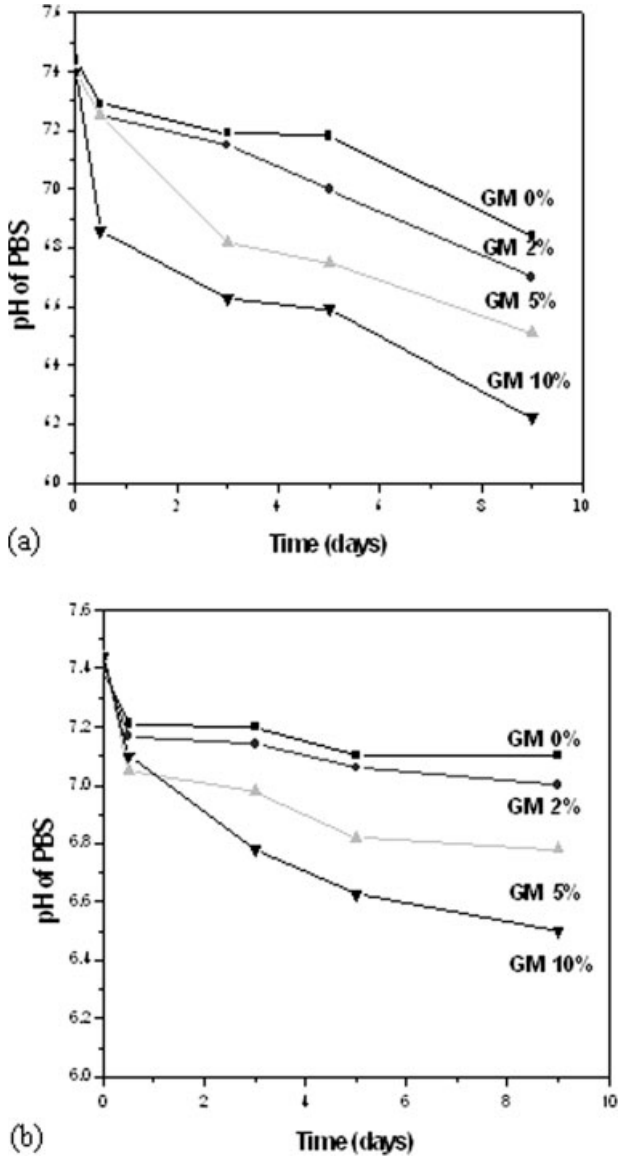

Figure 5 The pH variations of the microparticles in PBS in *in vitro*. (a) 260–350 μm and (b) 430–600 μm.

ally, the released GS from the GSMP showed the lag period regardless of particle sizes after initial burst.

However, GS release from the GSMP containing GM (10%) showed the sustained release patterns without lag periods as shown in Figure 3(b). Also, GS was released proportionately with the decrease of their particle sizes. In case of the smallest GSMP containing GM, the GS release showed the fastest rate. Moreover, other samples showed the desirable drug release patterns without lag period up to 7 days. From this result, it was shown that GM could improve the release of GS. The improved release patterns by GM can be explained that GM plays a role as a channeling agent, which can induce the release of GS from the matrix. Figure 4 shows the release of GS by grouping of percent of GM. In case of Figure 4(b), the GS release rate was faster than in Figure 4(a). Moreover, it showed faster release rate of GS at the initiation of release test. It can be explained that the release of GS could be accelerated by water with the decrease of microparticles size. In other words, the increase of surface area could promote the release of GS.

Variations of pH

The pH variation of the media was measured to estimate the effect of GM on the GS release from the microparticles. GM was changed into glycolic acid by water. This phenomenon could induce pH change of PBS. The pH of the media was decreased with the increase of incubation time, as shown in Figure 5. The increase of GM loading amount and decrease of the particle size extensively could decrease the pH of media, indicating the increase of concentration of glycolic acid. The decreased pH (acidic condition) of

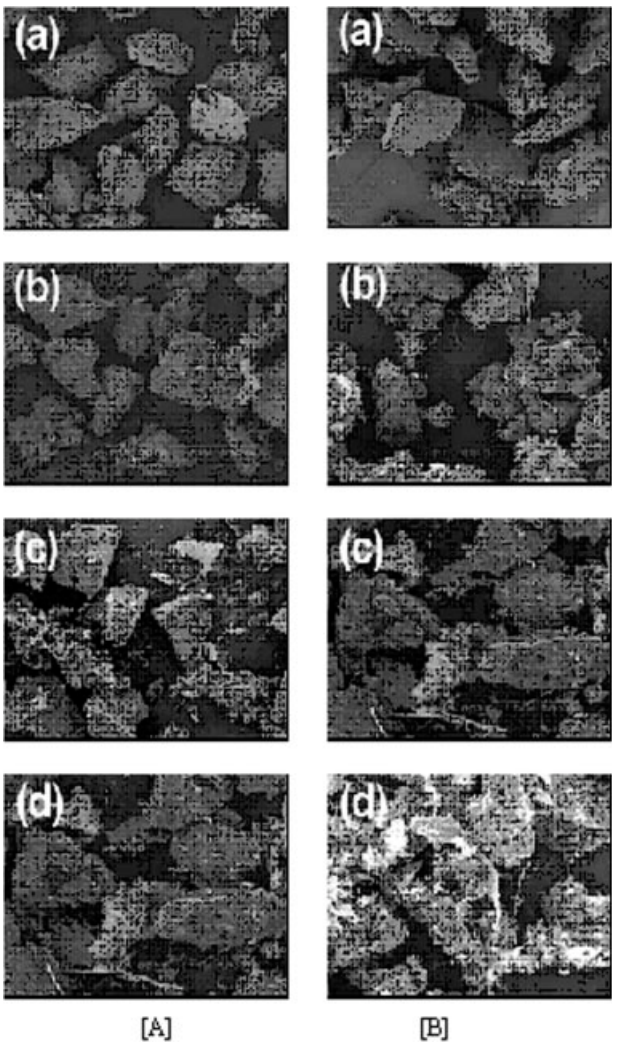

Figure 6 SEM of microparticles (260–350 μm) [A] GSMP, and [B] GSMPcontaining GM (10%) after *in vitro*. (a) 0.5 day, (b) 3 days, (c) 5 days, and (d) 7 days.

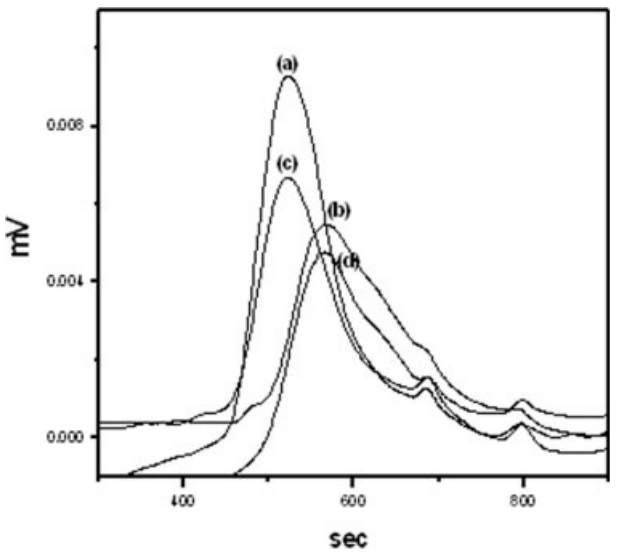

Figure 7 M_w changes of PLGA samples. (a) GSMP (after 0.5 day), (b) GSMP (after 7 days), (c) GSMP (GM 10% loaded, after 0.5 day), and (d) GSMP (GM 10% loaded, after 7 days).

media could contribute to the slight increase of hydrolysis rate of PLGA microparticles.[26]

Morphology of GSMP

Figure 6 shows the morphological changes of GSMP with (10%) and without GM, respectively. The microparticles showed the structural corruption. In case of GSMP containing GM (10%), the morphological changes showed the slightly faster structural changes than that of GSMP. This behavior could facilitate the faster drug release through PBS-filled channels. This behavior showed the anomalous result that is somewhat in correspondence with *in vitro* release patterns.

Measurement of molecular weight of PLGA

The changes of molecular weight of PLGA were investigated to determine the effect of GM, as a biodegradable and nontoxic additive, considering its initial loading amount. Figure 7 shows the result of GPC analysis. GSMP with GM (10%) and without GM showed the decreased molecular weights after 7 days of *in vitro* release test because of chain cleavages of PLGA. However, there was not a sharp difference in the change of molecular weights between GSMP and GSMP containing GM. From the result, it was found that GM could not affect the decrease of molecular weight of PLGA during the short time of the test.[5]

DSC analysis

Figure 8 shows the DSC thermal analysis on T_g of the various PLGA microparticles. Figure 8(a) shows the T_g of modified PLGA by melt-extrusion (before and after 7 days *in vitro*) in the first heating cycle. The T_g of the PLGA was shifted down to the below retention time incorporation to the study time. Figure 8(b) shows the T_g of PLGA, GS/PLGA, and GS/GM/PLGA microparticles (*in vitro* after 7 days). It shows the little changes in T_g. However, graphs were very similar each to other and quite similar to that of the original PLGA. It was observed in these graphs that the range of T_g was not influenced by drug and GM during the short time of the test. This result might show the nonbinding chemical property of each material. It could be concluded that the fabri-

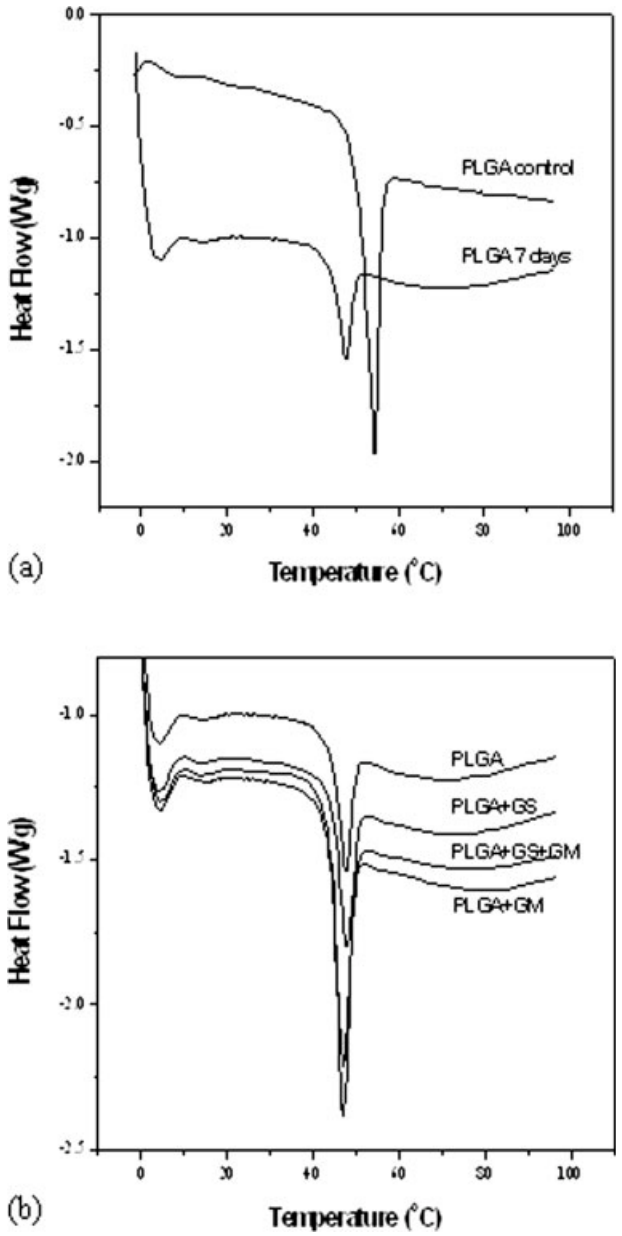

Figure 8 Glass transition changes of PLGA matrix. (a) Original PLGA (0 day and after *in vitro* 7 days) and (b) PLGA mixtures (after *in vitro* 7 days).

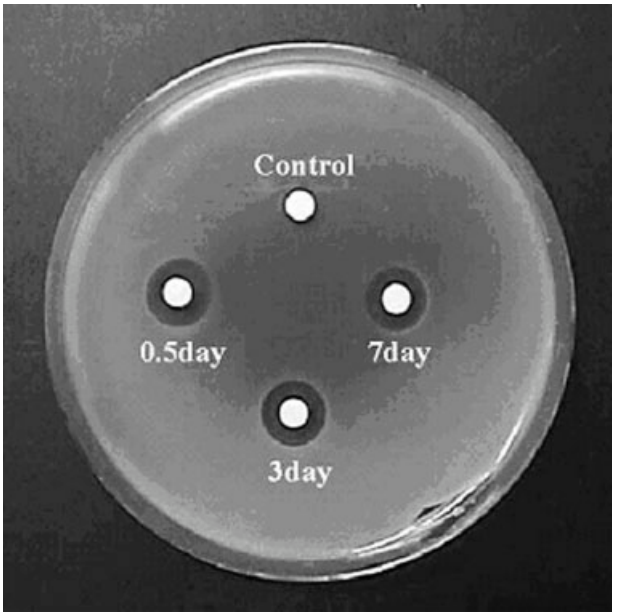

Figure 9 Picture of bacterial inhibition zone test using the GS solution (260–350 μm).

cated microparticles showed glass transition temperatures and their shift phenomena showed the degradation of PLGA regardless of the presence of additives.

Observation of antibacterial area

Bacterial inhibition zone test picture is shown in Figure 9. Control (blank) cloning disk did not show the antibiotic area. However, other samples (*in vitro* after 0.5, 3, and 7 days) showed the antibiotic area indicating the elimination of *Staphylococcus aureus* because of the antibiosis of released GS during the *in vitro* release test.

In this study, we developed GSMP containing GM with various loading amounts and particle sizes by means of melt-extrusion method. The prepared topically implantable GSMP containing GM has several advantages as below. First, it might reduce the side effects resulting from systemic administrations of GS like injection by means of topical administration. In the second place, the prepared microparticles do not need to use any organic solvents for the preparation. The third, the prepared GSMP containing GM has the desirable drug release patterns with the initial burst[5,12] by controlling the amount of GM and particle sizes. Fourth, any material after orthopedic surgery need not be removed.

From these advantages, it is clear that the prepared GSMP containing GM will possibly treat osteomyelitis and prevent secondary infections after orthopedic surgery. Consecutive studies including amount of dosage, toxicity of applied materials, and animal experiment are being carried out.

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