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Feature Article

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Local Drug Delivery System Using Biodegradable Polymers

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Abstract: For last five years, we are developing the novel local drug delivery devices using biodegradable polymers, especially polylactide (PLA) and poly(D,L-lactide-*co*-glycolide) (PLGA) due to its relatively good biocompatibility, easily controlled biodegradability, good processability and only FDA approved synthetic degradable polymers. The relationship between various kinds of drug [water soluble small molecule drugs : gentamicin sulfate (GS), fentanyl citrate (FC), BCNU, azidothymidine (AZT), pamidronate (ADP), 1,25$(OH)_2$ vitamin D_3, water insoluble small molecule drugs : fentanyl, ipriflavone (IP) and nifedipine, and water soluble large peptide molecule drug : nerve growth factor (NGF), and Japanese encephalitis virus (JEV)], different types of geometrical devices [microspheres (MSs), microcapsule, nanoparticle, wafers, pellet, beads, multiple-layered beads, implants, fiber, scaffolds, and films], and pharmacological activity are proposed and discussed for the application of pharmaceutics and tissue engineering. Also, local drug delivery devices proposed in this work are introduced in view of preparation method, drug release behavior, biocompatibility, pharmacological effect, and animal studies. In conclusion, we can control the drug release profiles varying with the preparation, formulation and geometrical parameters. Moreover, any types of drug were successfully applicable to achieve linear sustained release from short period (1~3 days) to long period (over 2 months). It is very important to design a suitable formulation for the wanting period of bioactive molecules loaded in biodegradable polymers for the local delivery of drug. The drug release is affected by many factors such as hydrophilicity of drug, electric charge of drug, drug loading amount, polymer molecular weight, the monomer composition, the size of implants, the applied fabrication techniques, and so on. It is well known that the commercialization of new drug needs a lot of cost of money (average: over 10 million US dollar per one drug) and time (average: above 9 years) whereas the development of DDS and high effective generic drug might be need relatively low investment with a short time period. Also, one core technology of DDS can be applicable to many drugs for the market needs. From these reasons, the DDS research on potent generic drugs might be suitable for less risk and high return.

Keywords : local drug delivery system, delivery devices, biodegradable polymers, pharmaceutics, tissue engineering.

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Introduction

It has been recognized that biodegradable polymers have become increasingly important in the development of drug delivery system (DDS) during the past two decades. Massive researches have been done to design appropriate devices like microspheres (MSs) and nanospheres, injectable form, wafer, tablet, and so on using synthetic and natural biodegradable polymers.[1-13] In the 1970s, simply and mainly long term controlled release devices of contraceptive steroids, local anesthetic, narcotic antagonist, anticancer and antimalarials drugs were developed to test the possibility of the pharmacological activities and elimination of the inconvenience of the repeated injection. Beginning of 1980s, rapid and innovative progress in the area of biotechnology, especially cell and cloning technology, made possible the successive and massive production of therapeutic peptides and proteins like cytokine, monoclonal antibody, hormone and growth factors. So, DDS using biodegradable polymers were extensively studied and commercialized for these peptides and proteins to achieve efficiency and to increase patient compliance. It can be avoided the difficulties associated with parental and oral delivery and patient compliance problems. Above DDS method would deliver the drug at a continuous rate, and reduce the dose-dependent toxicity by minimizing the fluctuation in plasma concentration.

Biodegradable polymers for local delivery system for DDS might be divided into natural and synthetic biomaterials as listed in Table I.[2,5,10,11] Among of these biodegradable polymers, one of the most significant candidates for the development of the biodegradable polymeric controlled release system is the poly(α-hydroxy acid)s family such as poly(glycolide) (PGA), poly(L-lactide) (PLA) and its copolymers as poly(D,L-lactide-*co*-glycolide) (PLGA; chemical structure as shown in Figure 1) which is only approved by the Food and Drug Administration (FDA) due to its controllable biodegradability and relatively good biocompatibility.[14-29] It provides many advantages such as regulating degradation period according to the molecular weight and mole fraction

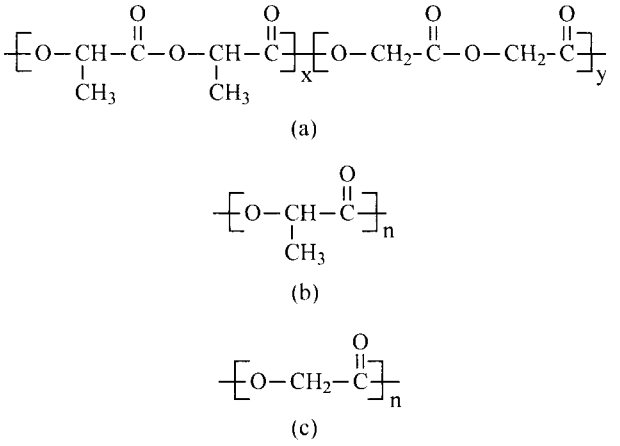

Figure 1. Chemical strucures of (a) PLGA, (b) PLA, and (c) PGA.

of lactide and glycolide in the copolymer, especially PLGA, producing toxicologically safe by-products that are further eliminated by the normal metabolic pathways. On the other hand, natural polymers might be varied in purity during the preparation process and the encapsulation process in which can be lead to denaturation of the polymer and the embedded drug.[11,12] This change sometimes might result in the variation in the quality of the DDS devices and immunogenic adverse reactions. From these reasons, these synthetic poly(α-hydroxy acid)s are preferable for the commercial products. Also, the wide acceptance of the poly(α-hydroxy acid)s as suture materials, made them an attractive candidate for biomedical applications like ligament reconstruction, tracheal replacement, ventral heniorraphy, surgical dressings, vascular grafts, nerve, dental, fracture repairs, and tissue engineering area.

Typical and successful commercial product is Lupron Depot® (leuprorelin acetate) and Decapeptil® (tryptorelin) composed of LH-RH agonist in biodegradable PLGA for the treatment of advanced prostatic cancer and endometriosis. Lupron Depot® is a once-a-month injection that is easily injected into the muscle or subcutis. It maintains persistent

Table I. Typical Biodegradable Polymers Used in DDS

Natural Biodegradable Polymers	Synthetic Biodegradable Polymers
- Polypeptides and proteins: Albumin, fibrinogen, gelatin, Collagen, etc. - Polysaccharides: Hyaluronic acid, starch, chitosan, etc. - Virus and living cells: Erythrocytes, fibroblast, Myoblasts, etc.	- Aliphatic polyesters of hydroxy acids: PLA, PGA, PLGA, poly(hydroxybutyric acid), poly(ε-caprolactone) - Polyorthoester - Poly(alkylcarbonate) - Polyaminoacids - Polyanhydrides - Polyacrylamides - Poly(alkyl-α-cyanoacrylate)s - Etc.

blood drug levels for 1 month due to zero-order drug release. PLGA, drug carrier, was completely biodegraded in the injection site for around 6~8 weeks after injection.[30] Another successful commercial product is Gliadel® wafer which composed of polyanhyride polymer and BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea) for the treatment of malignant brain tumor. This localized and controlled delivery device of the anticancer agent using biodegradable polymeric implants provides to solve the problem of low penetration of blood brain barrier (BBB).[4,30]

This article introduces our works on the development of various drug delivery applications with like MSs, microcapsule, nanoparticle, wafers, pellet, beads, multiple-layered beads, implants, fiber, scaffolds, and films applied using PLGA, PLA and PHVB biodegradable polymers during last 3 years.[32-81] Water soluble small molecule drugs [gentamicin sulfate (GS),[32,38-40,42,43,57,73,81] fentanyl citrate (FC),[34,37,51] BCNU,[45,53,58,59,65,66,68,70,71,76,80] pamidronate (ADP),[60,64,69] 5-fluorouracil (5-FU),[41,44,78] azidothymidine (AZT),[35] 1,25$(OH)_2$ vitamin D_3[55,67]], water insoluble small molecule drugs [fentanyl,[47,52,54,61,77] ipriflavone (IP)[72,75] and nifedipine[36,49]], and water soluble large molecule drug [nerve growth factor (NGF),[48,50,79] vascular endothelial growth factor,[46,56,62,63,77] brain derived neurotrophic factor and Japanese encephalitis virus (JEV)[33]] were applied for the development of local DDS devices.

Applications

JEV Vaccine Loaded PLGA MSs for the Oral Immunization. The extensive studies for biodegradable MSs with incorporated antigens as oral vaccine is of particular interest in making currently available vaccines more effective and in developing new vaccines against infections for which none currently exist. Advantages of biodegradable MSs for oral immunization are: (a) lower dosage requirement, (b) leading to a decreased probability of unwanted side effects and decreased effects, (c) localized or targeted delivery of antigen to antigen-presenting cell or the lymphatic system, (d) several antigen may be encapsulated, (e) facilitating the design of a formulation that can immunize an individual against more than on disease or against several epitopes of a given pathogen in a single injection, (f) reducing the number of vaccine dose required for successful vaccination to a single injection, (g) reducing the cost of immunization programs while increasing coverage, (h) entrapped antigens are protected from degradation in the gut, (i) possible of controlled or "pulsed" release of antigen from MSs after uptake, consequently, (j) improved patient compliance.[82]

The control of some fabrication parameters for the preparation for JEV vaccine as a model vaccine loaded PLGA MSs has been investigated such as the types of emulsifier (poly(vinyl alcohol) (PVA) and sodium dodecyl sulfate (SDS)), the concentrations of emulsifier, agitation speed, and the concentration of PLGA in W/O/W method. Also, the effect of surface morphology on biodegradation of PLGA has been observed. For the oral vaccination of JEV/PLGA MSs via the across the gastrointestinal tract as Peyer's patches, the size of MSs must be below around 15.0 μm. For the satisfaction of this size, the rate of agitation of 2,000 rpm could be minimum stirring speed in this study with 5.0 w/v% of PLGA concentration, and 100 mL of 1.0 wt% PVA. To investigate the effect of emulsifier types on the surface morphology of JEV vaccine loaded PLGA MSs, two kinds of emulsifiers as PVA and SDS were applied. The surface morphology of nonporous and porous structure was observed from SDS and PVA, respectively. From the assay of lactic acid and SEM observation as shown in Figure 2, it can be suggested that the rate of biodegradation of nonporous MSs was slower than that of porous surface morphology due to the lower surface area. In

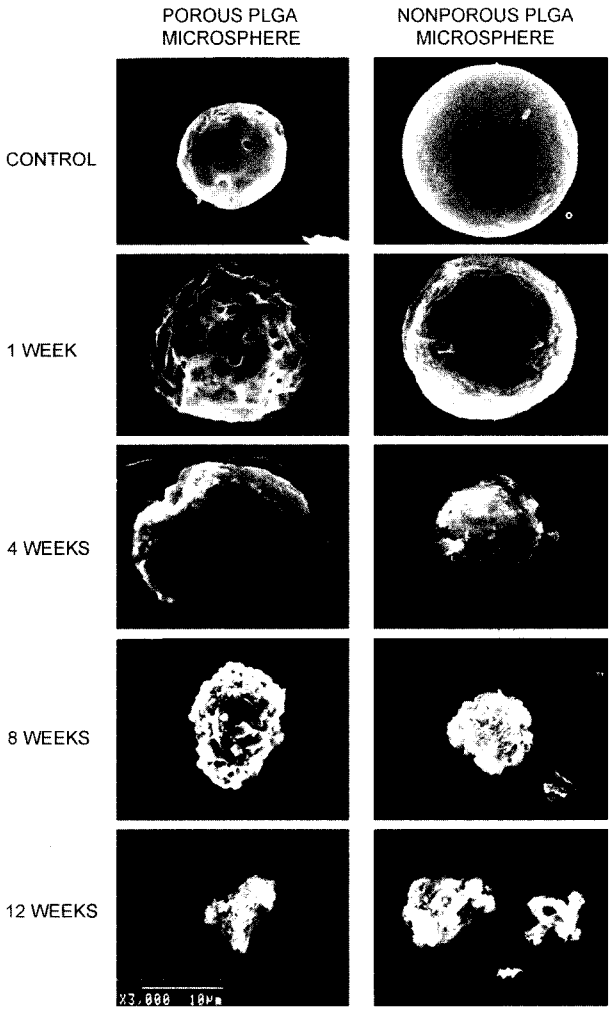

Figure 2. The biodegradaded morphologies of porous and nonporous JEV vaccine loaded PLGA MSs with M_w of 25,000 g/mole for 1, 4, 8, and 12 weeks (Original magnifications; ×3,000, scale bar; 10 μm).

conclusion, the formulation and the process variables play important roles in morphology of PLGA MSs, biodegradation of PLGA MSs and resulting the release pattern of drug.[33]

Different Types of GS Loaded Devices for the Treatment of Osteomeylitis. GS is one of the most potent antibiotics for bone infection such as osteomyelitis. Prolonged parental and oral antibiotic therapy for 4~6 weeks may be necessary for usual treatment. Some disadvantages of prolonged parental therapy by intraveneous 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 theraphy.[83] Above method would deliver the drug at a continuous rate, and reduce the dose dependant toxicity by minimizing the fluctuation in plasma concentration.

GS Loaded PLGA MSs: Controlled GS releasing MSs manufactured from biodegradable PLGA (75:25 by mole ratio) were prepared with an oil/oil solvent evaporation method. The MSs of different size (30~350 μm) were obtained with varying the experiment conditions, and the shape of MSs was smooth and spherical. The efficiency of encapsulation was over 81%. The effects of the preparation conditions on the size of MSs have been investigated. *In vitro* release studies showed that different release patterns and release rates could be achieved by simply modifying factors in the preparation conditions such as polymer concentration, surfactant concentration, molecular weight of PLGA and initial amount of drug. PLGA MSs with 20% of initial drug loading, 0.2% (w/w) of surfactant concentration and 50% (w/v) of PLGA concentration, were free from initial burst effect and a near-zero order sustained release was observed for over 60 days as shown in Figure 3. The release of GS loaded MSs with larger size like over 300 μm was more prolonged over 2 month, whereas small size batch like below 100 μm was observed burst effect. This study demonstrated that the release pattern of the drug from MSs could be improved by optimizing the preparation conditions of MSs.[32,37,40]

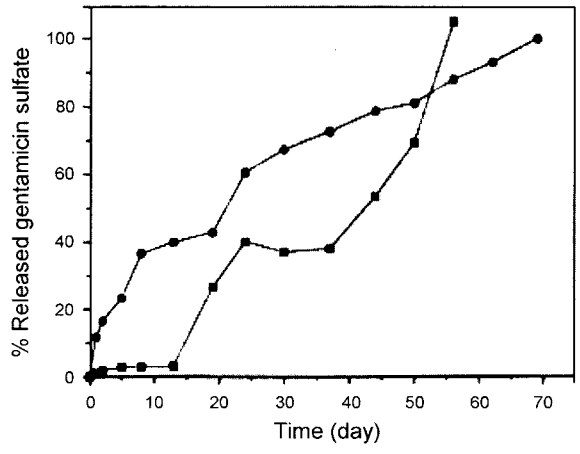

Figure 3. GS released behavior from PLGA MSs of different initial drug loading. ■: 3.9% and ●: 20% (M_w of PLGA: 24,000 g/mole, polymer concentration: 50% w/v).

GS Loaded Multilayered PLGA Matrix: Controlled GS releasing cylindrical matrix from biodegradable PLGA (75:25 by mole ratio, 20,000 and 90,000 g/mole) were prepared with simple heat compression method for the improvement of GS release pattern close to zero-order. The PLGA cylindrical matrix with various release patterns could be obtained by altering the molecular weight and its mixture ratio of PLGA. The addition of 90,000 to 20,000 g/mole reduced the release rate. In order to get more precise release pattern, multilayered such as two or three-layered cylindrical matrix were prepared with the gradient of GS concentration and the molecular weight of PLGA (Figure 4). These results indicated that a desirable release pattern could be obtained by an appropriate combination of PLGA molecular weight, its mixing ratio, the gradient of GS concentration and the gradient of PLGA molecular weight as shown in Figure 5. Mechanisms of the prolonged sustained release from the multilayered PLGA matrix over 60 days with zero-order rate was the different biodegradation duration with different molecular weight. Also it was observed that the release pattern of GS from the matrix could be improved by optimizing the preparation conditions of the multilayered PLGA matrix.[39]

Preparation of Biodegradable PHBV Wafer Containing GS. GS-loaded PHBV devices were prepared for controlled-release of GS. The effects of thickness, hydroxyvalerate (HV) content, initial drug-loading ratio, and additive content and types such as sodium SDS, microcrystalline cellulose, polyvinylpyrrolidone (PVP), and hydroxypropylcellulose (HPC) on the release profile have been investigated. The

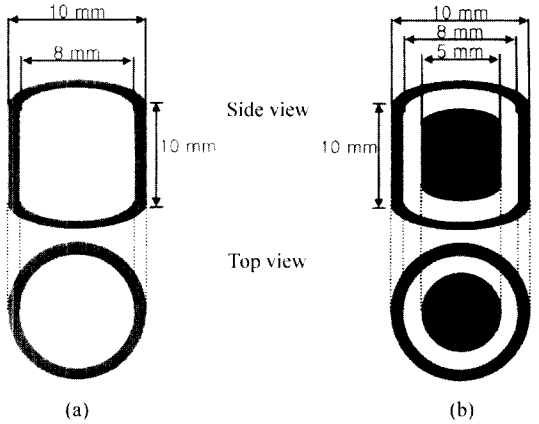

Figure 4. Schematic diagram of multilayered GS loaded PLGA cylindrical matrix. (a) two layered and (b) three layered.

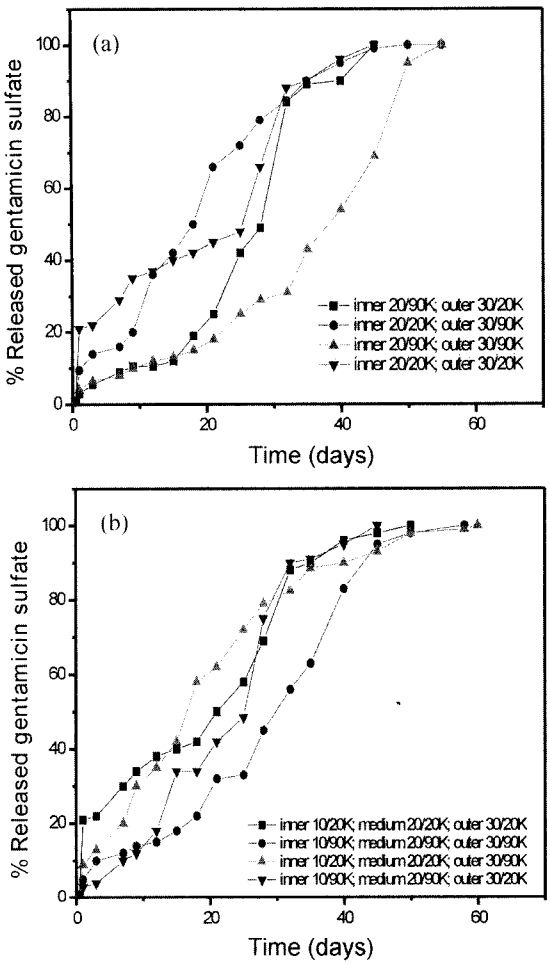

Figure 5. GS released behavior from multilayered GS loaded PLGA cylindrical matrix. (a) two layered, and (b) three layered using different molecular weight and amount ratio.

morphology of devices was examined with SEM before and after *in vitro* release; their highly porous surface and cross sectional were observed. It could be suggested that device would be affected by the packing of the HV and additive content, which would depend on their structure. A high performance liquid chromatography (HPLC) was used to detect and quantify the release of GS from the device. The drug release from all devices showed biphasic release patterns, and some matrices released the incorporated antibiotic throughout 30 days with a near-zero order release rate. 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 as shown in Figure 6. It might be suggested that the mechanism of GS release may be more predominant simple dissolution and diffusion of GS than erosion of PHBV in our system.

This study has demonstrated that the release pattern of the drug from the wafer fabricated by simple method could be improved by optimizing the preparation condition of wafers.

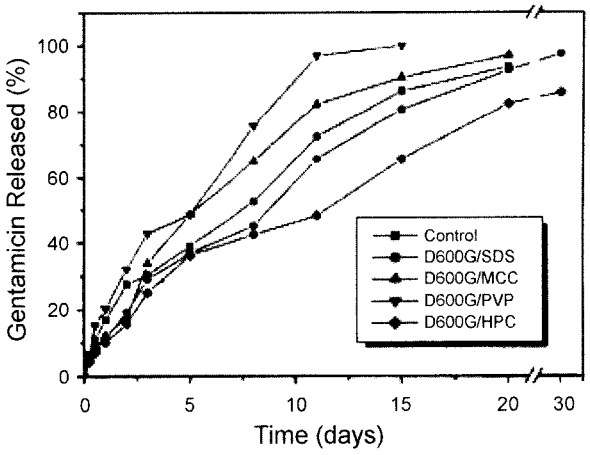

Figure 6. GS released behavior from PHBV wafers with various types of 5% additives (1 gram base with 3 mm thickness; 10% of GS initial loading; 15% of HV).

This direct compression method has the several merits; (1) it is applicable to unstable drugs such as peptides, because no heating or contact with the organic solvent, such as chlorinated solvents often used in the preparation of MSs 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.[38,41,43]

Porous PLA Scaffolds with GS Release System for the Application of Tissue Engineering. PLGA or PLA porous scaffolds are one of the most important elementary factors for *in vitro* cultivation of cell for the tissue engineered organ. One of the most significant problems of the PLGA and PLA scaffolds is the hydrophobic surface property resulting in the difficult penetration of culture media in the porous scaffolds and the source of infection site. That is, it is more desirable to endow with new functionality for the medical applications such as cell and tissue engineering and drug delivery systems. For example, hydrophobic surfaces of PLGA possess high interfacial free energy in aqueous solutions, which tend to unfavorably influence their cell-, tissue- and blood-compatibility in the initial stage of contact.[46,56,62,63]

PLA scaffold loaded with GS was prepared by emulsion freeze-drying method for the prevention of infection and the improvement of wettability, i.e., the cell- and tissue- compatibility. GS-loaded PLA scaffolds were characterized by SEM, mercury porosimetry and blue dye intrusion, and the GS release pattern was analyzed by HPLC. GS-loaded PLA scaffolds with porosity above 80%, medium pore size ranging from 30 to 57 μm (with larger pore diameters greater than 150 μm), and specific pore area in the range of 35 to 75 (m^2/g) were manufactured by varying processing parameter as GS concentration. It was observed that GS-loaded PLA

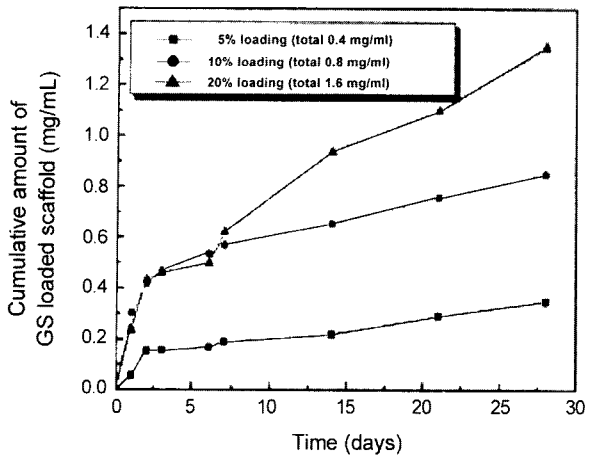

Figure 7. Cumulative amount of released GS from GS-loaded PLA scaffolds prepared by the emulsion freeze-drying process. ■; 5% GS, ●: 10% GS, and ◆ ; 20% GS.

scaffolds were highly porous with good interconnections between pores for allowing cell adhesion and growth. Figure 7 shows that the release pattern could be easily controlled by the controlling the preparation factors such as initial drug loading amount and polymer concentration and so on. Also, it can be observed the greatly improved wettability property by blue dye intrusion methods. GS/PLGA porous scaffolds may be applicable for scaffolds as structures that facilitate either tissue regeneration or repair during reconstructive operation.[42]

Glycolide Monomer Containing GS-loaded PLGA Microparticles by Melt Extrusion: For the achievement of more precise release pattern of GS, we developed GS-loaded PLGA microparticle (GSMP) containing glycolide monomer (GM) prepared by melt-extrusion method. After the preparation of polymer blend by melt extrusion, the powders of different sizes (90~1,000 μm) were obtained by freezer mill as shown in Figure 8. *In vitro* drug delivery release study was performed in pH 7.4 phosphate buffered saline (PBS). The drug release showed that different release rate could be achieved by simply modifying factors in the preparation conditions such as particle size and loading amount of GM. GSMP released from 2 to 7 days in case of the highest loading amount of GM (10%) and showed a near-zero-order with initial burst (Figure 9). The pH of medium was investigated to determine the effect of GM during the course of *in vitro* release test. GM affected to increase of GS release during the *in vitro* release test, which was the positive result of what would be expected based upon decrease of pH of medium. The morphological evaluations of samples were characterized by SEM. GM did not distinctive influence to

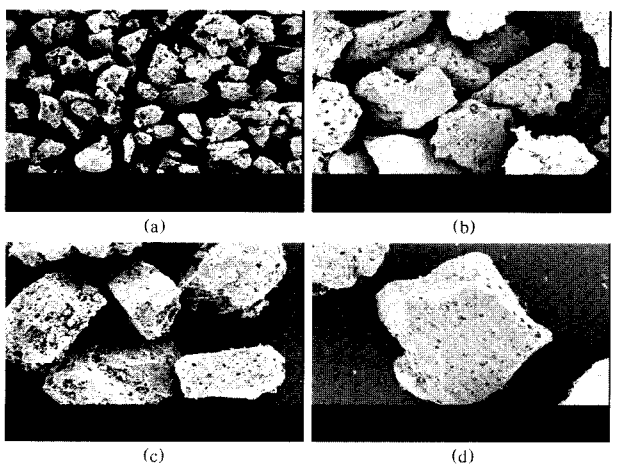

Figure 8. SEM microphotographs of GSMP microparticles separated by molecular sieve after freezer-mill fabricated by melt-extrusion method. (a) 90~180 μm, (b) 260~350 μm, (c) 430~600 μm, and (d) 710~1200 μm (original magnification; ×80).

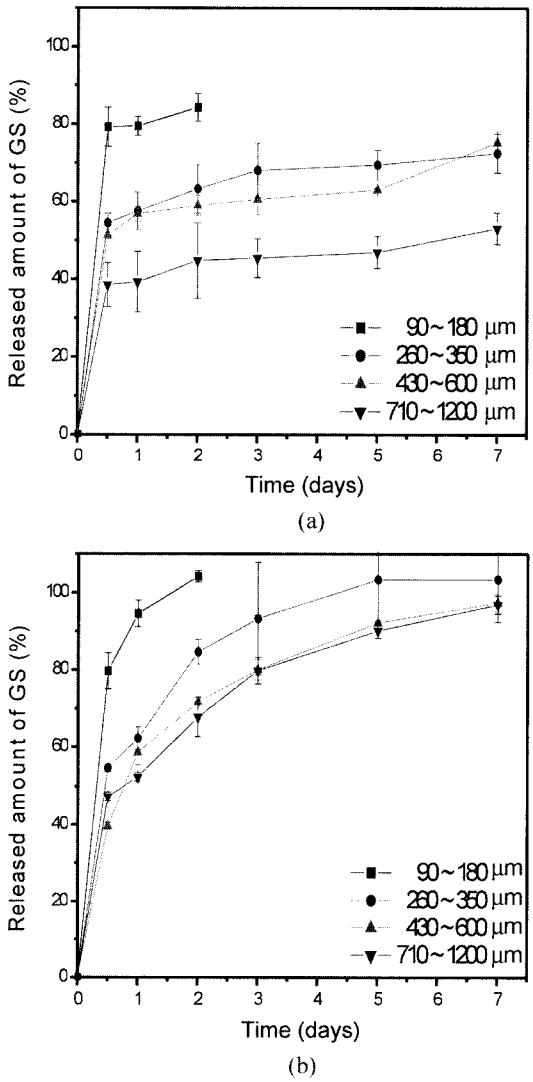

Figure 9. Cumulative release profiles of GS from GSMP in *in vitro* at 37 °C for 0~7 days. (a) GS 10% + GM 0% and (b) GS 10% + GM 10%.

change of morphology by the analysis of GPC and DSC, respectively. Bacterial inhibition zone test was established to identify antibiotic activity of GS (Figure 10).

From these results, we expected that containing GM would be a good dosage form with the sustained release pattern to deliver the antibiotic for the prevention of infections after surgery for 1 week or more. This locally sustained delivery form of scattering powders at infection site will be may be decreasing the side effects comparison to oral dosage forms with high dosage and frequent administration. Moreover, this delivery system has an advantage which does not need to remove the any materials after surgery due to its spontaneous degradation property by human body fluid.[43,57,73,81]

Preparation and Characterization of PLGA MSs for the Sustained Release of AZT. AZT (zidovudine, or azidothymidine) is a strong inhibitor of reverse transcriptase, and is known as effective in the treatment of acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Although orally administered AZT is rapidly absorbed from intestinal mucosa, it loses considerable potency during its first pass (40%) and then is rapidly eliminated from the body with a half-life of 1 hr. In addition, orally administered AZT often shows strong side effects on bone marrow resulting to develop leukemia, which may be attributable to an excessive plasma level of AZT immediately after administration. Therefore, an adequate zero-order release system must be required to decrease the high daily dose at AZT (5~10 mg/kg, every 4 hrs), to maintain an expected anti-viral effect which can be time-dependent, to reduce the strong side effects, and to improve patient compliance. The development of a sustained release device and formulation of AZT would be beneficial in comparison with the recent intermittent dose regimens.

Biodegradable MSs were prepared with PLGA (75:25 by mole ratio) by an oil/oil solvent evaporation method for the sustained release of anti-AIDS virus agent, AZT. The MSs of relatively narrow size distribution (7.6±3.6 μm) were obtained by controlling the fabrication conditions. The shape of MSs prepared was smooth and spherical. The efficiency of AZT loading into the PLGA MSs was over 93% compared to that below 15% for MSs by a conventional W/O/W method. The effects of preparation conditions on the morphology and *in vitro* AZT release pattern were investigated. *In vitro* release studies showed that different release pattern and release rates could be achieved by simply modifying factors in the fabrication conditions such as the type and amount of surfactant, initial amount of loaded drug, the temperature of solvent evaporation, and so on. The AZT-loaded PLGA MSs with 5% of initial drug loading, 25 °C of fabrication temperature, acetonitrile as a solvent, 1% of Span 80 as an emulsifier, and mineral oil as continuous phase appears to be a promising near zero-order release device for the reduction of the strong side effects and the improvement of the patient compliance (Figure 11). This study demonstrated that the morphology of PLGA MSs as well as *in vitro* release pattern of AZT could be improved by optimizing the fabrication conditions of the MSs.[35]

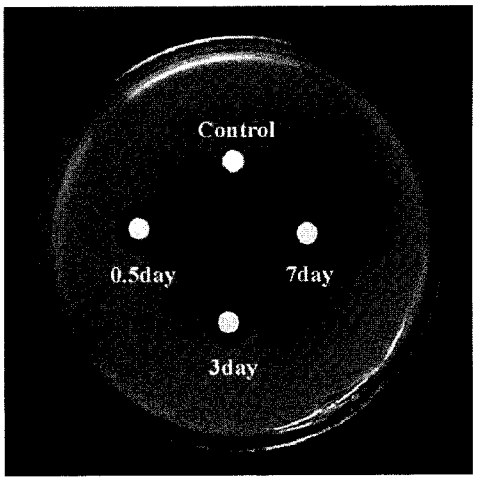

Figure 10. Picture of bacterial inhibition zone test using the released GS solution (260~350 μm).

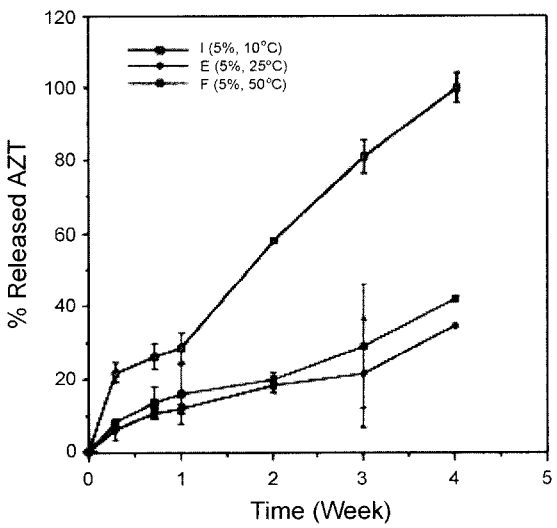

Figure 11. Effect of fabrication temperature on AZT released behavior from PLGA MSs prepared at different fabrication temperature with 5% of initial drug loading content. Batch I: 10 °C, E: 25 °C, and F: 50 °C.

Different Types of Fentanyl Loaded Devices for the Local Anesthesia. Fentanyl is a potent synthetic opiate commonly used for surgical analgesia and sedation. It is approximately 200 times more potent than morphine, has a rapid onset (1~2 min), and short duration of action (30~60 min). Because of its potency and quick onset, even a very small dose of fentanyl can lead to sudden death. Moreover, the expected concentration range of fentanyl is very low. A concentration of 1~3 ng fentanyl per mL of serum is effective therapeutic range for analgesia and toxicity is reached

between 3 and 5 ng/mL when fentanyl is abused. Therefore the detection of lower levels of the compound from analgesic doses is important. In addition, intraveneous administration of fentanyl results in a relatively short half-life, about 3.7 hrs in plasma, and simple parentral administration of fentanyl may not be fully effective, so frequent injections and continuous infusions are required to ensure adequate plasma levels. However, these methods have the disadvantage of potentially causing irreversible damage to nerve or surrounding tissues due to fluctuations in concentration and high levels of anesthetic. Additionally, anesthetic delivered in the form of pulse instead of zero-order kinetics may aggravate adverse reactions due to over dosage.[84-87] Therefore, a sustained release system is needed to prolong the action of local anesthetic as well as to avoid the inconvenience of patients and to maintain constant therapeutic levels. The development of long-acting local anesthetics is also needed for postoperative analgesia and control of chronic pain of cancer patients.

Preparation of Fentanyl-loaded PLGA MSs: Fentanyl-loaded biodegradable PLGA MSs were prepared to study the possibility for long acting local anesthesia. We developed the fentanyl base (FB, slightly water soluble)-loaded PLGA MSs by means of conventional O/W solvent evaporation method. The size of MSs was in the range of 10~150 μm. The lowest porous cross-sectional morphology and the highest encapsulation efficiency were obtained by using gelatin as an emulsifier. The influences of several preparation parameters, such as solvent types (MC and ethyl acetate), emulsifier types (gelatin and PVA), molecular weights and the concentrations of PLGA, and initial drug loading amount, etc, have been observed in the release pattern of fentanyl. The release of fentanyl in *in vitro* was more prolonged over 25 days, with close to zero order patterns by controlling the preparation parameters (Figure 12). We also investigated the physicochemical properties of fentanyl-loaded PLGA MSs by XRD and DSC.

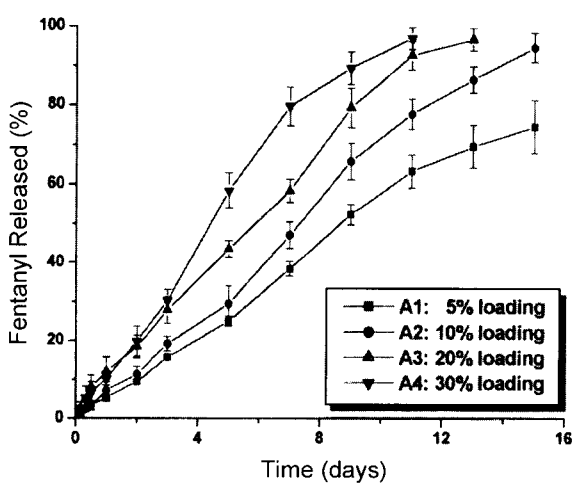

Figure 12. Effect of initial drug loading ratio on the fentanyl base release pattern (n = 3, 10 w/v% PLGA, 3 w/v% emulsifier and 10 mL ethylacetate).

From the results of preparation conditions, the encapsulation efficiencies of fentanyl were between 61.5 and 99.8%, depending on the particular formulation. The total MSs recovered amount varied between 52.8 and 87.6%. It was observed that the encapsulation efficiency and the yield decreased as a function of the increase in initial drug loading amount. As increasing of PLGA concentration from 3 to 20%, encapsulation efficiency was increased from 62.7 to 99.8%. Similar results were also obtained when decreasing the solvent volume. We assumed that the dominating loss of fentanyl must be due to transport of droplets of the O phase to the W phase. The increased viscosity in the O phase caused by the increased PLGA concentration or decreased solvent volume will decrease the loss transport of fentanyl and contribute to the enhanced entrapment efficiencies. Moreover, the O droplets containing fentanyl formed from the W phase were very small and the diffusion amount of fentanyl to the external phase during the solvent evaporation was relatively small, explaining the high encapsulation efficiency obtained. The pattern of drug release depends on various factors, such as initial drug loading ratio, polymer concentration, and solvent volume in W phase. Generally, small size and fast drug release are attributed to more water uptake, swelling ratio, and polymer degradation. In contrast, in our case, fentanyl release rate from the all batches decreased with decreasing MSs size. It might be suggested that the drug release profiles would be affected by the morphology of the MSs; increased concentration of O phase made porous stable MSs and resulted in denser MSs. In conclusion, this sustained, besides, constant localized release system can potentially provide anesthesia for a longer period than injection or topical administration.[47,52,77]

Influence of Processing Variable on Fentanyl Citrate-loaded PLGA MSs by Novel W/O/O Solvent Evaporation Method: Fentanyl citrate (FC), a highly water-soluble drug contrast to fentanyl base, was encapsulated within PLGA MSs by novel W/O/O double emulsion solvent evaporation method, to investigate the possibility for long-acting local anesthesia. Methanol, deionized water, or acetic acid as a solvent of FC and acetonitrile as that of PLGA were used, efficiently to disperse primary emulsion, respectively. Aluminum stearate, Tween 40 or gelatin was used as a surfactant for stabilizing the primary internal emulsion and Span 80 was also used for stabilizing second emulsion phase. Acetic acid and aluminum stearate were considered as an optimum solvent and surfactant in internal phase, respectively. The presence of aluminum stearate in internal oil phase was essential in forming stable emulsion droplets and affected stability of emulsion, without phase separation of FC and PLGA. The surface morphology of MSs was dense with no apparent pores and cross-sectional view had a porous struc-

ture with or without central hollow core. The influence of several preparation parameters such as surfactant types and concentrations, polymer concentrations and co-solvent volume ratio on the release pattern of FC was also observed. The release of FC was prolonged over 15 days with various release patterns by the control of preparation condition.[34,37,51]

A Local Delivery System of Fentanyl-loaded PLGA Oligomer Wafer: To obtain a sustained fentanyl delivery with effective and precise control fentanyl loaded wafer was designed using PLGA oligomer (PLGA 5005, ratio of lactide to glycolide = 50:50, 5000 g/mole, Wako Chem. Co. Ltd., Japan) by direct compression method. Fentanyl-loaded PLGA wafer with appropriate factors of drug loading (3~20%), thickness (0.9~4.5 mm), HPMC content (2~10%) could be reached controlled zero-order release (Figure 13). XRD and DSC analysis indicated the presence of crystalline drug in the wafer. The release of fentanyl from PLGA wafer was determined to be primarily diffusion controlled, but swelling and erosion also contributed to the release process.

Generally, the drug showed the biphasic release patterns, with an initial diffusion followed by a lag period before onset of the degradation phase. However, these wafers showed zero-order release because the degradation of PLGA matrix promoted the drug release followed by a diffusion process. *In vitro* release studies showed that different release patterns and rates could be achieve by simply modifying factors in the preparation conditions. The wafer degradation profiles were also investigated to understand the drug release mechanism. Gravimetric studies of mass loss of wafers during the incubation revealed that the weight loss increased apparently after 4 days and about 40% of mass loss was observed after 11 days fentanyl release. These results indicate that the polymer degradation was contributed to drug release followed by diffusion. In conclusion, this system showed very few initial burst and zero-order release profiles. The fentanyl-loaded PLGA wafers, especially oligomer molecular weight PLGA, appear to be a promising analgesic delivery device for the treatment of chronic pain without second operation for the removal of the implants after releasing of drug.[54,61]

Characteristic of Nifedipine-loaded PLGA Wafer. Nifedipine also was chosen as the model drug for the PLGA wafer for the local delivery device due to practically insoluble drug in water with solubility less than 10 μg/mL, a well known and most widely used coronary vasodilator from the group of dehydropyrine derivatives. Biodegradable wafers were prepared with PLGA oligomer (50:50 mole ratio, molecular weight 5,000 g/mole) by direct compression method for the sustained release of nifedipine to investigate the possibility of the treatment of hypertension. PLGA wafers were prepared by altering initial drug/polymer loading ratio, wafer thickness, and HPMC content, and their morphology and release pattern have been investigated. These wafers showed steady static release pattern for 11 days, and various biphasic release patterns could be obtained by altering the composition of wafers such as addition of matrix binder as HPMC to the PLGA wafer to reduce release rate of initial phase (Figure 14). The onset of polymer mass only occurred after 4 days and about 40% of mass loss was observed after 11 days nifedipine release. This system had advantage in terms of simplicity in design and controling of drug release rate and may be useful as an implantable dosage form.[36,49]

BCNU-loaded PLGA Wafer for the Treatment of the Malignant Brain Tumor. A major obstacles in the successful use of cytotoxic chemotherapeutic agents against brain tumors is the presence of the BBB that restricts permeability of the certain drug molecules within the brain and prevents diffusion of these agents into the brain tumor. BCNU is an

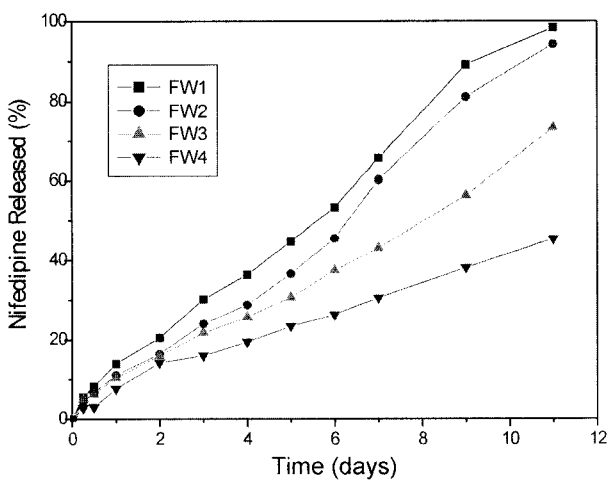

Figure 13. Effect of initial drug loading ratio on the fentanyl profile (5.0 × 0.9 mm^2) from the fentanyl-loaded PLGA oligomer wafers. FW1: 3%, FW2: 5%, FW3: 10% and FW4: 20%. Each point represents the mean of the at least three runs.

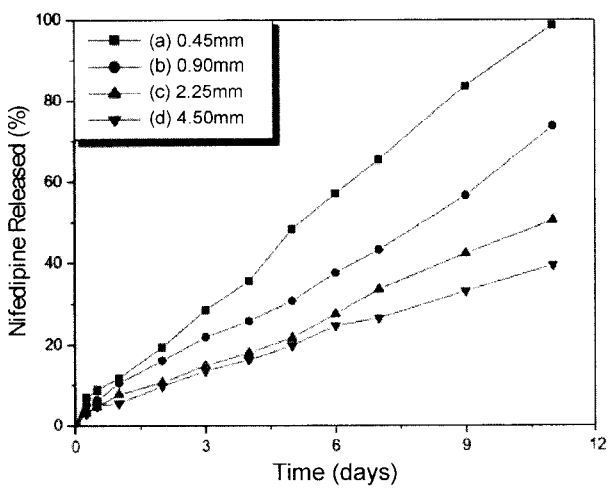

Figure 14. Effect of wafer thickness on the nifedipine release profiles at 10% drug initial content from the nifedipine loaded PLGA oligomer wafers.

important chemotherapeutic agent used for brain tumors, partially due to its ability to cross the BBB. However, BCNU must be administered in high systemic doses to achieve therapeutic brain levels due to its short half-life of about 20 min in plasma. Furthermore, prolonged systemic administration is associated with severe side effects such as bone marrow suppression, pulmonary fibrosis and hepatic toxicity.[4,30]

Development of implantable polymers that release chemotherapeutic agents directly into the central nervous system has had an impact on glioma therapy. This technology makes it possible to achieve very high local concentration of drugs while minimizing systemic toxicity and circumventing the need of a drug to cross the BBB. To date, one of the most outstanding results is BCNU-loaded polyanhydride wafer and recently won approval from FDA adjunct therapy for the treatment of brain tumors. Clinical trials with this controlled delivery polymer, Gliadel®, have shown an increase of median survival rate of patients, all of whom had failed prior therapy. Despite the clinical benefits achieved with Gliadel® are significant, improvement in survival was modest. Therefore, a clinical dose escalating study was recently carried out and proved that up to 20% loaded BCNU wafer was safe in current malignant glioma.

One possible approach to release BCNU over expanded period is the development of another biodegradable polymer system. The new system has longer degradation period than that of polyanhydride used in the implantable BCNU polymeric device. Modulation of BCNU release period could be achieved by increasing the ratio of carboxyohenoxypropane (CPP) to sebacic acid (SA) in the polyanhydride. However, the maximum release period using 50:50 CPP:SA copolymer was 18 days after a rapid initial burst of BCNU release within the first 24 hrs. As discussed above section, PLGA is well-known biodegradable polymer and biodegradable in brain tissue. We therefore designed BCNU loaded PLGA implant form for short-term to long-term delivery of BCNU as 3 days to 2 months period due to the advantage of PLGA compared than polyanhydride. Based on these good properties, injectable PLGA MSs that can release chemotherapeutics, cisplatin and BCNU, were prepared and their therapeutic efficacy was evaluated in the cavity wall of the debulked tumors.

BCNU was incorporated into PLGA by using spray-drying method. BCNU-loaded PLGA MSs characterized by SEM, powder XRD, and DSC. Homogeneous distribution of BCNU in PLGA MSs was confirmed by significant reduction of crystallinity of BCNU. MSs were fabricated into wafers with flat and smooth surface by direct compression method. *In vitro* release of BCNU in pH 7.4 PBS was prolonged up to 2 month using 20,000 and 90,000 g/mole PLGA molecular weight after short initial burst period (Figure 15). Release rate and 100% release period of BCNU were dependent on several parameters, such as molecular weight of PLGA (5,000 ~110,000 g/mole), concentration of PLGA (3~20 v/w%),

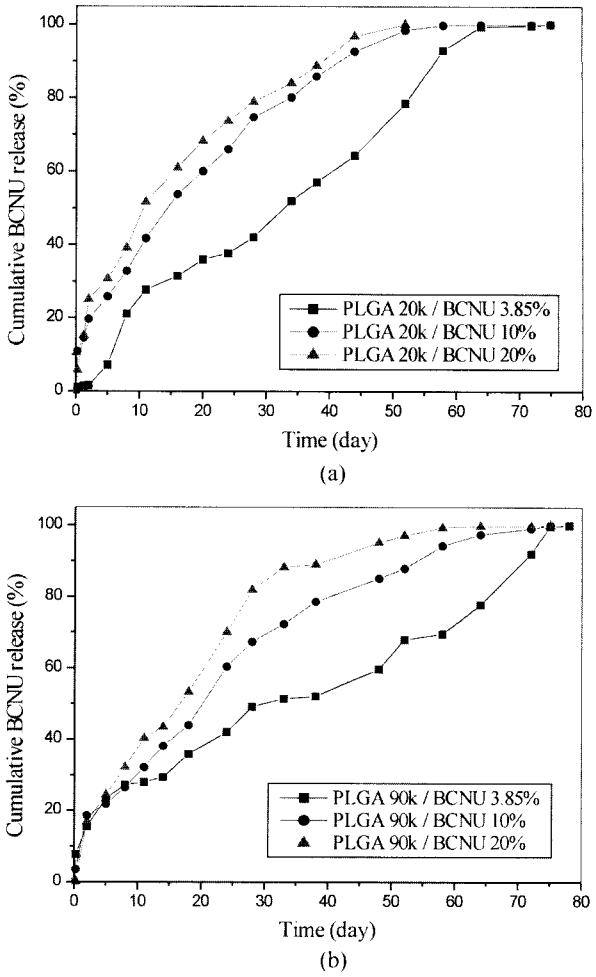

Figure 15. Effect of BCNU loading amount on BCNU release from PLGA wafers: (a) PLGA 20,000 g/mole and (b) 90,000 g/mole.

initial BCNU loading amount (3.85~40%) and additives such as salt and hydrophilic polymers (1~20%). Antitumor activity of BCNU-loaded PLGA wafer against XF 498 human CNS tumor cells continued over 1 month and, PLGA only did not affect the growth of the cells. Meanwhile, the cytotoxic activity of BCNU powder disappeared within 12 hrs as shown in Figure 16. These results strongly suggest that the BCNU/PLGA formulations increase release period of BCNU *in vivo* and also be useful in the development of implantable polymeric device for malignant glioma. Increasing the dose of BCNU in the wafer resulted in a more substantial regression of the tumor (Figure 17). The therapy with up to 30% BCNU implants were considered as tolerated. The study of the confirmation of these results using rat brain tumor model is in progress. In the near future work, more attention will be paid to implants which can enhance the penetration of BCNU in the brain tissue and to another drugs which can penetrate in the deep part of brain tissue, for example, 5-fluorouracil and interleukin, for enhancing the therapeutic efficacy of recurrent glioma. Studies related with

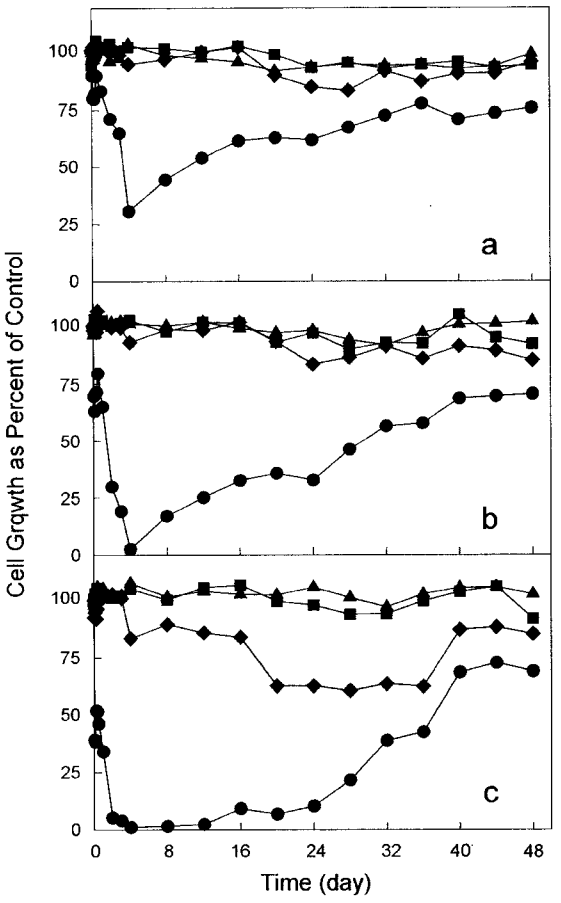

Figure 16. Cytotoxicity of BCNU-loaded PLGA wafer to the XF498 cells *in vitro*. The wafers were put in cell culture medium (A: 8 mL; B: 4 mL; and C: 2 mL), and the solutions of the samples were transfer to the cells after the various incubation time at 37 °C in the CO_2 incubator. Cell survival fractions were assessed after continuous exposure for 3 days by SRB assay. PLGA only (▲); 3.85% BCNU-loaded PLGA (■);10% BCNU-loaded PLGA (◆);20% BCNU-loaded PLGA (●).

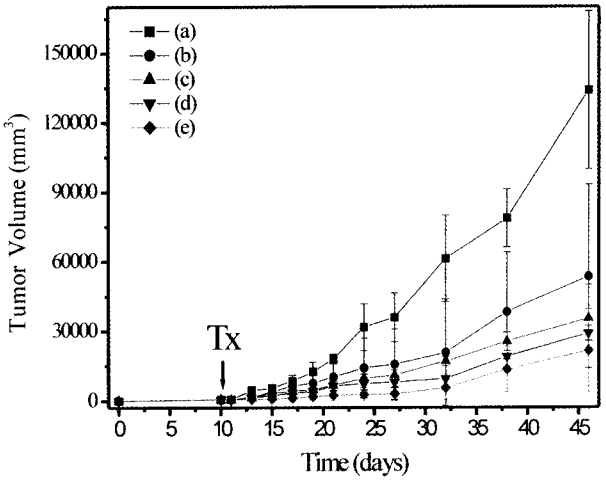

Figure 17. Tumor volume changes of Fisher rat treated with BCNU-loaded PLGA wafers; (a) control (non-treated), (b) 3.85, (c) 10, (d) 20, and (e) 30% BCNU initial drug content.

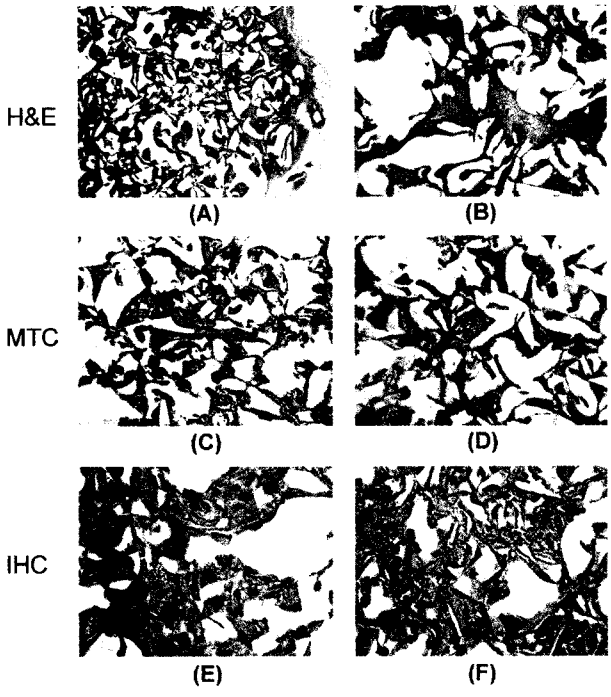

Figure 18. Histological evaluation of implanted VD_3/PLGA scaffolds; (A, C, E) PLGA (control) and (B, D, F) VD_3/PLGA scaffolds after 8 weeks implantation (original magnification: ×100).

the control of release pattern using additives, self-emulsifying drug delivery system, and protocol and standardization of animal model, are in progress.[45,53,58,59,65,66,68,70,71,76,80]

Preparation and Characterization of 1,25(OH)$_2$ Vitamin D$_3$ (VD$_3$)-loaded the Biodegradable PLGA Scaffolds for Tissue-engineered Bone. VD_3-loaded the biodegradable PLGA scaffolds are synthetic bone substitutes that promote bone formation by osteoconduction and osteoinduction.[88-91] Bioactive biomolecules-impregnated scaffolds were fabricated by solvent casting/salt leaching technique. PLGA was dissolved into methylene chloride and this solution was mixed with NaCl and VD_3, molded, and then leached in an aqueous solution. Porosities and pore sizes of VD_3/PLGA scaffolds were measured using mercury porosimetry. The scaffolds had relatively homogeneous pore structures throughout the matrix and showed an average pore size in the range from 35.4~114.1 μm and over 90% porosity. The release of sulfurhodamin B (SRB as model drug) was compared for PLGA scaffolds in relation with the drug loading amount, porosity, and pore size by spectrophotometer.

Two groups as PLGA only (control) and VD_3/PLGA scaffolds were implanted on the back of Sprague-Dawley rats to

investigate the effect of VD_3 on the osteoinduction and osteoconduction for 4 and 8 weeks as shown in Figure 18 for the each microphotograph for hematoxilin & eosin (H&E), Massons trichrome (MTC) and immunohistochemical (IHC) staining. The control could not observe that the evidence of new bone formation due to no biological activity and functionality of PLGA. However, we can observe the evidence of new bone formation by the bioactivity of the VD_3/PLGA scaffolds from the undifferentiated stem cells in the subcutaneous sites and other soft connective tissue sites having a preponderance of stem cells compared with PLGA scaffolds by MTC for collagen fiber formation and IHC staining for collagen Type I. From the results, this sustained, besides, constant localized released system can potentially provide VD_3 and encourage high activity of calcium and phosphate, which plays an important role for osteoconduction and osteoinduction. The VD_3/PLGA scaffolds may be used for the applications of bone generation. Studies on the detailed mechanism of osteoinduction and osteoconduction of VD_3/PLGA scaffolds, the quantification of osteoinduction such as calcium content and alkaline phosphatase activity are in progress.[55,67]

Treatment of Aural Cholestoma Using ADP-loaded PLGA Wafer as Local Delivery System. Implantable biodegradable wafers were prepared with ADP-loaded PLGA (75:25 mole ration by lactide to glycolide, molecular weight: 20,000 g/mole) by direct compression method for the sustained release of ADP to investigate the possibility for the treatment of bone resorption.[92-94] The release pattern of ADP/PLGA wafers were observed by HPLC and the wafers were implanted the Mongolian gerbils mastoid. The release rate of APD increased with increase of its initial loading amount as shown in Figure 19. We also measured the osteoclast index (Figure 20), i.e, number of osteoclast cell per total bone length and the surface area of osteoclast cell per total bone surface in experimental cholestoma, in which APD/PLGA wafers were implanted. The result indicated these wafers could reduce the osteoclast activities in experimental aural cholestoma. It may suggest the possibility for the treatment of bone resorption by implantable dosage form.[60,64,69]

Preparation of IP-loaded PLGA MSs and Scaffolds for Bone Regeneration. IP stimulates proliferation and differentiation of osteoblast and also enhances calcitonin secretion in the presence of estrogen. For the purpose of the stimulation of bone regeneration, IP/PLGA MSs was prepared by using conventional O/W solvent evaporation method. The size of MSs was in the range of 5~200 μm. The morphology and *in vitro* release amount of IP/PLGA MSs were characterized by SEM and HPLC, respectively. Also, the physicochemical properties of IP-loaded PLGA MSs were investigated by XRD and DSC. The highest encapsulation efficiency was obtained by using gelatin and PVA as emulsifiers. The morphology, size distribution, and *in vitro* release pattern of MSs were changed by several preparation parameters such as molecular weights (8,000, 20,000, 33,000, and 90,000 g/mole, Figure 21), polymer concentrations (2.5, 5, 10, and

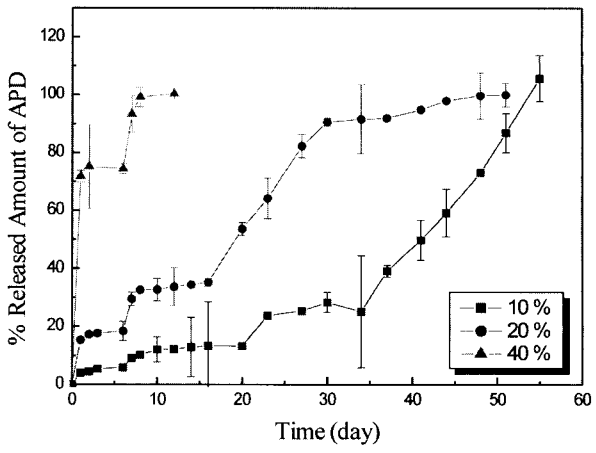

Figure 19. Percent release profiles of APD from APD/PLGA wafers with different loading amount.

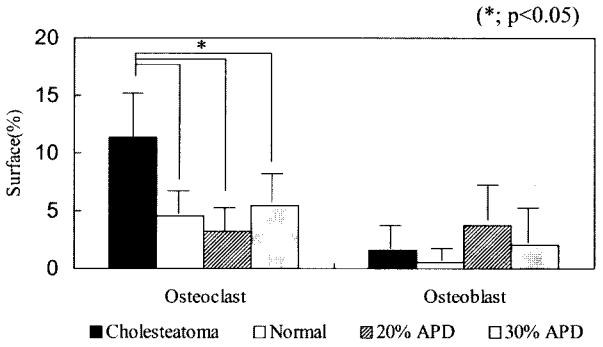

Figure 20. Effect of APD/PLGA wafers on osteoclast cell surface area per total bone surface. 20% APD; 20% APD loaded PLGA wafer and 30%; 30% APD loaded PLGA wafer.

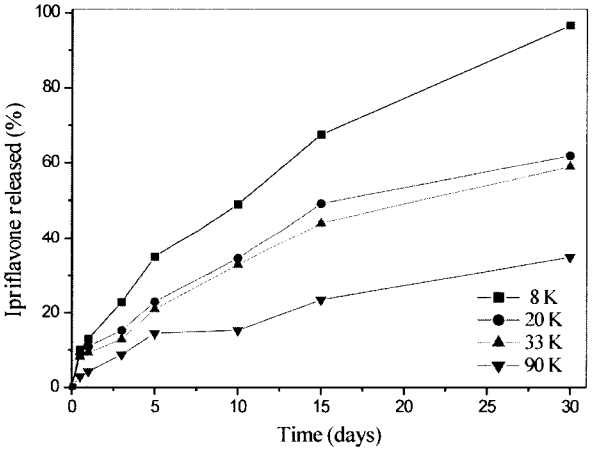

Figure 21. Effect of PLGA molecular weight using on IP release profiles.

20%), emulsifier types (PVA, gelatin and Tween 80), initial drug loading amount (5, 10, 20 and 30%), and stirring speed (250, 500 and 1,000 rpm). The release of IP in *in vitro* was more prolonged over 30 days, with closed to zero-order pattern by controlling the preparation parameter. It might be suggested that this DDS device is very useful the direct delivery of poorly water-soluble drug to malfunctioned site.[72]

Also, we developed the novel IP loaded PLGA scaffolds for the possibility of the application of the tissue engineered bone. IP/PLGA scaffolds were prepared by solvent/casting/salt leaching methods and were characterized by porosimeter, SEM, determination of residual salt amount, DSC, and XRD, respectively. IP/PLGA scaffolds were implanted into the back of athymic nude mouse to observe the effect of IP on the osteoinduction compared with control PLGA scaffolds. Thin sections were cut from paraffin embedded tissues and histological sections were stained H&E, von Kossa and IHC staining for Type I collagen and osteocalcin. It can be observed that the porosity was above 91.7% and the pore size was above 101 μm. Control scaffold and IP/PLGA scaffolds of 50% IP were implanted on the back of athymic nude mouse to observe the effect of IP on the induction of cells proliferation for 9 weeks as shown in Figure 22. The evidence of calcification, osteoblast, and osteoid from the undifferentiated stem cells in the subcutaneous sites and other soft connective tissue sites having preponderance of stem cells has been observed. In conclusion, it seems that IP plays an important role for bone induction in IP/PLGA local drug delivery device.[75]

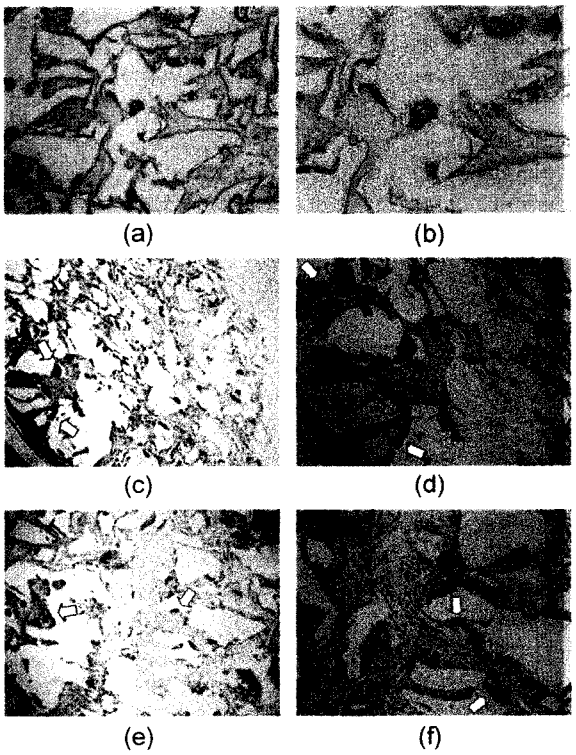

Figure 22. Photographs of histochemical staining for H&E after 9 weeks. (a) PLGA (×100), (b) PLGA (×200), (c) PLGA/IP (20% IP, ×100), (d) PLGA/IP (20% IP, ×200), (e) PLGA/IP (50% IP, ×100), and (f) PLGA/IP (50% IP, ×200)

NGF-loaded PLA and PLGA Scaffolds and Film for Tissue-engineered Nerve Regeneration. To optimize a scaffold for nerve regeneration, NGF used as a potential therapeutic agent to prevent the degeneration in Alzheimers disease patients,[95-99] loaded PLA scaffolds were prepared by emulsion freeze drying method.[48,50] Released amount of

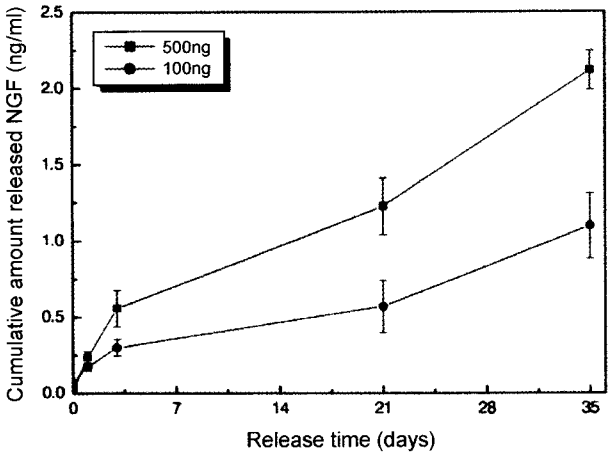

Figure 23. Cumulative amount of NGF released from NGF/PLA scaffolds of different initial loading.

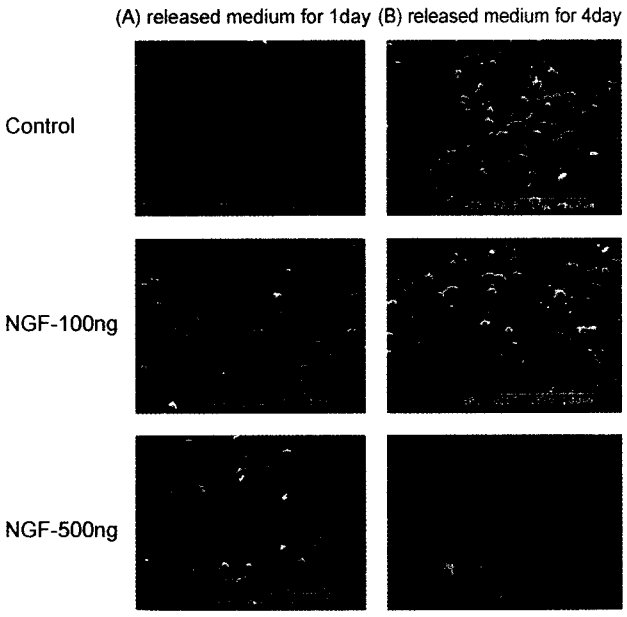

Figure 24. Effect of the NGF of release medium from NGF-loaded PLA scaffolds on the activity of PC-12 cell cultures. (A) released medium for 1 day and (B) released medium for 4 days. It could be observed the activated neurite sprouting from PC-12 by released NGF.

NGF from NGF-loaded PLA scaffolds were analyzed over a 4 weeks in PBS at 37 °C. The stability of NGF during the PLA scaffold preparation was evaluated by comparing the released amounts of total NGF as shown in Figure 23, assayed NGF enzyme-linked immunosorbant assay (ELISA). The released NGF stimulated neurite outgrowth of pheochromocytoma (PC-12) cells as shown in Figure 24. Changes of PLA weight percent in solution and protein loading level were assessed to provide formulations with the desired release rate and duration.

One of the significant disadvantages is the massive initial burst from the surface of NGF loaded microspheres and interconnected pores of the PLGA scaffolds and the massive loss during the preparation process resulting in no detection of accurate NGF concentration. In order to overcome these problems, we developed NGF-loaded biodegradable PLGA (the mole ratio of lactide to glycolide 75:25, molecular weight: 83,000 and 43,000 g/mole, respectively) film by novel and simple sandwich solvent casting method for the possibility of the application of regeneration of central nervous system.[79] Released behavior of NGF from NGF-loaded films was characterized by ELISA and degradation characteristics were observed by SEM and GPC. Also the bioactivity of released NGF was identified using a rat pheochromocytoma (PC-12) cell based bioassay. The release of NGF from the NGF-loaded PLGA films was prolonged over 35 days with zero-order rate and without initial burst and can be controlled by the variation of different molecular weight and different NGF loading amount as shown in Figure 25. After 7 days NGF released in PBS, PC-12 cell cultured on the NGF-loaded PLGA film for 3 days. The released NGF stimulated neurite sprouting in cultured PC-12 cells, that is to say, the remained NGF in the NGF/PLGA film at 37 °C for 7 days was still bioactive similar with NGF-loaded PLA scaffolds. These study might be suggested that NGF-loaded PLGA sandwich film can be released the desired period in delivery system and can be useful neuronal growth culture as nerve contact guidance tube for the application of neural tissue engineering.

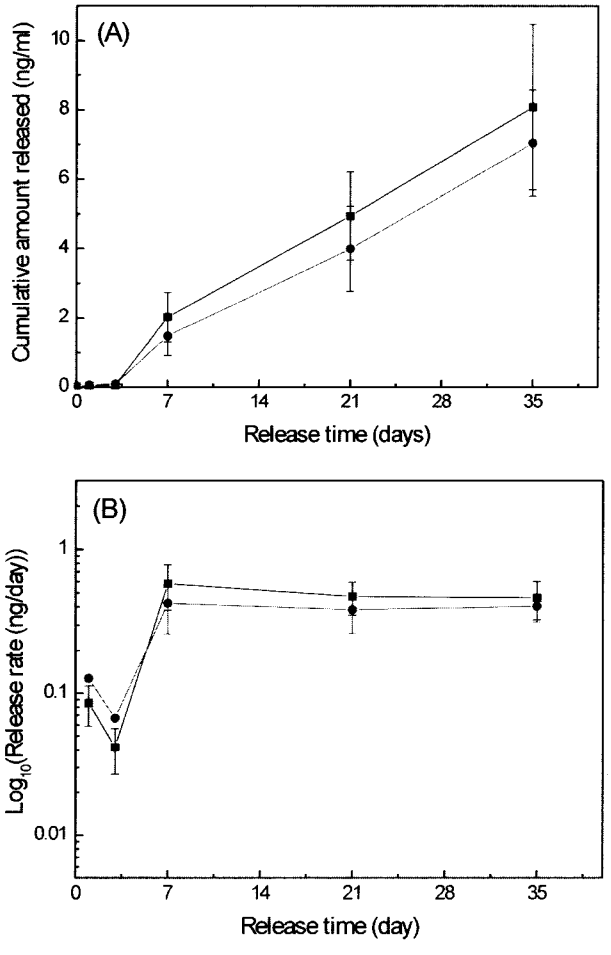

Figure 25. (A) Release profiles and (B) logarithmic plot of release rate for NGF from NGF-loaded PLGA films of 83,000 g/mole. (●) 25.4 ng and (■) 50.9 ng NGF/cm^2 PLGA.

Conclusions

Until recently, many biodegradable polymers come from natural and synthetic have been tested and applied for the development for drug carrier of DDS based on the following three mechanisms: mucosal absorption, controlled release and targeting. Among of these polymers, PLGA and PLA seems to be most desirable for the drug delivery device as many different types. Also, we can relatively and easily control the drug release profiles varying with the preparation, formulation and geometrical parameters. Moreover, any types of drug such as water-soluble, water-insoluble, small, large molecule, negatively or positively charged, and so on were successfully applicable to achieve linear sustained release from short period (1~3 days) to long period (over 2 months), in other words, it is very important to design a suitable formulation for the predetermined releasing period of bioactive molecules loaded biodegradable devices. The form of device can be MSs, microcapsule, nanoparticle, wafers, pellet, beads, multiple-layered beads, implants, fiber, scaffolds, and films for the purpose of local delivery in the research area of drug delivery and tissue engineering. It is also considered the drug release is affected by many factors such as hydrophilicity of drug, electric charge of drug, drug loading amount, polymer molecular weight, the monomer composition, the size of implants, the applied fabrication techniques, and so on.

It is well known that the development of new drug needs lots of money (average: over 10 million US dollar per one drug) and long time (average: above 9 years) whereas the development of DDS for potent generic drug might be need relatively small investment and short time. Also, one core technology can be applicable to many drugs to meet the market within time frame. From these reasons, the research

on DDS for potent generic drug has less risk and high return than new drug development. For the next DDS generation, DDS will provide more complex release control such as a stimulus control, sensor control, and targeting using "intelligent" PLGA or PLA biodegradable materials responsive to external stimli and more complex devices mimicking viruses or living cells such as drug self-producing system without need for a periodic drug supply must be necessary.

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