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Preparation of poly(DL-lactide-*co*-glycolide) microspheres encapsulating all-trans retinoic acid

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Abstract

Poly(DL-lactide-*co*-glycolide) (PLGA) microspheres containing all-trans retinoic acid (atRA) were prepared by o/w solvent evaporation method and various preparation parameters, such as poly(vinyl alcohol) (PVA) concentration in aqueous solution, PVA MW, drug weight, solvent, polymer MW, and polymer weight, on the characteristics of microspheres and drug release were investigated. PVA concentration in water phase was a critical factor in making microspheres consistently with smooth surface and round shape. In our study, at least 2% (w/v) of PVA in aqueous solution was necessary for making microspheres with round shape. The particle size of microspheres ranged 10–100 μm. AtRA was slowly released from PLGA microspheres over 30 days. Sterilization of microspheres by ethylene oxide (EO) gas at 37 °C did not significantly affect the characteristics of drug release or its morphology. Cell growth inhibition of atRA was affected by preparation process of microspheres rather than the EO-gas sterilization process. These results indicate that PLGA microspheres containing atRA are acceptable for controlled release devices for use in the treatment of brain tumor.

Keywords: All-trans retinoic acid; Poly(DL-lactide-*co*-glycolide); Controlled release; Biodegradable microspheres; Brain tumor

1. Introduction

Malignant gliomas are commonly treated with a combination of surgery, radiation therapy, and systemic chemotherapy. Two factors are considered as major limitations for chemotherapy of brain tumor (Gutmann et al., 2000). First of all, blood–brain barrier (BBB) has been considered as a major factor of limitation of drug delivery to the brain for treatment of brain tumor (Pardridge et al., 1991; Pardridge, 1998), restricting the penetration of agents given systemically. BBB is formed by the tight endothelial cell junctions of capillaries within the brain and limits penetration of drugs into the brain. Anticancer agents are poorly delivered to the brain tumor site in effective concentrations, resulting in ineffective treatment for brain tumors. Another reason for failure of inhibition of brain tumor recurrence is the low response of infiltrated

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foci of tumor cells to the anticancer agents. Since malignant gliomas are highly invasive, this makes it difficult to remove or treat the infiltrated foci locally. These are the major obstacles that prevent effective chemotherapy thus resulting in response rates lower than 20% of short duration (DeAngelis et al., 1998). Recurrences are usually observed after only short periods (8–10 months), more effective therapies must be developed, including biological agents that are not intrinsically toxic to the brain tissue surrounding the tumor and that inhibit invasion of brain tumor cells.

In this view, there has been considerable effort to develop controlled release implant systems containing traditional anticancer drugs such as 1,3-bis(2-chloroethyl)-1-nitrourea (BCNU), carmustine, paclitaxel, etc. (Hochberg et al., 1985; Brem et al., 1991; Menei et al., 1996; Walter et al., 1994). By being implanted directly into the cavity wall of debulked tumors, these polymeric drugs bypass the BBB and exposure high concentration of therapeutic agents at the target site (Brem et al., 1991). An attractive alternative that would permit delivery directly to the site of residual tumor is the use of polymers such as poly(DL-lactide-*co*-glycolide) (PLGA) that are formed into small diameter microspheres. Microspheres have been proven to be efficient systems for delivery of a wide range of chemotherapeutic drugs to the brain (Ike et al., 1992; Menei et al., 1999). They can be injected safely as a suspension allowing drug delivery to virtually any brain region (Emerich et al., 1999). Since PLGA may be considered biocompatible to the brain tissue (Menei et al., 1993), anti-tumor agents such as 5-fluorouracil, BCNU, carmustine, taxol, and carboplatin were entrapped into the polymer microspheres for sustained drug release treat brain tumor (Menei et al., 1996; Emerich et al., 2000; Walter et al., 1994; Olivi et al., 1996). However, most of these trials did not show remarkable increases in overall survival rates because these trials were not inhibited invasive behavior of the brain tumor effectively.

It is known that retinoic acid (RA) and its analog, the retinoids, regulate cell behavior during development and play key roles in cell fate determination, cell division, and cell differentiation (Morris-Kay, 1992). RA has been observed to promote neuronal survival, drive astrocytic differentiation, and inhibit differentiation of O2A progenitor cells in the spinal cord (Noll and Miller, 1994). Especially, retinoids and related compounds such as all-trans retinoic acid (atRA) may have therapeutic value in the treatment of human malignant gliomas (Defer et al., 1997) since retinoids strongly inhibit proliferation and migration in primary cultures of human glioblastomas multiforme (Rotan, 1991; Bouterfa et al., 2000), suggesting that atRA is an adequate chemotherapeutic agent to inhibit local invasion of human malignant gliomas. Also, atRA is effective in the treatment of epithelial and hematological malignancies such as breast cancer (Kalmekerian et al., 1994), head and neck cancer (Giannini et al., 1997), ovarian adenocarcinoma (Krupitza et al., 1995), and acute promyelocytic leukemia (APL) (Huang et al., 1988). However, in spite of its pronounced effects, some side effects such as retinoid acute resistance, hypertriglyceridemia, mucocutaneous dryness, and headache were reported (Conley et al., 1997; Frankel et al., 1992; Muindi et al., 1992) limits the clinical applications of atRA. The cancer relapsed in many patients after a brief remission despite continued atRA treatments, i.e. patients became resistant to further atRA exposure. It was reported that cancer recurrence might be due to the decrease of the plasma atRA concentration (Muindi et al., 1992). The several authors reported rapid decrease of half-life of atRA with continuous oral administration or intravenous injection (Achkar et al., 1994; Hirota et al., 1997; Shelly et al., 1982).

In other words, it was reported that various kinds of formulation for atRA to overcome the side effects and rapid decrease of half-life is necessary. Giordano et al. (1993) reported that atRA-loaded microspheres was effective in reducing the incidence of tractional retinal detachment by a sustained release of atRA. Liposome-encapsulated atRA showed higher and maintained longer serum tretinoin concentrations rather than oral atRA, resulting liposomal-atRA was effective to newly diagnosed APL (Estey et al., 1999). Solid lipid nanoparticles are reported as a useful formulation to solve the poor aqueous solubility (Szuts and Harosi, 1991) of atRA and able to use it by intravenous injection (Lim and Kim, 2002). Choi et al. (2001) obtained the pseudo-zero order release profiles of atRA for 5 weeks by encapsulating into biodegradable microspheres and control the release rate by the ratio of poly(L-lactide) (PLA) and PLA-poly(ethylene glycol) (PEG) block copolymer. Ezpeleta et al. (1996)

reported that atRA was encapsulated into gliadin nanoparticles with high loading efficiency.

The aim of this study is to make microspheres of atRA to provide stabilization and sustained release of components that inhibit invasion of human malignant gliomas in vitro, which has received scant mention in literature. Although atRA has been proved to be effective against several malignancies in human clinical trials, the cancer relapsed after a brief remission in many patients who were treated with atRA. Therefore, anti-invasive drug delivery to human malignant gliomas is thought to be a more ideal approach in the treatment of brain tumors. Microspheres for brain drug delivery has several advantages such as reduced toxicity of anti-tumoral agents, overcoming the BBB, effective delivery of the drug to the tumor, and controlled release of drug into the brain for a long time. Microspheres containing atRA was studied with the various formulation factors and their efficacy to the cell line of human malignant gliomas after sterilization by ethylene oxide (EO) gas. AtRA-encapsulated biodegradable microspheres are considered to be an useful approach in increasing the drug efficacy and reduce the side-effects.

2. Materials and methods

2.1. Materials

PLGA 50/50 (Resomer RG503H) was purchased from Boehringer Ingelheim, Pharma Co., Germany. Polyvinyl alcohol (PVA, #500, #1500) was purchased from Yakuri Pure Chemicals Co., Japan. DL-lactide and glycolide was purchased from Polyscience Inc., USA. Dichloromethane (DCM), chloroform, ethyl acetate (EA) acetone, methanol, and tetrahydrofuran (THF) are purchased from Aldrich Chem. Co. Ltd., USA. α-Minimum essential medium (α-MEM) was purchased from GIBCO (Invitrogen Co., USA). AtRA, stannous octoate, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Co. Ltd. All other chemicals and reagents were used as extra reagent grade at all of the experiments. PLGA 50/50 with different molecular weight (lower molecular weight than Resomer RG503H) was synthesized as reported previously (Nah et al., 1998).

Table 1
Characterization of PLGA

	Molecular weight[a]		Polydispersity (Mw/Mn)
	Mw	Mn	
Low MW	9600	7500	1.28
Medium MW	20700	15600	1.33
High MW[b]	41500	30900	1.34

Mw, weight average molecular weight; Mn, number average molecular weight.
[a] Measured by GPC described in experimental section.
[b] PLGA of high MW is Resomer RG503H.

2.2. Gel permeation chromatography (GPC) measurement

Molecular weight (MW) of PLGA was measured with a Waters liquid chromatography system coupled with a Waters 410 differential refractometer using Waters Styragel™ HR1, HR2, and HR4 columns at a flow rate of 1 ml/min. THF was used as an eluent. The average MW of polymer evaluated with polystyrene as a standard. MW of PLGA is listed in Table 1.

2.3. Preparation of PLGA microspheres containing atRA

PLGA microspheres containing atRA was prepared as follows: 200 mg of PLGA and 20 mg of atRA was dissolved in 5 ml DCM and poured into 100 ml of water containing PVA (2% w/v). Oil in water (o/w) emulsion was stirred at 1500 rpm for 1.0 h. The resulting solution was harvested by centrifugation at 4000 rpm and washed five times with distilled water following freeze-dried for 2 days. The preparation conditions and variables are listed in Table 2. All procedures for preparation was carried out at darkened condition to avoid drug degradation by light.

2.4. Observation using scanning electron microscopy (SEM)

The morphology of microspheres was observed with SEM (Jeol JSM-5400). Microspheres were placed on a double-sided tape attached onto graphite surface. The sample was coated with gold/palladium using an Ion Sputter (Jeol JFC-1100). Coating was provided at 20 mA for 4 min. Observation was performed at 25 kV.

Table 2
Formulation factor for the preparation of PLGA microspheres containing atRA

	Variations	Drug contents (wt.%)	Loading efficiency (wt.%)	Particle size (μm ± S.D.[a])	Particle morphology
PVA content					
V1	1.0 wt.%	5.0	52.6	57.375 ± 0.258	Irregular
V2[b]	2.0 wt.%	5.2	54.9	37.350 ± 0.413	Sphere
V3	5.0 wt.%	2.64	27.1	17.851 ± 0.483	Sphere
PVA MW[c]					
V2	500	5.2	54.9	37.350 ± 0.413	Sphere
Vm1	1500	4.54	47.6	19.747 ± 0.403	Sphere
atRA weight					
D1	0.2% (w/v)	1.66	33.8	17.802 ± 0.423	Sphere
V2	0.4% (w/v)	5.2	54.9	37.350 ± 0.413	Sphere
D2	0.8% (w/v)	8.83	48.4	93.417 ± 0.405	Irregular
Solvent					
V2	DCM	5.2	54.9	37.350 ± 0.413	Sphere
S1	Chloroform	2.4	24.6	10.004 ± 0.413	Sphere
S2	EA	4.68	49.1	54.726 ± 0.347	Sphere
Polymer MW[d]					
P1	Low MW	3.43	35.5	14.135 ± 0.533	Sphere
V2	Medium MW	5.2	54.9	37.350 ± 0.413	Sphere
P2	High MW	5.02	52.9	30.870 ± 0.496	Sphere
Polymer weight					
V2	4% (w/v)	5.2	54.9	37.350 ± 0.413	Sphere
W1	10% (w/v)	2.3	58.8	29.016 ± 0.395	Sphere

[a] Mean of three measurements.
[b] Control is as follows: 200 mg (4% (w/v) in organic solvent) of PLGA (MW = 20,000) and 20 mg (0.4% (w/v) in organic solvent) of atRA was dissolved in 5 ml of DCM and poured into 100 ml of water (PVA concentration: 2.0 wt.%). Oil-in-water emulsion was stirred at 1500 rpm for 1 h 30 min with reduced pressure.
[c] 500 and 1500 of PVA are number of repeating unit of vinyl alcohol.
[d] MW is described in Table 1.

2.5. Measurement of particle size

Particle size of PLGA microspheres containing atRA was measured with Photon Correlation Spectroscopy (Shimadzu SALD 2001, Shimadzu Co. Ltd., Japan).

2.6. Drug release studies

Drug contents in microspheres are determined as follows: 10 mg of freeze-dried microspheres were dissolved in acetone and measured at 365 nm with UV spectrophotometer (UV-1200, Shimadzu Co. Ltd.). Ten milligrams of PLGA was used as a blank test during measurement of drug contents.

Drug release test was performed as follows: 10 mg of microspheres was distributed into 200 ml of phosphate buffered saline (PBS, 0.01 M, pH 7.4). Release test was performed at 37 °C with stirring rate of 50 rpm. The release medium was exchanged everyday to maintain the sink condition. The exchange of medium was varied according to the series of sample to prevent saturation of drug in the release medium. At predetermined time schedule, vial containing release medium was hold to settle down the microspheres for 10 min and the microspheres harvested by filtration. Harvested microspheres at scheduled time point were washed three times with distilled water and lyophilized for 2 days. Lyophilized microspheres were carefully weighed and dissolved in acetone to evaluate the remained drug in the microspheres by UV spectrophotometer at 365 nm. All experiments were triplicate. The equation of drug contents and loading efficiency were as

follows:

$$\text{Drug contents} = \frac{\text{amount of atRA in the microspheres}}{\text{weight of microspheres}} \times 100$$

$$\text{Loading efficiency} = \frac{\text{residual amount of atRA in the microspheres}}{\text{feeding amount of atRA}} \times 100$$

2.7. Ethylene oxide (EO) gas sterilization of microspheres

EO-gas sterilization of microspheres encapsulating atRA was carried out using a 3M SterVac 5 XL (3M, St. Paul, MN). Microspheres were sterilized by the cool cycle at 37 °C. Sterilization has consisted of three steps, such as preconditioning, gas exposure, and fresh air purging. The precondition step establishes chamber vacuum, temperature, and humidity. For humidification of the chamber, stream was injected three times for 33 min. Prior to gas exposure step, initial vacuum was allowed to reach 170 mbar and humidity was set above 30%. In the gas exposure step, EO-gas exposure time was approximately 220 min. The pressure in the sterilizer chamber was maintained at 700 ± 5 mbar during the initial gas exposure step and at 620 ± 5 mbar during the final gas exposure step. And the residual EO gas in the sterilized samples was removed by the fresh air purge for over 8 h at the 37 °C. The sterilized microspheres were sealed in glass vials and stored at 4 °C before use.

2.8. Cell culture study

Malignant glioma cell line, U87MG, is obtained from American Type Culture Collection (ATCC, USA). Cells were maintained at α-MEM containing 10% fetal bovine serum in a CO_2 incubator (5% CO_2 at 37 °C).

The effect of atRA encapsulated in microspheres on cell growth is determined using a MTT cell proliferation assay. Microspheres containing atRA is dissolved in 100% DMSO as a stock solution and then diluted further using α-MEM. U87MG, malignant glioma cell line, is seeded at a density of 5×10^3 per well in 96-well plates with α-MEM containing 10% fetal bovine serum and incubate overnight in a CO_2 incubator (5% CO_2 at 37 °C). After that, fresh medium containing atRA are added. After a 4-day incubation, the cell proliferation assay, MTT Cell Titer 96, is performed. Absorbance was measured at 560 nm using a microtiter plate reader (Thermomax microplate reader Molecular Devices).

3. Result and discussion

3.1. Preparation of microspheres containing atRA

Generally, characteristics of microspheres such as particle size, morphology, and drug contents were changed by various kinds of experimental condition (Choi et al., 2002). PLGA microspheres containing atRA were prepared by conventional o/w emulsion-solvent evaporation method and their results summarized in Table 2. As shown in Table 2, particle size, loading efficiency, and morphology of the microspheres showed significant variation according to the formulation variables. PVA content in the water phase of o/w emulsion could affect the particle morphology and size. As shown in Table 2, particle size decreased according to the increased concentration of PVA in the water phase. Loading efficiency decreased at 5% (w/v) of PVA. These results might be due to that PVA in water phase increased the hydrophilic environment and stabilized the emulsion droplets, inducing decreased particle size and loading efficiency. Normally, stability of o/w emulsion increased according to the increased concentration of PVA in the water phase against coalescence and then increased PVA concentration in the water phase resulted in smaller particle size. Especially PVA concentration below 1% (w/v) resulted in irregular particles with big debris as shown in Fig. 1. Above 2% (w/v) PVA, microspheres showed spherical shape although surface morphology was still rough. Therefore, 2% (w/v) PVA in water phase was used in the following experiments. PVA MW also affected the particle size only giving minor changes to the loading efficiency. AtRA weight significantly affected the particle size, i.e. the higher the atRA weight the larger the particle size. However, increased atRA weight above 0.4% (w/v) did not induce increased loading efficiency and 0.8% (w/v)

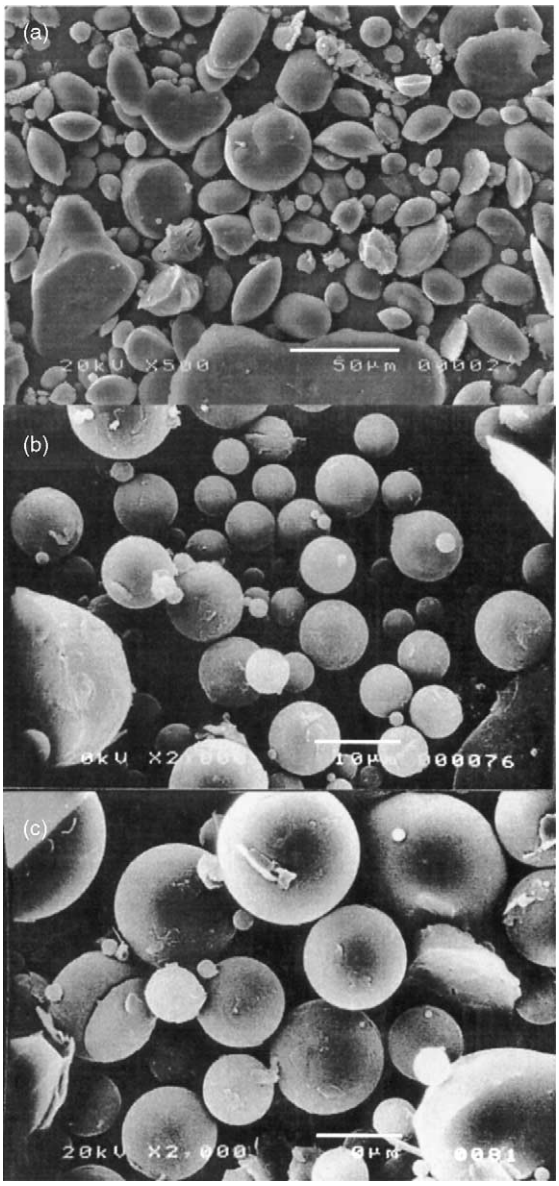

Fig. 1. SEM photographs of PLGA microspheres containing atRA against PVA concentration. (a) 1% (w/v), (b) 2% (w/v), (c) 5% (w/v) in Table 2.

resulted in the irregular morphology of particles with debris. From these results, PVA concentration and atRA weight owed significant variation to the microsphere morphology and characteristics. The solvent used also affected the microsphere characteristics. When chloroform was used, particle size and loading efficiency was decreased. EA resulted in bigger particle size than DCM but drug content was not significantly changed. When higher MW of polymer was used, particle size and loading efficiency was not significantly changed whereas they were decreased at low MW of polymer. Jalil and Nixon (1990) also reported that lowering the microcapsule diameter was observed with respect to lower polymer molecular weight. Polymer weight in the organic phase was slightly affected the loading efficiency and particle size.

3.2. Release of atRA from the PLGA microspheres

Due to the low solubility of atRA in aqueous solution, the release medium exchanged every day and drug release amount was assessed by drug remained in the microspheres. To evaluate the influence PVA concentration on the drug release, various concentrations of PVA were studied as shown in Fig. 2. As shown in Fig. 2(a), the higher the PVA concentrations in water phase the higher the release rate of atRA from the microspheres. Initial burst effect showed during about 10 days. After that atRA release from microspheres revealed pseudo-zero order kinetics until about 28 days. Fig. 2(b) showed the effect of PVA MW on the drug release from microspheres. The higher MW of PVA induced more rapid release of drug and it also released as a pseudo-zero order kinetics for 28 days.

Fig. 3 showed the effect of atRA weight on the release rate from microspheres. As shown in Fig. 3, the higher the drug weights the lower the release rate. These phenomena were reported by several authors (Gref et al., 1994; Jeong et al., 1998; Peracchia et al., 1997). It was reported that hydrophobic drug was crystallized inside the nanoparticles or microspheres and phase separation occurs at higher drug contents in the particles whereas it is presented as a molecular dispersion inside the microspheres at the lower drug contents. Resultantly, crystallized or aggregated hydrophobic drug should be dissolved more slowly and diffused into the outer aqueous phase than that of molecular dispersion. It was suggested that atRA in lower drug contents could finely dispersed in the PLGA microsphere matrix whereas higher one entrapped drugs in the PLGA matrix like network and existed as a crystallized form (Gref et al., 1994).

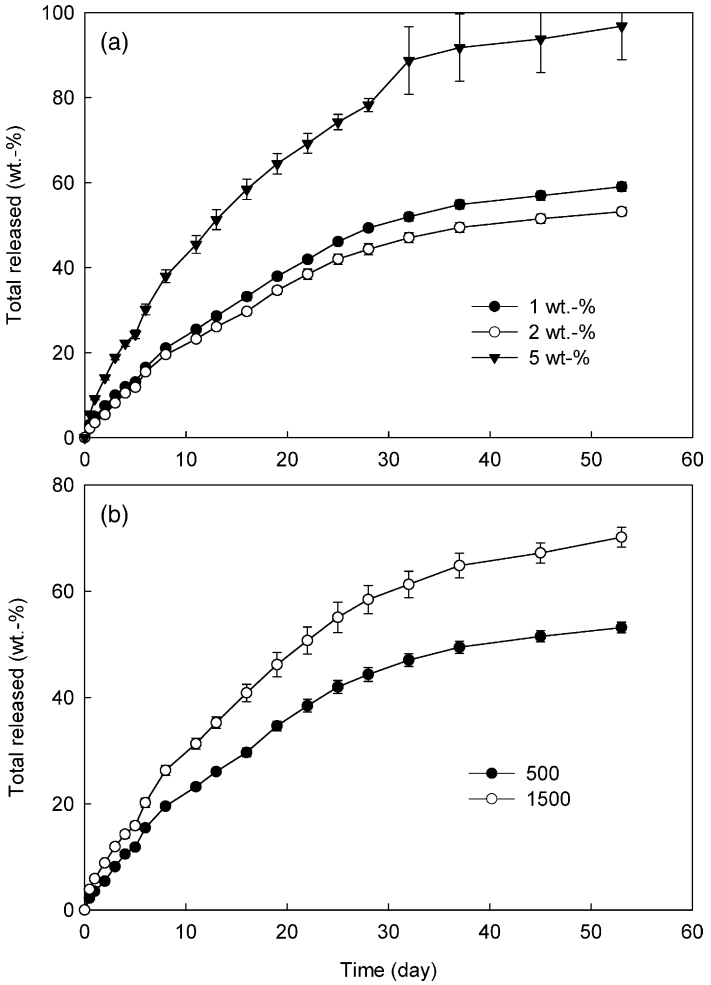

Fig. 2. The effect of PVA concentration (a) and PVA molecular weight (b) on the atRA release characteristics from PLGA microspheres. Drug contents of microspheres are described in Table 2 ($n = 3$).

Crystallized drug were released by simple dissolution and diffusion (Guyot and Fawaz, 1998).

Fig. 4 shows the effect of solvent used on the release rate of atRA from microspheres. When chloroform was used, drug release rate was faster than that of DCM and EA. It showed pseudo-zero order release kinetics until about 50 days while drug release rate of microspheres prepared by EA was slightly rapid than DCM. AtRA was continuously released from microspheres for 40 days with pseudo-zero order kinetics.

Fig. 5 shows the effect of polymer MW and weight on the release rate of atRA from PLGA microspheres. As shown in Fig. 5(a), the drug release rate between medium and high MW polymers were not significantly changed while low MW polymers showed more rapid drug release. The effects of amount of polymer added in organic phase were studied. As shown in Fig. 5(b), the increased weight of polymer (W1) induced the increased release rate of drug from the microspheres. This result was consisted with other results as previously reported (Choi et al., 2002). Choi et al. reported that increased weight of polymer resulted in larger particle size, higher loading efficiency, and increased drug release rate. Indeed, increased drug release rate by increasing polymer weight was due to the lower drug contents although loading efficiency was increased.

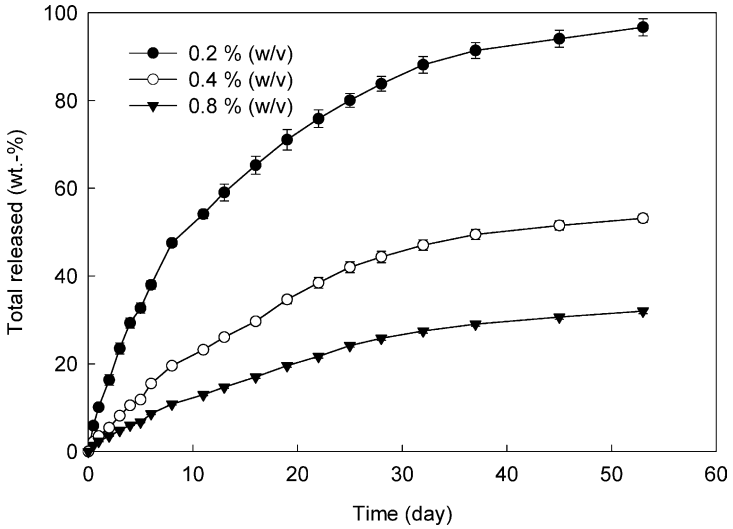

Fig. 3. The effect of atRA weight in the organic phase on the atRA release characteristics from PLGA microspheres. Drug contents of microspheres are described in Table 2 ($n = 3$).

Estey et al. (Estey et al., 1996, 1999) reported that atRA-encapsulated liposome apparently resulted in greater exposure to tretinoin and for a longer time. Positively charged liposomes are reported to be effective to increase the skin permeation of atRA (Montenegro et al., 1996). Lim and Kim (2002) reported that aqueous solubility and stability of atRA increased greatly by use of 2-hydroxypropyl-β-cyclodextrin and can make it as a parenteral and/or oral formulation. It was reported that liposomal-atRA was shown in significantly better pharmacokinetic profile than non-liposomal atRA with low side-effects (Ozpolat and Lopez-Berestein, 2002). Giordano et al. (1993) reported that a single injection of atRA-loaded

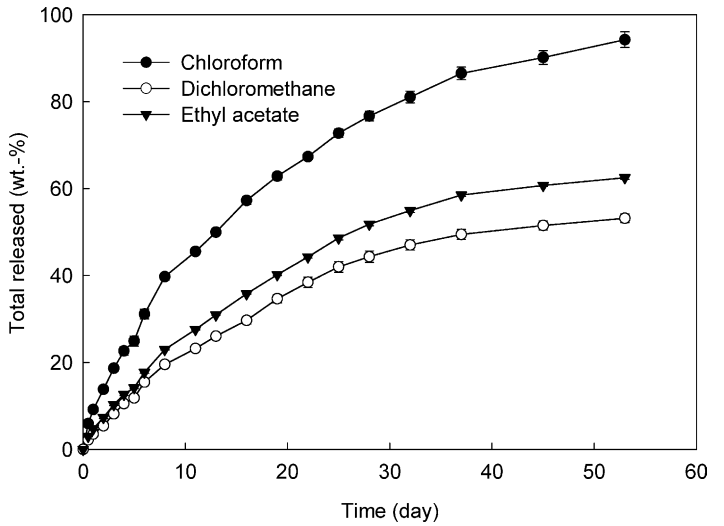

Fig. 4. The effect of solvent used for microsphere fabrication on the atRA release characteristics from PLGA microspheres. Drug contents of microspheres are described in Table 2 ($n = 3$).

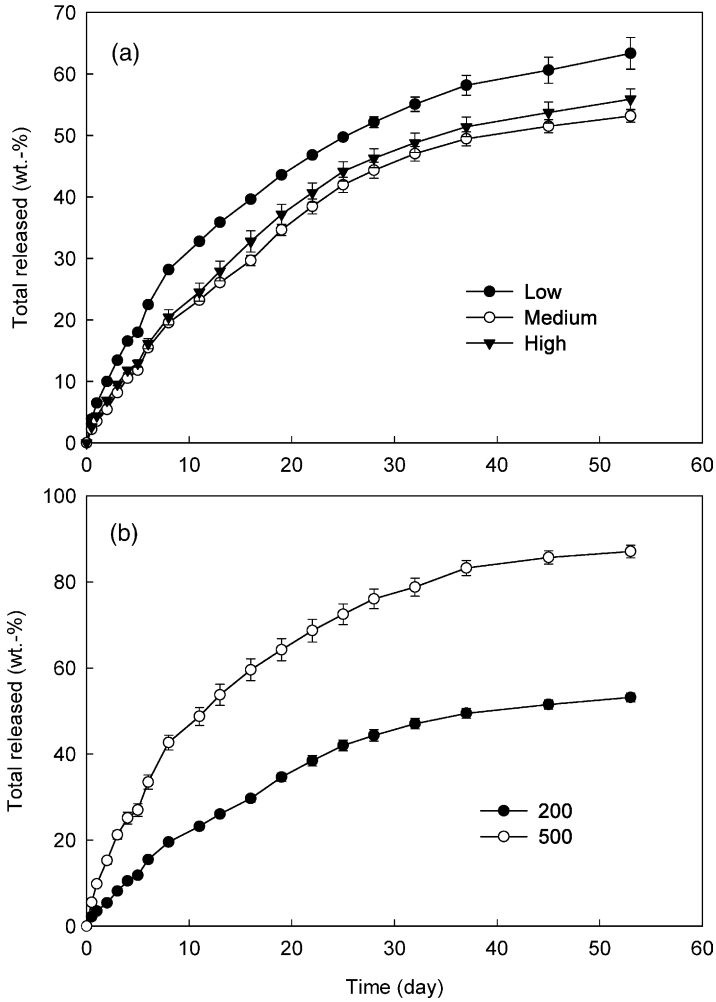

Fig. 5. The effect of polymer MW (a) and polymer weight in the organic phase (b) on the atRA release characteristics from PLGA microspheres. Drug contents of microspheres are described in Table 2 ($n = 3$).

microspheres was effective in reducing the incidence of tractional retinal detachment after 2 months in a rabbit model of proliferative vitreoretinopathy.

Resultantly, sustained formulation such as microspheres is considered to be useful device to overcome disadvantages of atRA and increase the half-life of drugs.

3.3. Effect of sterilization of microspheres containing atRA with ethylene oxide gas

Since sterilization is important process for clinical use of biodegradable microspheres, EO-gas sterilization is critical factor for use of microspheres. From the results of sterilization process, EO-gas sterilization did not affect to drug and microsphere stability and sterilized microspheres can be used for clinical use. For use in clinical application of microspheres containing atRA, sterilization is essential to prevent contamination problem. There are several sterilization methods such as steam, dry heat, EO gas and γ-irradiation. Among them, steam and dry heat sterilization are carried out at high temperature and cause severe degradation of microspheres, resulting decreased therapeutic activity of drug. In this respect, ethylene oxide gas and γ-irradiation have been preferred for the sterilization

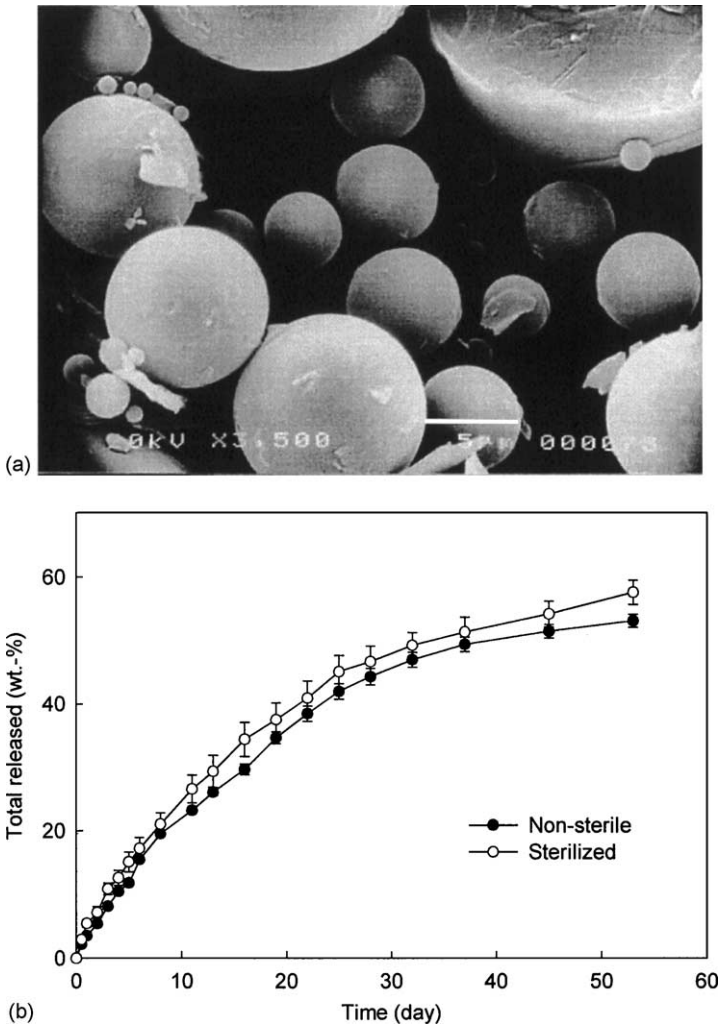

Fig. 6. SEM photographs of PLGA microspheres (V2 in Table 2) (a) and changes of release characteristics (b) after EO-gas sterilization.

of microspheres containing therapeutic agents. EO-gas sterilization requires simpler equipment and lower cost than γ-irradiation. Since molecular weight of polymer and morphology of microspheres are important factor for drug release kinetics and properties of microspheres, EO-gas sterilization does not affect the molecular weight of polymer whereas γ-irradiation significantly reduces it (Choi et al., 2001).

To investigate the effect of EO-gas sterilization, sterilization process by EO for microspheres containing atRA was carried out at cooled cycle of 37 °C to prevent aggregation of microspheres and to maintain therapeutic properties of atRA. Fig. 6 shows morphology of PLGA microspheres containing atRA observed by SEM after EO-gas sterilization. As shown in Fig. 6(a), morphology of microspheres was not significantly changed by EO-gas sterilization, indicating EO-gas sterilization did not affect to microsphere properties and polymer. The changes of drug release after EO-gas sterilization are shown in Fig. 6(b). As expected, drug release rate was not significantly different before and after EO-gas sterilization, although it was slightly increase by EO-gas sterilization. Particle size of microspheres after EO-gas sterilization could be negligible (36.9 μm).

To investigate the stability of atRA in microspheres during EO-gas sterilization, cell growth inhibition using U87MG cell lines was performed by MTT test using microspheres before and after EO-gas sterilization. To test cell growth inhibition, sterilized microspheres was dissolved in DMSO and diluted with α-MEM (DMSO concentration was below 0.1% (w/v)). Fig. 7(a) shows the effect of EO-gas sterilization of microspheres on the cell growth inhibition of U87MG cells. The cell growth inhibition by microspheres containing atRA after EO-gas sterilization was slightly changed compared to atRA. However, cell cytotoxicity of microspheres containing atRA was not significantly changed by EO-gas sterilization, indicating that atRA was affected by process of microsphere preparation rather than EO-gas sterilization process. As shown in Fig. 7(b), empty microspheres was not affected to the cell cytotoxicity of U87MG.

These results indicate that cell growth inhibition properties of atRA are not changed by biodegradable polymer, PLGA and EO-gas sterilization did not

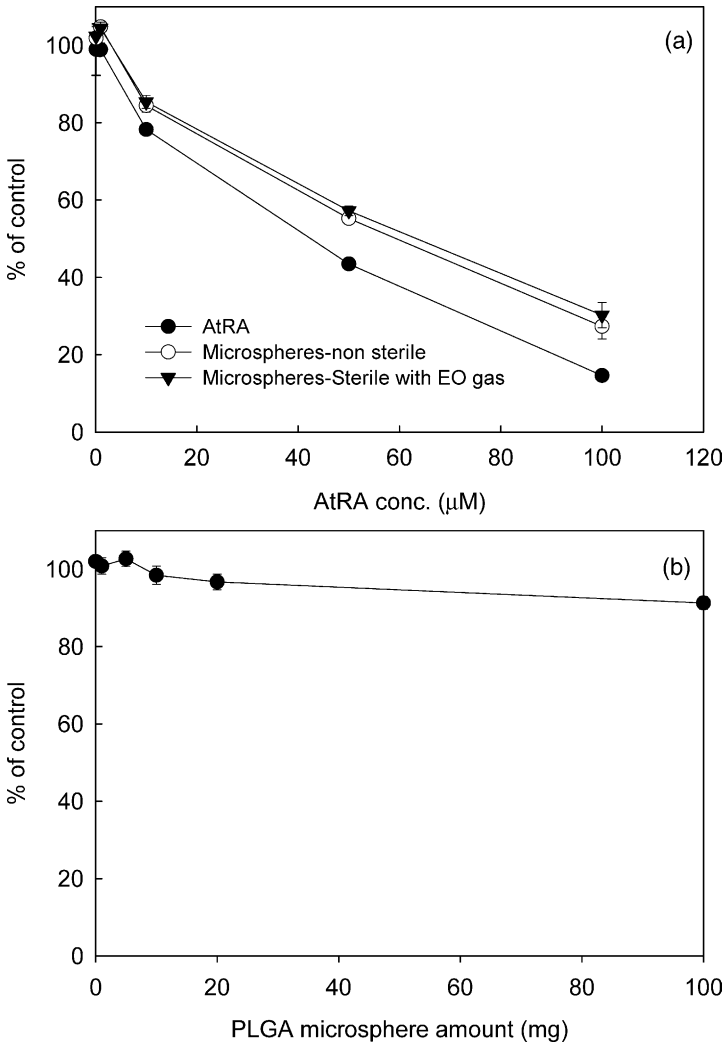

Fig. 7. Cell growth inhibition of U87MG by atRA and sterilized PLGA microspheres containing atRA (a) and empty microspheres (b) after 4 days. The value was mean of eight wells and expressed as S.E.

affect to drug and microsphere stability and sterilized microspheres can be used for clinical use.

4. Conclusion

PLGA microspheres containing atRA was successively prepared by o/w emulsion solvent evaporation process. In the preparation variables, PVA concentration was one of the important factors to make a spherical microspheres. Particle size and loading efficiency was decreased according to the increased concentration of PVA and higher MW of PVA. Drug release was faster at the 5% (w/v) of PVA and higher MW. Increased atRA weight induced increased particle size and decreased drug release. 0.2% (w/v) of atRA resulted pseudo-zero order release kinetics about for 50 days. Chloroform was resulted in smaller particle size but loading efficiency also decreased. Low MW of PLGA resulted in decreased particle size and loading efficiency while medium and high MW of PLGA was not significantly changed whereas drug release rate against polymer MW was not significantly changed. The higher the polymer weights the higher the release rate of the drug from the microspheres. Among formulation variables, V2 was considered as a suitable formulation in further research. PLGA microspheres were sterilized by EO gas at cooled cycle of 37 °C. After sterilization process, drug release and morphology of microspheres were significantly not changed. In results of cell growth inhibition of atRA, therapeutic activity of atRA was affected by preparation process of microspheres rather than EO-gas sterilization process. These results indicate that PLGA microspheres containing atRA is acceptable for controlled release devices for use in treatment of brain tumor due to their size and drug release profiles.

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