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Preparation of 5-Fluorouracil-Loaded Poly(L-lactide-*co*-glycolide) Wafer and Evaluation of *In Vitro* Release Behavior

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Abstract: The controlled delivery of anticancer agents using biodegradable polymeric implant has been developed to solve the problem of penetration of blood brain barrier and severe systemic toxicity. This study was performed to prepare 5-FU-loaded poly(L-lactide-*co*-glycolide) (PLGA) wafer fabricated microparticles prepared by two different method and to evaluate their release profile for the application of the treatment of brain tumor. 5-FU-loaded PLGA microparticles were characterized by scanning electron microscopy (SEM), powder X-ray diffraction (XRD), and differential scanning calorimetry (DSC). SEM observation of the 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method showed that 5-FU was almost surrounded by PLGA and significant reduction of crystallinity of 5-FU was confirmed by XRD. In case of release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing, the release profile of 5-FU followed near first order release kinetics. In contrast to the above result, release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method followed near zero order release kinetics. These results indicate that preparation method of the 5-FU-loaded PLGA microparticles to fabricate into wafers was contributed to drug release profile.

Keywords: brain tumor, 5-FU, PLGA, wafer, rotary solvent evaporation method, release profile.

Introduction

Brain tumor has been studied for a long time to achieve complete therapeutic cure. Although patients suffering from glioblastomas take surgical resection, external-beam radiotherapy, and systemic chemotherapy, median survival is still less than 1 year.[1] Successful delivery of cytotoxic chemotherapeutic agent to the brain tumor via a systemic route is very difficult due to the presence of BBB.[2,3] The localized and controlled delivery of anticancer agents using biodegradable polymeric implant has also been developed as an alternative way to solve the problem of low penetration of BBB. Presently anticancer agent has been delivered using device that biodegradable polymers have been employed to achieve sustained release system such as liposome, microsphere, and wafer.[4-9]

Synthetic biodegradable polymers have been extensively studied for implantable devices. Poly(L-lactide-*co*-glycolide) (PLGA) has been approved for drug delivery use by the Food and Drug Administration, so it has been used for the study of a controlled release system over the past decade. It provides many advantages such as regulating varying degradation period according to mole fraction of lactide and glycolide,[10] producing biocompatible and toxicologically safe by-products that are further eliminated by the normal metabolic pathways. It has been widely used as carriers in controlled-release delivery systems[11-16] and in tissue engineering area[17-21] due to above reasons.

In the previous our study,[22-25] 1,3-bis(2-chloroethyl)-1-nitrosourea (carmustine, BCNU)-loaded PLGA wafer has been studied for brain tumor. BCNU is one of the commonly used chemotherapeutic agents, partially due to its ability to cross the BBB.[26] Long-term delivery of BCNU,[22] the BCNU release behaviour with the changes of various dimension of wafer and additives,[23,24] cytotoxicity against various tumor cell lines,[25] and *in vivo* anti-tumor activity were

This paper is dedicated to Professor Won Jei Cho's retirement.

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investigated. But it has been reported that BCNU presents a very short penetration distance because it gets drained out of the system before being able to diffuse to any appreciable distance.[27] This was correlated with its transvascular permeability. Nevertheless, 5-fluorouracil (5-FU) exhibits a poorer transvascular permeability than BCNU. Thus, even if its hydrophilic characteristics prevents the molecule from diffusing extensively, it is more likely to stay a longer period of time in the tumor vicinity, with rising concentrations. Hence, its local efficacy may increase.[28]

In this study, 5-FU was fabricated into PLGA wafer for the purpose of improvement of transvascular permeability of BCNU. 5-FU is water soluble and an antimetabolite of the pyrimidine analog type that is widely used alone or in combination chemotherapy regimens for the treatment of advanced gastrointestinal tract cancer, breast cancer and several other types of cancer.[29] This study was performed to prepare 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing and rotary solvent evaporation method[30,31] and to evaluate their release profile by high performance liquid chromatography (HPLC) for the possibility of the treatment of brain tumor. The physical characteristics of 5-FU-loaded PLGA microparticles were studied using powder X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

Experimental

Materials. 5-FU was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and PLGA (Resomer® RG 502H; PLGA 50/50 mole ratio by lactide to glycolide; molecular weight, 8000 g/mole) was obtained from Boehringer Ingelheim (Germany). Acetone (Duck San Chem. Co, Korea) and methylene chloride (MC, Tedia, Japan) were used. All other chemicals were reagent grade. Deionized water was prepared by a Milli-Q purification system from Millipore (Molsheim, France).

Preparation of 5-FU-Loaded Microparticles and Wafers. 5-FU-loaded PLGA microparticles were prepared by two method. The first method was mechanical mixing that 5-FU and PLGA were mixed by vortexer. The second method was rotary solvent evaporation method. Briefly 5-FU and PLGA were co-dissolved in acetone at room temperature. Amount of 5-FU against PLGA was varied from 10% to 30%. After acetone was evaporated by rotary evaporator (Rotavapor, Büchi, Switzerland) at 30 °C, the aggregated solid was collected. The aggregated solid was put into the vaccum oven for overnight to remove residual solvent. The microparticles from aggregated solid were prepared by freezer mill (SPEX 6750, USA) for 10 min in liquid nitrogen. 5-FU-loaded PLGA microparticles prepared from above two method were used to prepare wafer by compression molding. Microparticles (10 mg) were compression-molded into wafer using Carver Press (MH-50Y CAP 50 tons, Japan) at 20 kg_f/cm^2 for 1 second at room temperature. Wafer was 3 mm (diameter) × 1 mm (thickness) in size with a flat surface and stored at 0 °C until use.

Determination of Drug Content. The drug content was determined in 5-FU-loaded PLGA microparticles. Ten mgs of 5-FU-loaded PLGA microparticls were dissolved in 1 mL of MC. PLGA was precipitated by adding 9 mL of water and after centrifugation, 100 μL of aliquots of supernatant were analyzed by HPLC.

Physicochemical Characteristics. XRD (D/MAX III, Rigaku, Japan) and DSC (Mettler TA 4,000, AC, USA) were used to determine crystallinity and thermal characteristics of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles. The samples were placed in a quartz sample holder and scanned from 1.5 to 60° at a scanning rate of 4°/min. DSC analyses were carried out at a nitrogen flow of 50 mL/min and a heating rate 10 °C/min from 20 to 330 °C. The endothermic energy was derived by gravimetrically measuring the peak areas.

Morphology Observation. Scanning electron microscopy (SEM, model S-2250N, Hitachi, Japan) was used to examine the morphology of 5-FU-loaded PLGA microparticle and wafer. All samples for SEM were mounted on metal stub double-sided tape and coated with platinum for 90 sec under argon atmosphere using plasma sputter (SC 500 K, Emscope, UK).

***In Vitro* Release Test.** Release of 5-FU from PLGA wafer was monitored for a period of incubation in phosphate buffered saline (PBS, pH 7.4) at 37 °C. Wafers were placed in 50 mL of PBS in a glass vial with constant shaking at 60 rpm. At specific time following incubation specific amount of PBS was taken out from the vial with pipette and same volume of fresh PBS was replaced. All samples were analyzed using HPLC system equipped with a Model P-2000 pump, a Model AS-3000 autosampler, and a Model UV-1000 UV detector at 266 nm (Thermo Separation Products, Fermont, CA, USA). The column used was μ-Bondapak™ C_{18} (4 × 300 mm, Grom, Germany). Mobile phase was acetate buffer solution, pH 4.7 and flow rate was adjusted 1.0 mL/min.

Results and Discussion

Physicochemical Characteristics. The chemical structures of 5-FU and PLGA used in this study are shown in Figure 1. To investigate the crystallinity and thermal properties of 5-FU in PLGA matrix, 5-FU-loaded microparticles were characterized by XRD and DSC. Figure 2 shows the XRD spectra of 5-FU, PLGA, and 5-FU-loaded microparticles prepared by two method. XRD patterns showed that 5-FU was crystalline, showing characteristic peaks at $2\theta = 28°$ while PLGA was amorphous. The crystalline 5-FU was detected in 5-FU-loaded PLGA microparticles prepared by mechanical mixing and the crystalline peak was increased with increasing the drug loading amount. The crystalline 5-FU was also

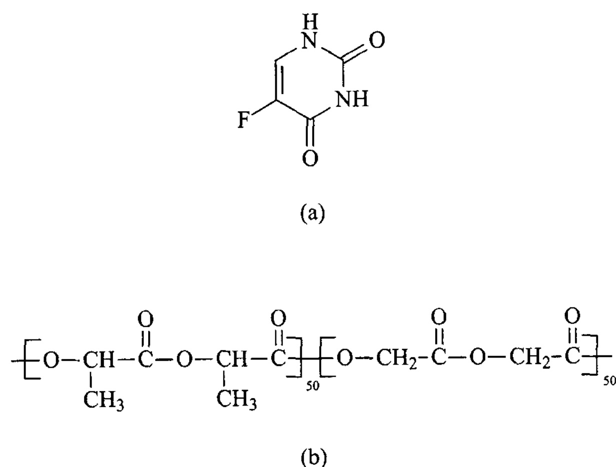

Figure 1. Chemical structures of (a) 5-FU and (b) PLGA (50/50).

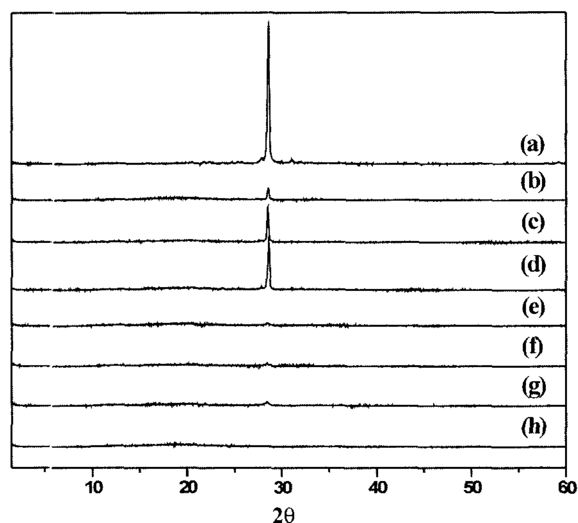

Figure 2. X-ray diffraction patterns of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles. (a) 5-FU; (b) 5-FU 10%; (c) 5-FU 20%; (d) 5-FU 30% prepared by mechanical mixing; (e) 5-FU 10%; (f) 5-FU 20%; (g) 5-FU 30% prepared by rotary solvent evaporation method; (h) PLGA.

detected in 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method but the crystalline peak was much smaller than crystalline peak of 5-FU-loaded PLGA microparticles prepared by mechanical mixing.

Figure 3 shows DSC thermograms of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method. T_m of 5-FU was detected same as reported temperature [32] in the paper and high endothermic peak was shown due to high crystallinity. Table I shows T_m and enthalpies (ΔH_m, J/g) of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method. ΔH_m of 5-FU was 195 J/g. ΔH_m of 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation

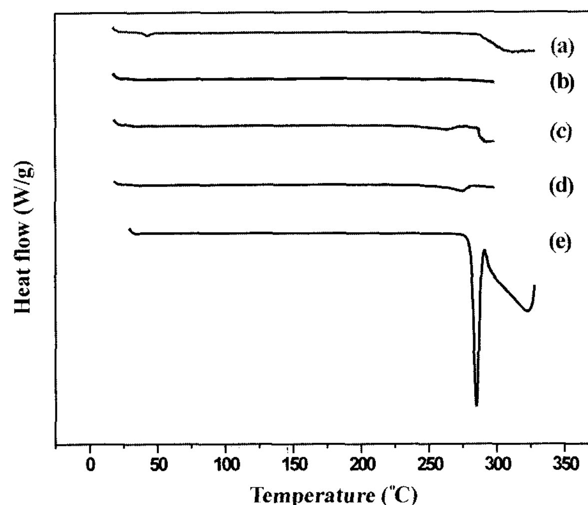

Figure 3. DSC thermograms of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles. (a) PLGA 8K; (b) 5-FU 10%; (c) 5-FU 20%; (d) 5-FU 30% prepared by rotary solvent evaporation method; (e) 5-FU.

Table I. Thermal Properties of 5-FU, PLGA and 5-FU-Loaded PLGA Microparticles Prepared by Rotary Evaporation Method

Sample	T_m (°C)	H_m (J/g)
5-FU	284.78	195.0
PLGA 8K	-	-
5-FU 10%/PLGA 8K	240.77	56.1
5-FU 20%/PLGA 8K	263.34	106.3
5-FU 30%/PLGA 8K	274.59	144.3

method was smaller than ΔH_m of 5-FU. From XRD patterns and DSC thermograms, it could be suggested that crystallinity of 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method was decreased by PLGA. The change of crystallinity of 5-FU-loaded PLGA microparticles according to preparation method could affect the release behavior.[33,34]

Determination of Drug Content and *In Vitro* Release Test. Drug content in 5-FU-loaded PLGA microparticles prepared from two method was from 95 to 105% and it could confirm that 5-FU was homogeneously dispersed in PLGA matrix. Figure 4 shows release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing. The release rate of 5-FU increased with the increase of 5-FU loading amount and the release profiles of 5-FU irrespective of 5-FU loading amount followed near first order release kinetics. After 6 hrs, it showed initial burst. Initial burst also increased with the increase of 5-FU loading amount. The reason of initial burst could be thought due to diffusional release and dissolution of drug

particles on the surface of wafers and a higher drug loading amount resulted in a much drug particles on the surface of wafers. A period of slow release followed from approximately 12 hrs to 10 days during which release rate increased gradually. This release pattern is mainly dependent on the diffusion of the drug through the polymer matrix that has many channels due to the polymer degradation after the water uptake.

Figure 5 shows release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method. In contrast to the release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing, release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method followed near zero order release kinetics. The release pattern was

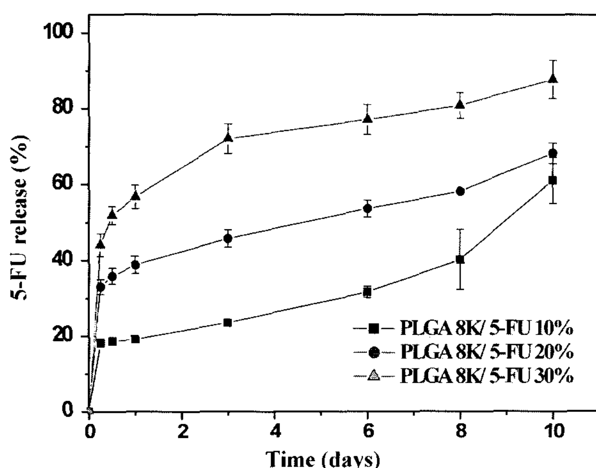

Figure 4. Release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing.

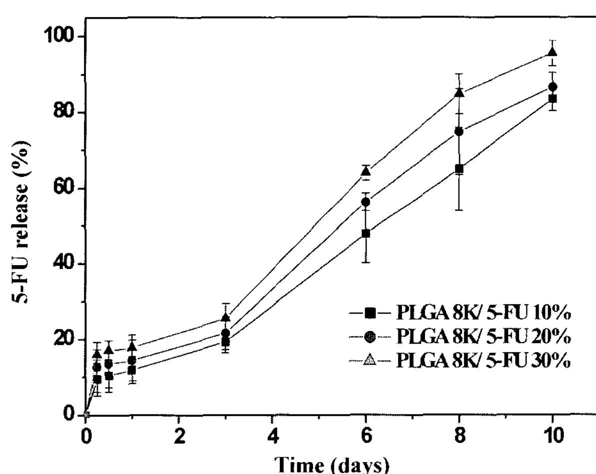

Figure 5. Release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method.

almost identical regardless of loading amount of the drug. After 6 hrs, an initial burst irrespective of drug loading amount was less than 20%. The reason that initial burst of 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method was much smaller than 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing might be explained that there was relatively few drug particles near the surface of the wafer because drug particle was almost surrounded by PLGA. A period of slow release followed from approximately 12 hrs to 3 days which release rate increased gradually and a period of fast release followed from approximately 3 to 10 days. After initial burst, slow release is governed by duration of the hydration of polymer surrounded drug, which is considered as the first step in degradation of PLGA. After 3 days, continuous erosion of the polymer surrounded drug by hydrolysis to generate bigger pores and channels for continuous diffusion of drug from the wafer was compensating the fast drug release.[35]

Morphology Observation of Microparticles. Figure 6 shows SEM microphotographs of 5-FU, PLGA, and 5-FU-loaded PLGA microparticles prepared by two method. 5-FU crystals were found and PLGA amorphous was found ((a) and (b) respectively). 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method showed almost 5-FU surrounded by PLGA ((c)~(f)). Whereas 5-FU-loaded PLGA microparticles prepared by mechanical mixing showed there were present 5-FU and PLGA separately ((g)~(i)). The difference of morphology of 5-FU-loaded PLGA microparticles prepared by two method could affect the release behavior.

Morphology Observation of Wafers. Figure 7 shows SEM microphotographs of the surface and cross-sectional morphology of 12 hrs and 8 days after *in vitro* release test for 5-FU 10%-loaded PLGA wafer fabricated microparticles prepared by two method. The surface and cross-sectional morphology before PBS exposure were smooth and non-porous (not shown). After 12 hrs, surface had many cracks and cross-section became porous. In case of 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing comparing with 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method, surface of the wafer had bigger channels that there was more drug particles and more initial drug release in the surface. After 8 days, there was more porous and continued growth in pore size in the surface and cross-section of the wafer. 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary evaporation method had more porous and bigger pore size compared with 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing. This result suggests that 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary evaporation method showed more release of 5-FU during this point than 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechan-

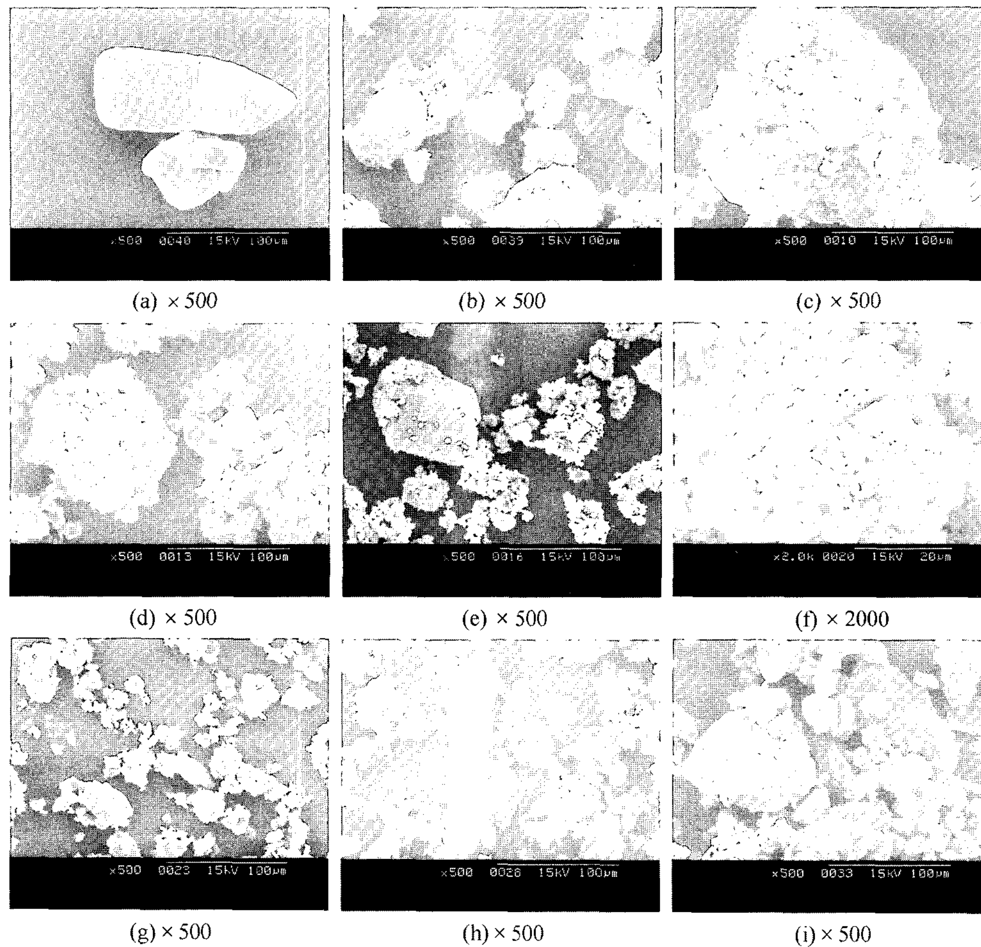

Figure 6. SEM micrographs of 5-FU-loaded PLGA microparticles. (a) 5-FU; (b) PLGA 8K; (c) 5-FU 10%/PLGA 8K; (d) 5-FU 20%/ PLGA 8K; (e) 5-FU 30%/PLGA 8K; (f) 5-FU 30%/PLGA 8K microparticles prepared by rotary solvent evaporation method; (g) 5-FU 10%/P.̣GA 8K; (h) 5-FU 20%/PLGA 8K; (i) 5-FU 30%/PLGA 8K microparticles prepared by mechanical mixing.

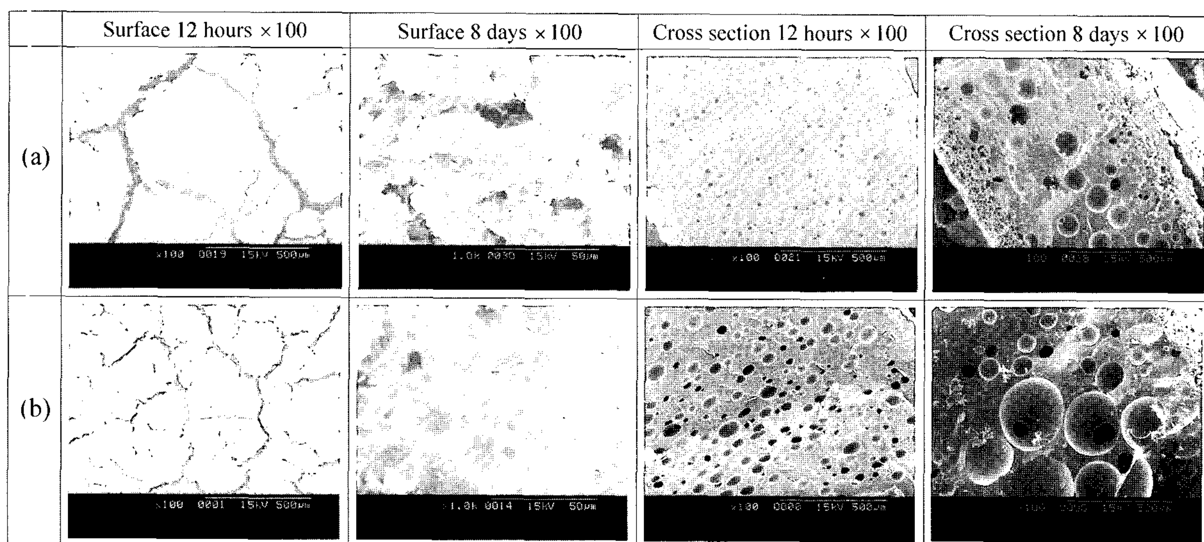

Figure 7. SEM micrographs of 5-FU 10%/PLGA wafer prepared (a) by mechanical mixing and (b) by rotary solvent evaporation method.

ical mixing due to more porous and bigger pore size in the wafer.

Conclusions

5-FU-loaded PLGA microparticles were prepared and compared by mechanical mixing and rotary solvent evaporation method. SEM observation of the 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method showed that 5-FU was almost surrounded by PLGA. From XRD patterns and DSC thermograms, it could be suggested that crystallinity of 5-FU-loaded PLGA microparticles prepared by rotary solvent evaporation method was decreased by PLGA. In case of release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by mechanical mixing, the release rate of 5-FU increased with the increase of 5-FU loading amount, and the release profile of 5-FU irrespective of 5-FU loading amount followed near first order release kinetics after initial burst. In contrast to above result, release profile of 5-FU from 5-FU-loaded PLGA wafer fabricated microparticles prepared by rotary solvent evaporation method followed near zero order release kinetics after small initial burst less than 20%. It can be suggested that preparation method of the 5-FU-loaded PLGA microparticles to fabricate into wafers was a very important role for drug release profile. Study on the cytotoxicity test against 9 L gliosarcoma cell is in progress.

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