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Effect of lactide/glycolide monomers on release behaviors of gentamicin sulfate-loaded PLGA discs

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Abstract

In order to develop the desirable drug release patterns such as no lag time and exact zero-order release rate, gentamicin sulfate (GS)-loaded poly(D,L-lactide-co-glycolide) (PLGA) discs containing lactide monomer (LM) or glycolide monomer (GM) were prepared. LM or GM was applied for the controlling drug release pattern due to its non-toxic and biodegradable nature. Water absorption, mass loss, pH change, and morphology of discs were examined to study the effect of LM or GM addition. GS release showed near zero-order profile in the GS-loaded polymeric discs prepared in the presence of LM or GM (10%). The channel of GS-loaded PLGA containing LM or GM was formed by the dissolution of LM or GM. Water uptake of disc increased till 21 days from the beginning of the test. The pH variations of media declined in the same manner with the result of mass loss. The antibiosis of GS was also confirmed by bacterial inhibition zone test using the prepared polymeric discs. From these results, we expected that the polymeric discs containing LM or GM would be a good dosage form as a topically implantable device which can get rid of lag period from PLGA matrix.

Keywords: Gentamicin sulfate; Polymeric disc; LM or GM; Antibiosis; Controlled release; Zero-order

1. Introduction

Osteomyelitis can occur in bone and its marrow after knee or hip replacement surgery mainly by pyrogenic microorganism, *Staphylococcus aureus* (Benaerts et al., 1999; Cho et al., 1999; Stephens et al., 2000; Soriano and Evora, 2000; Posyniak et al., 2001). It can be generally medicated by administration of gentamicin sulfate (GS) (Fig. 1) with systemic dosage forms. However, systemic dosage forms are difficult to manifest a desirable treatment effect of the antibiotic due to insufficient concentration of GS by poor blood supply at the deed-seated infection site (Khang et al., 2000). For example, administration of GS through injection needs it to be injected three times a day to achieve the desirable treatment effect at the infection site because of short half-life of GS in the plasma (Soriano and Evora, 2000). Therefore,

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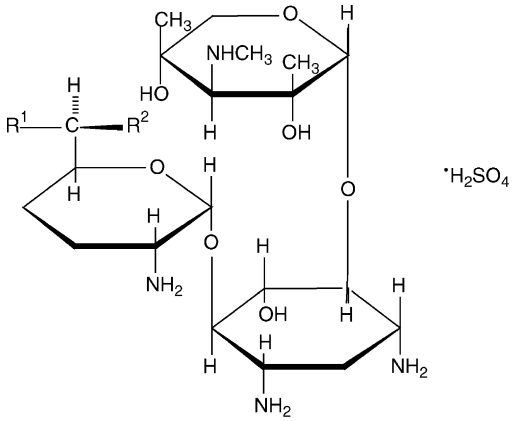

Gentamicin sulfate C_1 ; R^1=CH_3, R^2=$NHCH_3$

Gentamicin sulfate C_2 ; R^1=CH, R^2=NH_2

Gentamicin sulfate C_{1a}; R^1=H, R^2=NH_2

Fig. 1. Chemical structure of GS.

this systemic administration of GS needs to increase dosage in order to maintain the effective level at the infection site. This excess administration can also induce various side effects such as nausea, vomiting, headache, skin eruption, ototoxicity, and nephrotoxicity (Khang et al., 2000; Soriano and Evora, 2000).

Many efforts have been made to overcome those problems which might occur in systemic administration through the development of sustained drug delivery system (Mauduit et al., 1993; Khang et al., 2000; Hedberg et al., 2002; Yun et al., 2002). The sustained drug delivery system would deliver the drug at a continuous rate and reduce the dose-dependent toxicity by minimizing the fluctuations in plasma concentration (Schmidt et al., 1995). 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 be removed after release of GS because of non-biodegradability of PMMA (Seligson and Henry, 1993; Padilla et al., 2002). Therefore, much attention has been given to the development of sustained drug delivery system using poly(D,L-lactide-co-glycolide) (PLGA), already approved by FDA, since they are naturally biodegraded to glycolic acid and lactic acid in vitro and/or in vivo (Bhardwaj and Blanchard, 1998; Choi et al., 2002). Consequently, no surgical intervention or procedures are required for their removal. Considering the advantage of PLGA, we have recently examined the GS-loaded PLGA devices as sustained drug delivery system, for example, microspheres (Cho et al., 2000; Yun et al., 2002a), film (Choi et al., 2001), wafer (Khang et al., 2000; Yoo et al., 2003), double layered cylindrical tablets (Son et al., 2000), scaffold (Choi et al., 2001), microparticles (Yoo et al., 2004a), and so on. In the previous study, we used NaCl and PVP as additives to obtain the favorable drug release patterns without lag periods from GS-loaded PLGA matrix which occasionally might occur in PLGA devices (Yoo et al., 2004b). However, these additives did not contribute desirable drug release patterns such as no lag time and exact zero-order release rate.

Currently, our work focused on the lactide/glycolide monomers (LM/GM) as additives to achieve desirable drug release patterns and further pursued on the possibility for using of LM or GM as non-toxic and biodegradable materials blended with PLGA for the manufacture of disc for locally implantable administration of GS. LM or GM, i.e., main composition of PLGA, strongly suggested that it has several advantages such as moderate solubility in water for the controlled drug diffusion and biocompatibility as well as non-toxic and biodegradable property as mentioned above.

The aim of this study is the development of the GS-loaded polymeric disc containing LM and GM as candidate of desirable drug delivery system which is able to maintain sustained GS release over 1 month without lag periods. Also, the acting effects of GM or LM from GS-loaded PLGA were evaluated.

2. Materials and methods

2.1. Materials

GS was obtained from Dong Shin Pharmaceutics Co. Ltd. (Seoul, Korea). LM, GM, and poly (D,L-lactide-co-glycolide) (PLGA, Resomer® RG 503 H; 50:50, mole ratio of lactide to glycolide; molecular weight, 33,000 g/mole) were purchased from Boerhinger Ingelheim (Ingelheim, Germany).

Table 1
Manufacturing formulation of discs

Batch no.	GS (%)	LM (%)	GM (%)	PLGA (%)
1	10	0	0	90
2	10	10	0	80
3	10	30	0	60
4	10	0	10	80
5	10	0	30	60

o-Phtaldialdehyde (OPA) was purchased from Sigma (St. Louis, MO, USA). 1-Heptanesulfonate was purchased from Fluka Chem. Co. Ltd. (GmbH, Germany). Methanol (Burdick & Jackson, USA), glacial acetic acid (Tedia, USA), and isopropylalchol (IPA, Jin Chem. Co. Ltd., Korea) were used as pure grade without purification. Beef extract, tryptone peptone, casitone, dextrose, nutrient agar, and brucella broth were purchased from Difco Laboratories (Detroit, USA). Water was obtained by a Mill-Q purification system from Millpore (Molsheim, France). All other chemicals were of analytical grade and used with distilled purification.

2.2. Fabrication of disc

The conventional direct compression method was applied to fabricate the GS-loaded polymeric discs by means of 5 mm radius of mold. Briefly, GS, PLGA, LM, and GM as various compositions (Table 1) were physically mixed by means of pipette, vortex mixer, and shaker (Wrist Action, Pittsburgh, USA). The mixed powders were weighed (10 mg) and pressed (MH-50Y, CAP 50 t, Japan) under 20 kgf/cm^2 for 5 s.

2.3. Measurement of drug content

Three of the fabricated samples were firstly put into methylene chloride (MC) to dissolve PLGA, and then distilled water (DW) was added in the solution to dissolve GS. The solutions were maintained in sonicator (Fisher Scientific, FS 110®, USA) to entirely dissolve each material for 5 min. Supernatants were centrifuged at 13,000 rpm. A portion of the solutions was mixed with IPA and OPA (1:1:0.4 as volume ratio) for derivatization, and then the initial loading amount of GS was determined by high performance liquid chromatography (HPLC) method based upon our previous study (Yoo et al., 2003).

2.4. In vitro release test

In vitro release test was performed by placing the discs into vials and adding 10 ml of phosphate buffered saline solution (PBS, pH 7.4). The sample vials were shaken in sealing condition to avoid partial evaporation of the fluids at 37 °C in a shaking bath at 110 rpm/min. At the scheduled time, the release media were periodically taken out from the vials with pipette and same volume of the fresh medium was replaced. All analyses were carried out with each sample taken out from three vials. The cumulative drug concentration was plotted against release time.

2.5. Quantitive analysis of GS

The amount of released GS from the discs was determined by HPLC analysis. The HPLC system consisted of UV detector (UV-1000, Spectra System, USA), pump (Bischoff, Switherland), 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/l). 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 monitored at 330 nm.

2.6. Measurement of water uptake/mass loss of disc

All samples were taken out from the vials at a set time followed by the removal of water from the discs using soft wipes (Kimwipes®, Yuhan-Kimberly, Korea). The obtained discs were weighed to determine the water uptake of the disc during in vitro release test before being freeze-dried by the freeze-dryer (Ilshin®, Ilshin Lab Co. Ltd., Korea) for 3 days. The dried discs were also weighed to determine the mass loss from original disc.

2.7. Determination of pH variation of media during degradation test

At the set time, the pH variations of the PBS taken out from the solutions with discs were measured by a pH meter (Corning 340, USA)

Table 2
Classification and formulation of microbiological culture media for bacterial inhibition zone test

Materials	Type of media (g/100 ml of water)		
	Liquid	Solid	Soft
Beef extract	0	0.15	0.75
Tryptone peptone	0	0.6	0.3
Casitone	0	0.4	0.2
Dextrose	0	0.1	0.05
Nutrient agar	0	0.5	0.75
Brucella broth	0.8	0	0

equipped with a combined glass electrode at room temperature.

2.8. Morphology observation

Scanning electron microscope (SEM, S-2250N, Hitachi, Japan) was used to examine the morphological change of the discs during, before, and after in vitro release test as well as to reveal the degradation patterns of PLGA. To observe SEM, 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.

2.9. Bacterial inhibition zone test

Cultivation and transfer of *S. aureus* were carried out to identify the antibiosis of GS in vivo. Briefly, the subcultured pure *S. aureus* was incubated for 1 day at 37 °C. From the matured *S. aureus*, one of the colonies was extracted and then cultured in liquid medium for 1 day at 37 °C. The prepared soft medium (3 ml) and the liquid medium (1 ml) containing the cultured colony were poured into the solid medium (15 ml) (Table 2). And then, some of cloning discs were soaked in GS solutions taken from the in vitro test vials (0, 3, 7, 12, and 21 days). The wet cloning discs were put onto the separate agar-dishes to identify the antibiosis of GS. After 1 day, antibiotic area of GS was observed and pictured. Antibiotic areas of GS were measured by means of a zone reader (Fisher Scientific, USA).

3. Results and discussion

3.1. Characterization of polymeric discs

The conventional direct compression method was applied to prepare the GS-loaded PLGA disc containing LM or GM at room temperature. There are no distinctive differences for the prepared PLGA discs indicating that main properties of disc depend on only PLGA, not additives.

3.2. Quantitative analysis of GS from polymeric discs

It is very important to quantitatively analyze GS released from the GS-loaded PLGA disc prepared in the presence of LM or GM. Hence, much effort has been paid to analyze GS, quantitatively. However, most of the analysis methods were complex and had time-consuming steps. First of all, we investigated to optimize analysis condition of GS using HPLC. The concentration of GS (10–100 μg/ml) was monitored with HPLC after derivatization using OPA for 15 min because GS alone did not show the signal in UV detector. The stability of GS solution using OPA was examined to establish precise analysis condition. As shown in Fig. 2, GS concentration after OPA derivatization decreased dramatically as keeping

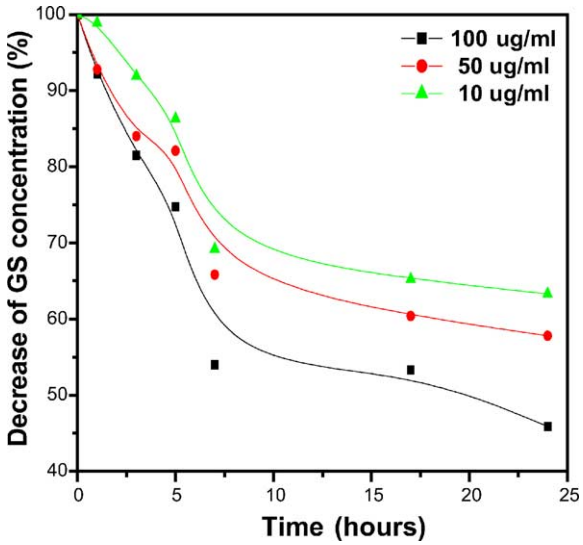

Fig. 2. Changes in GS concentration after OPA derivatization.

time at room temperature increased due to instability of OPA. In case of the highest concentration of GS standard solution, the decrease rate of concentration was faster than the other samples. Therefore, the concentration of GS could be detected by immediate analysis of the solution reacted with OPA. The prepared polymeric discs showed drug contents from 98 to 102% indicating that GS was loaded in quantitative content.

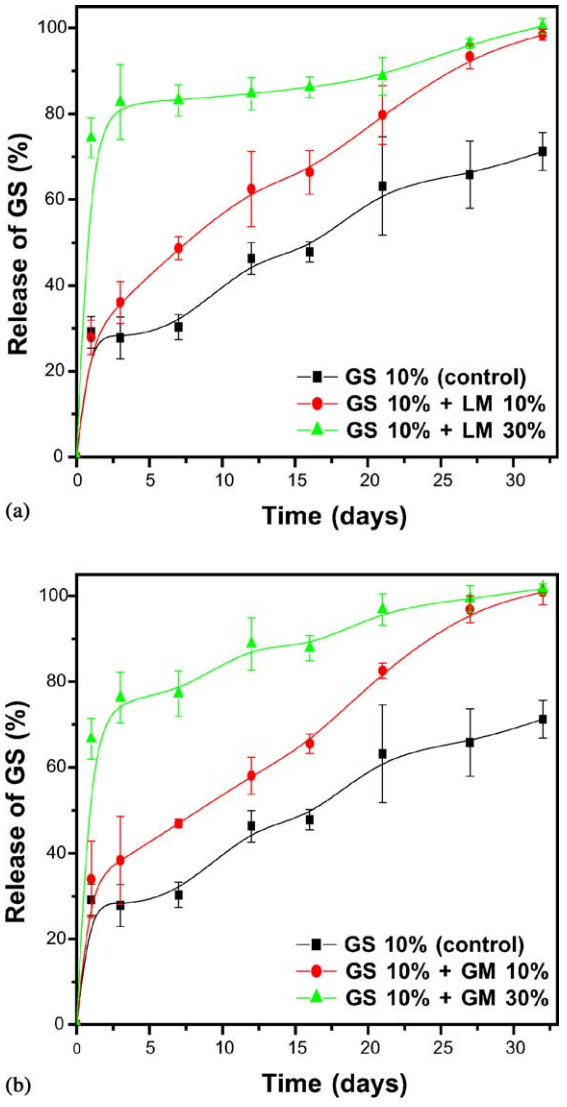

Fig. 3. Effect of monomers on in vitro release profiles of GS: (a) the disc containing LM and (b) the disc containing GM.

3.3. GS release from polymeric disc

The experiment of GS release from the prepared discs was performed at 37 °C under shaking (110 rpm). Fig. 3 shows the GS release profiles obtained for 30 days. The disc in the absence of LM or GM as control showed tri-modal release profiles (Fig. 4). At day 1, the released amount of GS from the control disc reached around 30% implying that the initial burst of GS was due to the release of drug from the disc surface. This is agreed well with the result of microparticles in our previous study (Yoo et al., 2004a). The GS release was almost plate for 7 days. The increase of GS release was again detected by accepting the medium into the polymeric disc along a channel after 7 days; maybe, the small channel should be formed through the degradation of PLGA (Yoo et al., 2004b). Meanwhile, the disc in the presence of LM or GM showed faster release of GS than control. Furthermore, the increase of LM or GM concentration showed the fast release of GS. This result indicated that LM or GM acted as a channeling agent to form the channel into polymeric disc. From Fig. 3, we found that almost zero-order release profiles can be achieved by the addition of 10% monomer. Moreover, this sustained GS release was maintained over 1 month without lag periods.

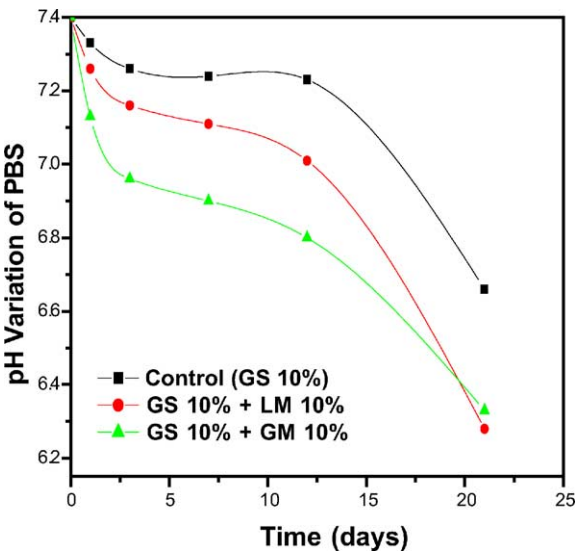

Fig. 4. Measurement of pH variations of media.

3.4. Variation of pH

LM or GM can act as additive change to lactic acid (LA) or glycolic acid (GA), respectively, by cleavage of ester bond through hydrolysis. The change induces decrease of media pH (Yoo et al., 2004b). Hence, the variation of pH of PBS as medium was measured to estimate the effect of LM or GM in the GS release from the discs. Fig. 4 shows the pH changes measured in the absence and presence of LM or GM (10%) for 21 days. With the increase in time, pH of the discs containing monomers dropped towards acidic faster than that of the control disc. The pH drop of the discs containing GM, moreover, showed faster rate than that of the discs containing LM. It strongly suggested that the change of GM to GA is faster than that of LM due to hydrophobicity. The decreased pH of media could be contributed to the increase of hydrolysis rate of PLGA resulting in the increase of channels on matrix (Yoo et al., 2004b).

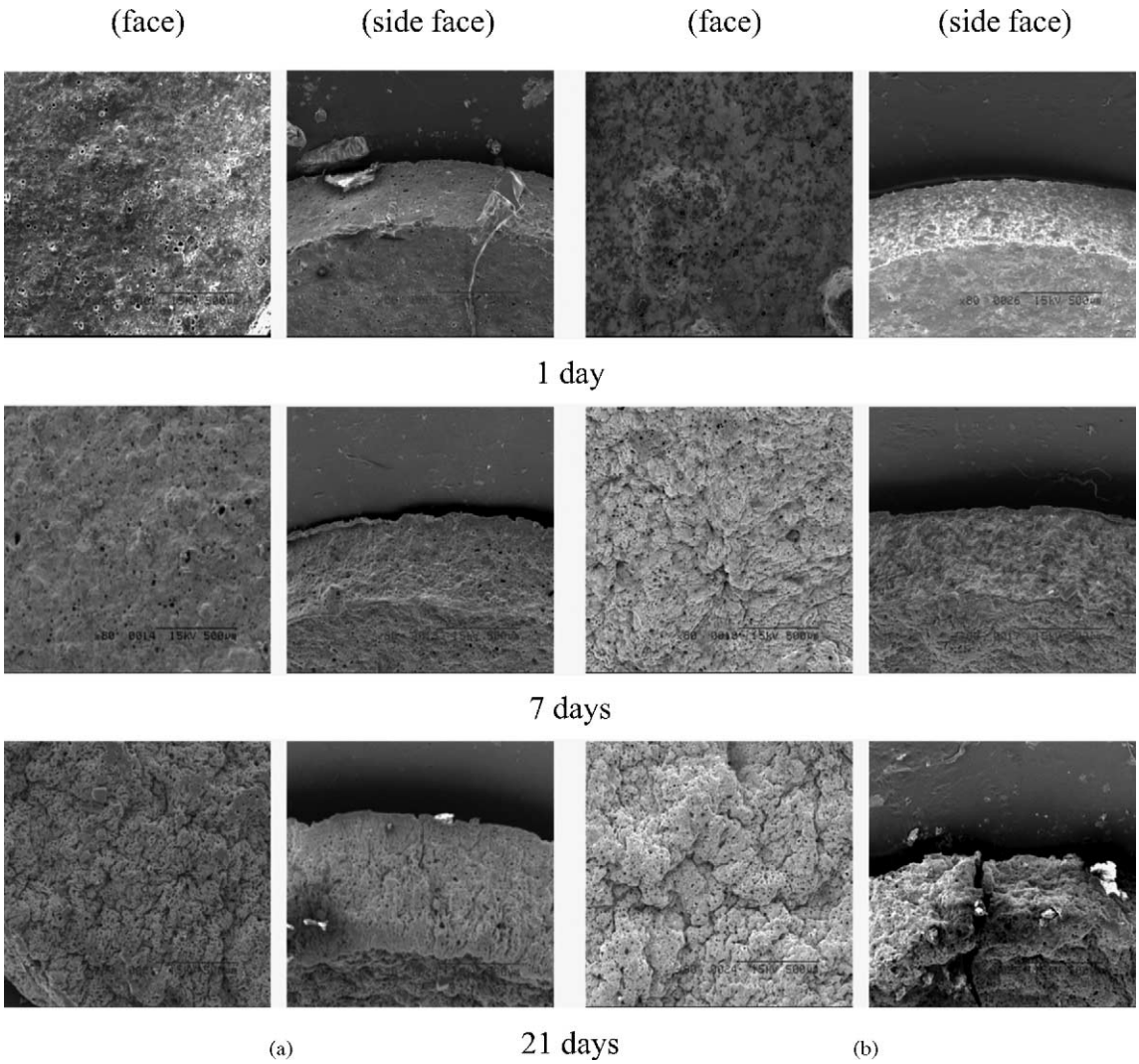

Fig. 5. SEM pictures of: (a) the control disc and (b) the disc containing GM (10%): in vitro after 1, 7, and 21days (left, face; right, side face; original magnification, 80×).

3.5. Morphological evaluation

SEM measured to observe the change of polymer matrix. Fig. 5 shows the morphological changes of the polymeric discs without and with GM (10%), respectively. With the time increase, the discs showed the structural metamorphosis. There are not large differences between the control disc and the disc containing GM after 1 day. The difference of structural metamorphosis is observed after 7 days. The disc containing monomers compared with control disc showed the more structural metamorphosis indicated the formation of channel by GM. Therefore, this changed structure could induce the faster drug release through PBS-filled channels.

3.6. Water uptake of discs

The water uptake of discs was measured to examine the changes of properties of discs in the absence and presence of LM or GM. As shown in Fig. 6, water uptake of the discs containing LM or GM (in case of 10% monomers loaded) was increased faster than that of the control disc. After 7 days, water absorption amount of the control disc was 20% but for the discs containing monomers, it was approximately 60%. LM or GM could make pores easily on the discs by dissolving them and the formed pores could induce high water absorption into the disc indicating that LM or GM acted as channeling agent. Meanwhile, the control disc had to make pores on the disc by dissolution of only drug supposing that PLGA degraded at similar rate after 7 days.

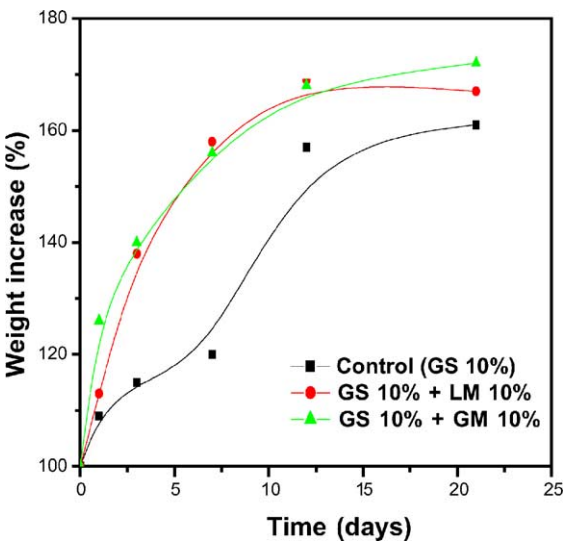

Fig. 6. Changes of water uptake of discs.

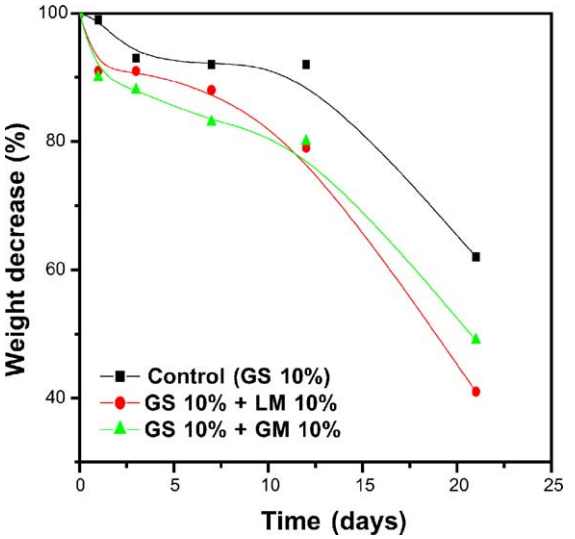

Fig. 7. Changes of mass loss of discs.

3.7. Mass loss of discs

Fig. 7 shows the mass loss of the discs to evaluate the degradation of PLGA. As same manner with the result of water absorption test, the mass loss of the dried discs containing monomers declined dramatically. It was a result of faster incoming of water into the disc due to dissolution of monomers which could make more possible channels. After 21 days of dissolution test, the disc containing 10% GM was decreased to 49% of its own weight, meanwhile, the control disc was decreased approximately to 63%. However, there was no significant weight loss of polymer matrix considering the initial loading amount of monomers. This result strongly indicates that the added LM or GM induced the formation of channels.

3.8. Observation of anti-bacterial area

In order to convict the effect of GS on the Gram-negative bacteria, e.g., *S. aureus*, bacterial inhibition zone test was performed under the condition of

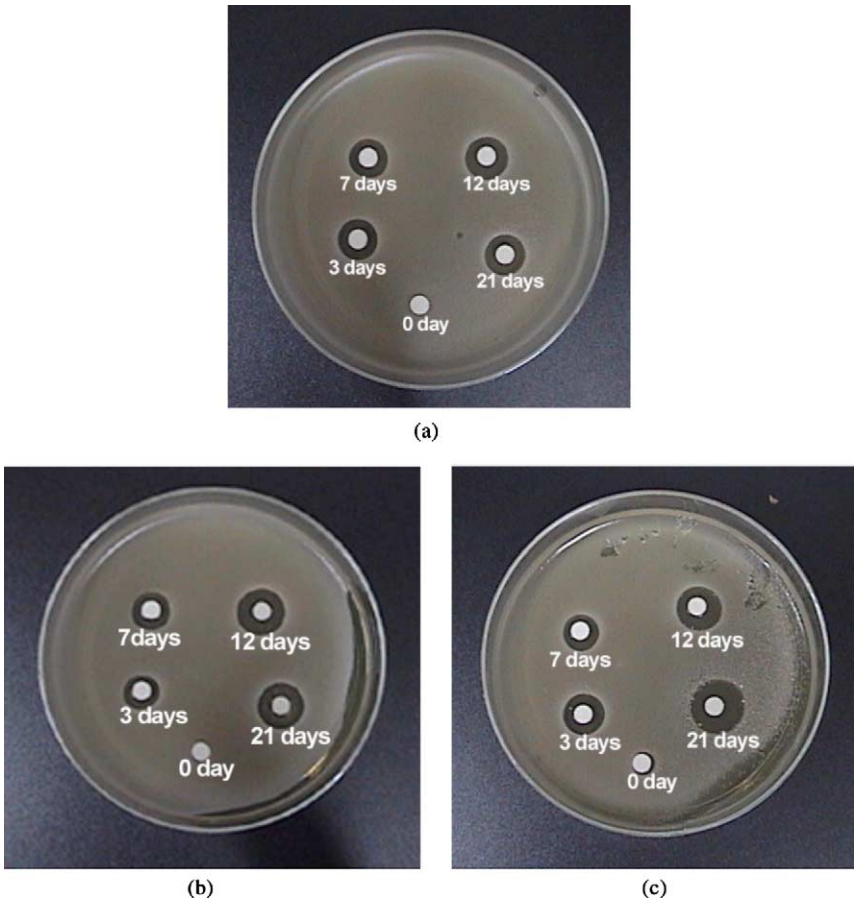

Fig. 8. Pictures of anti-bacterial areas by GS: (a) the control disc; (b) the disc containing LM (10%); and (c) the disc containing GM (10%).

asepsis. Fig. 8 shows the results of bacterial zone test by using *S. aureus*. On day 0 of cloning, disc could not make the anti-bacterial area. However, other samples taken from the vials of GS-loaded PLGA without and with GM or LM (in vitro after 3, 7, 12, and 21 days) showed the anti-bacterial areas indicating the elimination of *S. aureus* through the action of the released GS. They did not gradually show the activity of GS up to 7 days according to the amount of released GS. However, after 7 days, the discs containing monomers showed the reasonable results with respect to antibiosis of GS. We found that the addition of GM or LM could control the release profiles of GS. Through this experiment, GS showed the anti-bacterial areas against *S. aureus* which can suppress the activation of bacteria.

4. Conclusion

In this study, we prepared the polymeric discs containing LM or GM which had a good mixing property with PLGA. From the result of in vitro test, we found that the release profiles of GS-loaded polymeric discs in the presence of 10% monomers showed nearly zero-order kinetics up to over 1 month with the initial burst.

GS release patterns relied upon the concentration of LM or GM. LM or GM acted as good channeling agents in the GS-loaded polymeric discs. The initial burst may be a positive result which could immediately act on Gram-negative bacteria, e.g., *S. aureus* at the first time of administration (Schmidt et al., 1995). From the result of bacterial inhibition zone test, we

observed the desirable antibiosis of GS by using in vitro release solutions. It showed significant antibiotic activity of GS indicating the elimination of *S. aureus*.

We expected that GS-loaded polymeric discs containing LM or GM would have the possibility of the treatment effect to the osteomyelitis and the prevention of secondary infections after orthopedic surgery for 1 month or more. Moreover, this locally implantable device might decrease the side effects in comparison to other dosage forms by its sustained release and local delivery at the infection site. Consecutive studies including amount of dosage and animal experiment are being carried out.

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