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PEG modulated release of etanidazole from implantable PLGA/PDLA discs

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Abstract

In this work, etanidazole (one type of hypoxic radiosensitizer) is encapsulated into spray dried poly(D,L-lactide-co-glycolide) (PLGA) microspheres and then compressed into discs for controlled release applications. Etanidazole is characterized by intracellular glutathione depletion and glutathione transferases inhibition, thereby enhancing sensitivity to radiation. It is also cytotoxic to tumor cells and can chemosensitize some alkylating agents by activating their tumor cell killing capabilities. We observed the release characteristics of etanidazole in the dosage forms of microspheres and discs, subjected to different preparation conditions. The release characteristics, morphology changes, particle size, and encapsulation efficiency of microspheres are also investigated. The release rate of etanidazole from implantable discs (13 mm in diameter, 1 mm in thickness, fabricated by a press) is much lower than microspheres due to the reduced specific surface. After the initial burst of 1% release for the first day, the cumulative release within the first week is less than 2% until a secondary burst of release (caused by polymer degradation) occurs after one month. Some key preparation conditions such as drug loadings, disc thickness and diameter, and compression pressure can affect the initial burst of etanidazole from the discs. However, none of them can significantly make the release more uniform. In contrast, the incorporation of polyethylene glycol (PEG) can greatly enhance the release rate of discs and also reduces the secondary burst effect, thereby achieving a sustained release for about 2 months.

Keywords: Discs; Spray dryer; Microspheres; Etanidazole; Polyethylene glycol (PEG)

1. Introduction

Radiosensitizers are chemical or pharmacological agents that can increase the lethal effect of radiation to the cancer cells while possessing the least influence on normal tissues. In general, they are classified into two main types: halogenated pyrimidines and hypoxic cell sensitizers [1,2]. Hypoxic cell radiosensitizers have attracted significant interests both in lab scale and clinical trials. It is proposed that the existence of poorly vascularized areas in the growing tumor mass lead to a proportion of cells being remote from capillaries, forming hypoxic cells [3]. The tumor cell response to the X-ray radiation is mostly controlled by the presence of hypoxic cells (10–20% in animal model) in tumor, which are more resistant to radiation than well-oxygenated ones. Hypoxic cell sensitizers can mimic the effect of oxygen, forming toxic DNA radicals to damage cellular DNA and prevent DNA repair [4], thereby enhancing radiosensitization effect.

Etanidazole (ETA), the second generation of hypoxic cell radiosensitizers, was developed accordingly in an attempt to reduce the toxicity of misonidazole (the first generation of 2-nitroimidazole) [5]. Etanidazole has a shorter half-life than misonidazole. Its partition coefficient in octanol/water is 0.046, much lower than the value of 0.43 for misonidazole [6]. However their radiosensitization effect and permeability are indistinguishable from each other. Etanidazole has been shown to have less neurotoxicity, and hence could have cumulative administration doses three to four folds higher than those of misonidazole [7]. Some researches also conclude that etanidazole, misonidazole and other nitroimidazoles are effective radiosensitizers even at extreme dosage levels [8]. However, the clinical trials for etanidazole in conjunction with fractionated radiation have displayed some disappointing results [9,10]. An

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European randomized trial of etanidazole combined with radiotherapy in head and neck carcinomas also demonstrated that adding ETA to conventional radiotherapy didn't afford any benefits for patients, although most of the side effects were only related to nausea, vomiting and allergies; grade I neurotoxcity was mostly observed [11]. The failure in the survival rate improvement may be partly due to systemic toxicity when the drug is administered over a long time [12–14].

Radiosensitizers are generally administered via systemic methods in clinical trials. However, these drugs have short half-lives, and are easily metabolized within the body or excreted by kidney. In particular, in order to allow the drug to reach the targeted area and achieve the desired chemotherapeutic level, a large quantity of drug is usually employed, leading to toxic side effects. On the other hand, drug uptake concentration in direct contact with the tumor cells and the exposure time are key factors for the effective radiosensitization. A sustained release intratumoral drug delivery system, such as a biodegradable polymer implant, would provide a localized drug release to the tumor over a prolonged time period. This motivates the development of controlled release systems for radiosensitizers to eliminate the unexpected side effects and enhanced effectiveness [2,12,15–17].

In the post-surgery therapies of brain tumors, drug carrying discs are sometimes used [18]. To fabricate these discs, powders are generally obtained first, and then compressed into devices with different sizes. Several techniques have been employed to fabricate powders. For instance, mills or pestles are used to grind the polymer or drug into fine particles, mixed them together [12,19] and then compressed into desired discs or extruded via heating. Alternatively, a tumbling blender can be utilized to produce homogenous drug-polymer mixtures before compression into discs [20]. However, this process is subjected to the constraint that minimal damage to the polymer and the encapsulated drug should be conferred since heating or mechanical force is involved. In addition, it is important that microspheres or microparticles are produced first before the compression-molding process is applied [18,21].

Commonly used methods to fabricate microspheres include solvent evaporation/solvent extraction method, phase separation method as well as spray drying method. Solvent evaporation/solvent extraction method is no longer suitable for the fabrication of etanidazole loaded microspheres since etanidazole is a highly hydrophilic drug with a relatively low molecular weight (214.2 Da) [22]. A large amount of drug can be lost to the aqueous phase while the microspheres are produced, resulting in significantly lower encapsulation efficiency. Phase separation, an anhydrous process, is capable of fabricating highly water-soluble drugs with high drug entrapment efficiency. Since a large amount of organic solvent is required, it is difficult to remove the residual organic solvent from the microspheres. Furthermore, particles obtained via a phase separation technique are heavily aggregated with a wide size distribution [23]. In contrast, spray drying is quite convenient and allows the use of mild conditions and the scaling up of the process. In addition, it depends less on the properties of drug and polymer as compared to the two other methods mentioned above. Moreover, the microspheres generated can achieve reasonably high encapsulation efficiency and product yield [24,25].

Etanidazole has been tested for drug delivery by using PCPP: SA 20:80 as a carrier [2]. The release of etanidazole from discs can only persist for a few hours due to its high hydrophilicity. Another implantable device in the form of rods was prepared by heat extrusion [12] and was inserted in the tumor-bearing mice for radiosensitization test. Significant increase of TGD was observed in the intra-tumoral cases. This result indicates that implantable system could improve treatment outcomes in hypoxic tumors [12].

Although a few phase III clinical trials of etanidazole have not provided sufficient evidence for its overall survival rate in the treatment of tumors by systemic administration, it is still promising to develop and test a controlled release system aiming at enhanced drug delivery efficacy. In this work, the controlled release of etanidazole from spray dried PLGA microspheres is studied. The microspheres are subsequently compressed into the dosage form of implantable discs. The key parameters to fabricate discs such as disc size and thickness, drug loading, polymer type, and compression pressure are investigated. Finally the modulation of the release behavior by polyethylene glycol (PEG) is discussed.

2. Materials and methods

2.1. Materials

Biodegradable polymers PLGA 65:35 (MW 40,000–75,000), PLGA 85:15 (MW 90,000–126,000), Poly(DL-lactide) (PDLA, MW 106,000), etanidazole (MW 214) and polyethylene glycol (PEG) with different molecular weights were purchased from Sigma-Aldrich (St. Louis, USA). Ethylacetate (EA) and dichloromethane (DCM) of reagent grade were acquired from Merck (Darmstadt, Germany) and Fisher Scientific Chemicals (Fair Lawn, NJ, USA), respectively.

2.2. Microspheres and discs preparation

PLGA polymers and etanidazole were dissolved in a solvent (dichloromethane or ethylacetate) to form a solution of a certain polymer concentration (defined as

polymer weight/volume of the solvent used, g/ml). The polymer solution was spray dried in a Büchi 191 Mini Spray Drier (Flawil, Switzerland) through a nozzle of 0.7 mm. The process parameters were given as: inlet temperature 55°C, outlet temperature 40–46°C, aspirator ratio 100%, compressed air flow rate 700Nl/h, polymer solution feed rate 20% (roughly equivalent to 4 ml/min when 3% PLGA 65:35 DCM solution was used), polymer concentration 3% (g/ml) unless otherwise noted. The theoretical drug loadings (defined as the ratio of the drug used with respect to the total weight of the polymer and drug) were always kept at 1% unless otherwise noted. Collected microspheres were weighed and stored in the desiccator under vacuum condition at least 24 h before use. To fabricate PEG-incorporated microspheres, dichloromethane (DCM) was used as the solvent instead of EA since PEG does not dissolve in EA. The yield of the microspheres is defined as the ratio of the actual weight of the microspheres obtained to the theoretical weight of the polymer and drug used.

The weighed, spray-dried microspheres were compressed into discs of different sizes by using a hydraulic hand press (GRASEBY SPECAC, Orpington Kent, Britain) and a die of corresponding size. 25 mg of microspheres was used for fabricating a small disc with the diameter of 5 mm while 170 mg for a larger disc with the diameter of 13 mm. Both discs had a thickness of 1 mm unless noted otherwise. A pressure of 2 tons per square meter was applied onto the microspheres for 3 min. Compressed discs were stored in vacuum before use.

2.3. Particle size and distribution

The particle size and its distribution were measured by a laser light scattering technique (90 Plus Particle size analyzer, Brookhaven Instruments Corporation, Holtsville, New York, USA). Dry microspheres were suspended in ultra-pure water in the presence of 1.0% w/w Tween 80 (Sigma Chemical Company, St. Louis, MO, USA), and then sonicated in the water bath for 3 min to disaggregate the microspheres. Tween 80 was employed to keep the particles from re-aggregation. Loaded particles were diluted by ultra pure water to make sure the particles count rate was within the range of 200–700 kcps. For every sample, five runs were conducted with the duration of 1 min for each. The measurement was carried out at 23°C with the final result being given as the average of the five analyses for each sample.

2.4. Encapsulation efficiency

The actual content of etanidazole in the microspheres was determined by the following procedure: 5–10 mg microspheres were dissolved in 1 ml dichloromethane (DCM) and shaken for about half an hour, then 10 ml phosphate buffer solution (PBS) (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2, Pierce, Rockford, IL, USA) was added into the DCM solution and shaken vigorously for about 1 h. The PBS buffer solution containing the extracted drug was clarified by centrifugation at 4000 rpm for 15 min. The drug content of each sample was determined using reversed phase HPLC. Encapsulation efficiency is calculated as the ratio of actual to the theoretical loading of the drug in the microspheres.

2.5. Morphology studies

The morphology of microspheres was examined by Scanning Electron Microscopy (XL 30 SEM Philips, Eindhoven, The Netherlands). The microspheres were mounted onto a copper cylinder (10 mm in diameter, 10 mm in height) by using a double-sided adhesive tape. The specimens were coated at a current of 10 mA for 4 min using the Ion Sputtering Device (JFC-1100E, Jeol, Japan). The discs were incubated in the buffer solution at 37°C, retrieved at different stages and vacuum-dried. Their surface morphology was characterized by SEM, following the similar procedures for microspheres.

2.6. In vitro release study

In vitro release tests were carried out in triplicate at 37°C phosphate buffer solution. 5–10 mg accurately weighed microspheres was suspended in a 10 ml vial containing 1.5–2 ml 0.05% Tween 80 (Sigma Chemical Company, St. Louis, MO, USA) PBS buffer solution. Sample tubes were placed in a shaking water bath with a horizontally shaking speed of 110 rpm. At different hours following the incubation, the test tubes were retrieved from solutions and fresh phosphate buffer was replaced. The cumulative percentage of drug released for the first half an hour after incubation was calculated as the initial burst for the microspheres. The drug content in PBS was analyzed using HPLC system (Perkin-Elmer Corporation, CT, USA) and Inertsil ODS-3 column (4.6×200 mm, 5 μm, GL Science, Tokyo, Japan) at ambient temperature. Mobile phase consisted of 95% water and 5% acetonitrile delivered at a flow rate of 1 ml/min, and UV detector was operated at a wavelength of 324 nm [2,22].

Compressed discs were also incubated at 10 ml 0.05% Tween 80 (Sigma Chemical Company, St. Louis, MO, USA) phosphate buffer solution. Every week a sample was taken and the buffer was refreshed. On the first and the third day of the first week, release samples were also taken subject to HPLC analysis similar to microspheres. The initial burst of the discs is defined as the cumulative release of drug from discs after the disc is incubated in the PBS for one day.

2.7. Differential scanning calorimetry (DSC)

The measurement of the glass transition temperature (T_g) of the microspheres was carried out by DSC 200 (Netzsch, Thermische Analyse, Germany). Approximately 7–10 mg microspheres was weighed into aluminum pans and hermetically sealed. The samples were heated from 0°C to 180°C at a rate of 10°C/min under nitrogen atmosphere. A covered, empty pan was used as a reference. The results obtained from the first heating were recorded.

3. Results and discussion

3.1. Polymer type

Microspheres made of different polymers were fabricated to be the raw materials for implantable discs. Table 1 gives the yield, particle size and encapsulation efficiency data. All the yields are more than 40%, and the drug encapsulation efficiencies are more than 85%. Particle size and distribution are found to decrease in the order of PLGA 65:35, PLGA 85:15 and PDLA. As shown in Fig. 1, PLGA 65:35 microspheres have the fastest release rate, 90% of the total drug is released within the first 2 days. PLGA 85:15 releases much slower, taking about ten days for 90% of the drug to be released. In addition, PLGA 85:15 has an initial burst lower than PLGA 65:35. PDLA microspheres has the slowest release rate, persisting for over one month. Its initial burst is also the lowest in all the three polymers. The release of etanidazole from discs is quite different from that of microspheres: the release rate is considerably reduced after the formation of discs, and two characteristic stages are observed in the release profile (Fig. 2). The first stage is characterized by the initial burst followed by a relatively long period of slow release rate. The initial burst of all three polymers is only 1% for the first day. After the initial burst, a very slow and nearly leveled off release is found and lasts a long period of time until a second burst occurs. It takes 1 month, 2 months, and 3.5 months for PLGA 65:35, PLGA 85:15 and PDLA, respectively, to incur the second burst. At the first stage, the slow release rate is mainly dominated by the diffusion of the drug from the discs. Since the specific area of the compressed disc is much smaller than the microspheres, its release rate is therefore restrained. In the second stage, the second burst appears followed by a slow release rate until the release goes to

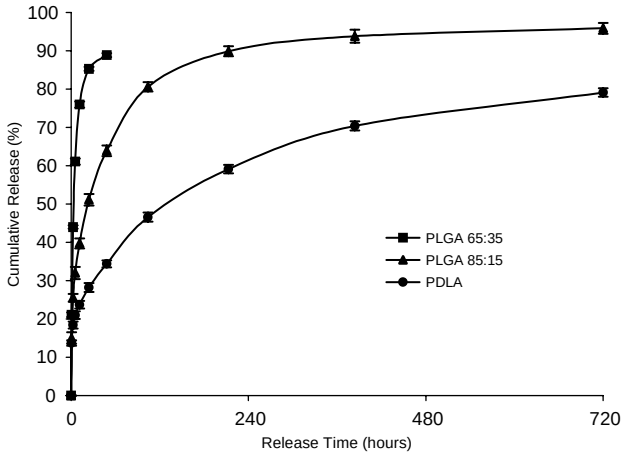

Fig. 1. Release profile of microspheres for different polymers. Fabrication conditions of microspheres are given in Table 1. The figure represents geometric mean ± s.e. for all points.

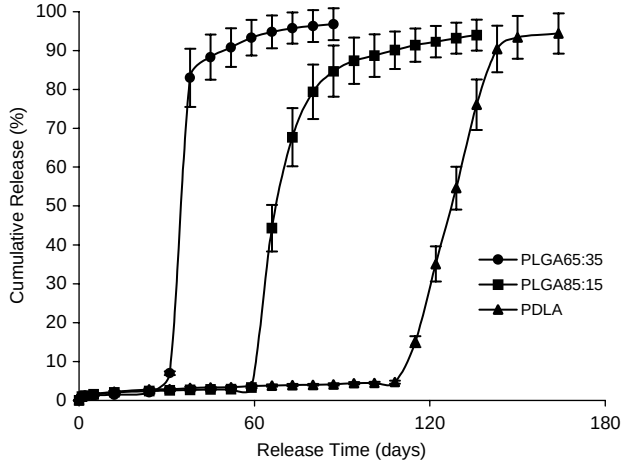

Fig. 2. Release profile of discs made of different polymers (disc diameter = 13 mm, thickness = 1 mm). Fabrication conditions of microspheres are given in Table 1. Discs compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

Table 1
Microspheres of different polymers

Polymer type	Yield (%)	Size (μm)	Polydispersity	Encapsulation efficiency (%)
PLGA 65:35	41.9	1.77 ± 0.12	0.23 ± 0.05	92.9 ± 4.8
PLGA 85:15	43.7	1.50 ± 0.10	0.20 ± 0.05	98.4 ± 1.0
PDLA	48.3	1.34 ± 0.14	0.13 ± 0.07	88.3 ± 1.1

Fabrication conditions: outlet temperature 55°C, pump feed rate 20%, compressed air flow rate 700 Nl/h, aspirator ratio 100%, drug loading 1%, polymer concentration 3% (g/ml), solvent EA.

Table 2
PLGA 65:35 microspheres fabricated at different drug loadings

Drug loading (%)	Yield (%)	Size (μm)	Polydispersity	Encapsulation efficiency (%)
1	41.9	1.77±0.12	0.23±0.05	92.9±4.8
5	42.2	2.14±0.07	0.355±0.04	94.4±3.9
9	38.2	2.47±0.14	0.369±0.03	80.2±1.4

Fabrication conditions: inlet temperature 55°C, pump feed rate 20%, compresses air flow rate 700 Nl/h, aspirator ratio 100%, solvent EA, polymer concentration 3% (g/ml).

completion. The time duration for the second burst varies with different polymers. PLGA 65:35 takes one week to reach 90% of total release at the onset of the second burst. However, PLGA 85:35 takes about 3 weeks, and PDLA takes about 5 weeks to achieve the second burst. After the second burst, a very slow decrease in the release rate is observed. This is mainly caused by the decreased concentration gradient due to the diffusion of the drug left in the discs. From Fig. 2, it is seen that the release of etanidazole from PLGA 65:35 discs persists for about 2 months and satisfies the basic requirement for one radiotherapy course, while PLGA 85:15 and PDLA discs have a too long degradation time (3–5 months). However, it is disappointing that the drug release from all the discs is not uniform, and therefore has no practical use unless further efforts are made to modify the release characteristics.

The second burst may be induced by the degradation of polymer. When the polymer molecular weight decreases (caused by degradation) to a critical limit, the physical properties of the discs will be greatly altered, and hence the diffusion of the etanidazole from the disc becomes easier. A big second burst may be due to the large amount of drug kept within the discs as well as the high solubility of etanidazole in water. During the release experiments, samples were collected from the water bath. It was found that the disc changed its color from white to colorless, transparent, and sticky shortly before the second burst occurred. The shape of the disc was also changed due to its loss of integrity, and hence the original round and regular surface could no longer be observed. It is evident that the degradation has led to the occurrence of the second burst. The coincidence in the release profile with the onset of the second sharp burst reveals that during this period the release is mainly caused by polymer degradation. The lag time (before the second burst) can be an index for the degradation properties of different polymers. Henceforth, PLGA 65:35 degrades the fastest, and this is followed by PLGA 85:15. PDLA has the lowest degradation rate. The duration of the second burst may be related to both the degradation rate of the polymer and its molecular weight.

The microspheres obtained from the spray dryer have a very small particle size around 2 μm. After being compressed into discs, the specific area (surface area per unit volume) is reduced significantly such that there is only limited surface for the release of etanidazole. The overall release characteristics might be improved if larger particles were employed to fabricated discs, provided other fabrication conditions remain the same. This is because larger particles may produce a larger specific area as compared with small particles due to the increased voidage in the disc structure.

Based on the discussion above, it is believed that the release of etanidazole from disc is controlled jointly by both diffusion and degradation. Due to the reduced specific area, the diffusion has a very small effect on the total release. Degradation plays a much more significant role on the cumulative release of etanidazole from discs.

3.2. Drug loading

As shown earlier, PLGA 65:35 discs can achieve a sustained release of etanidazole for about 2 months. Based on this favorable release property, we chose PLGA 65:35 as the basic materials for subsequent investigations. Microspheres with different drug loadings were fabricated using the biodegradable polymer PLGA 65:35. The yield, particle size and encapsulation efficiency of microspheres were studied and given in Table 2. It is clear that particle size increases with increasing drug loading. Within the range of 1–5% drug loadings, encapsulation efficiency seems to be less affected by the drug loading. The yield of the first two types of microspheres is more than 40%. In contrast, a very high drug loading (9%) may show a different story in which a lower encapsulation efficiency is obtained.

The release behaviors of microspheres and discs at three different drug loadings are illustrated in Figs. 3 and 4, respectively. The drug release rates are similar from microspheres with 1% and 5% drug loadings, while 9% drug loading results in a much higher initial burst and a faster release rate. As compared with microspheres, the drug release rate and initial burst of the compressed discs are greatly reduced. This may be due to the greatly decreased specific surface area. However, after the initial burst of 6% release for the first day, the cumulative release within the following four weeks is less than 5% until a secondary burst of release occurs after one month. Discs with 1% and 5% drug loadings have no appreciable difference in the

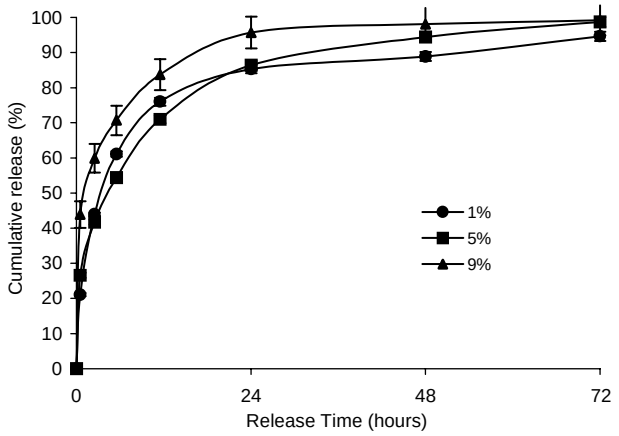

Fig. 3. Release profile of PLGA 65:35 microspheres with different drug loadings. Fabrication conditions of microspheres are given in Table 2. The figure represents geometric mean ± s.e. for all points.

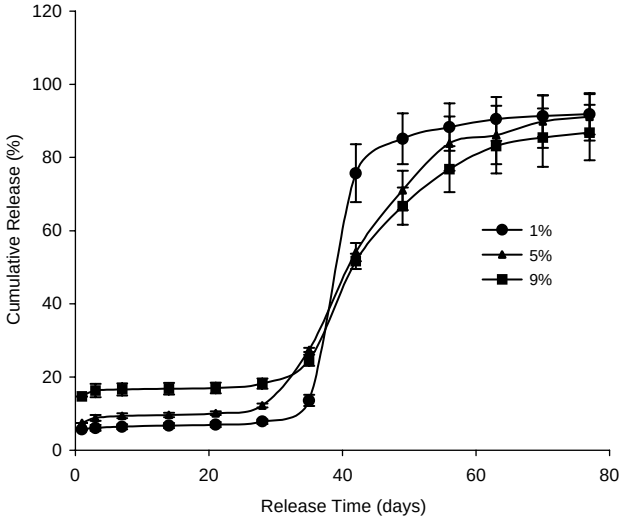

Fig. 4. Release profile of PLGA 65:35 discs with different drug loadings (disc diameter = 5 mm, thickness = 1 mm). Fabrication conditions of microspheres are given in Table 2. Discs compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

release behavior, while a higher drug loading produces a higher initial burst. In the meantime, the second burst is reduced with increasing drug loading, this may be caused by the higher initial burst and the resultant lower drug content in the disc. However, the trends of the subsequent release profile are quite similar and less dependent on the drug loadings. No improvement of the release behavior is observed by employing a higher drug loading.

3.3. Diameter of discs

Fig. 2 is the release characteristics of a larger disc with a diameter of 13 mm, while Fig. 4 gives the corresponding result of a smaller disc (5 mm in diameter). It is found that reducing the diameter of the disc can increase the initial burst from 1% to 6%, however, the overall trend for the release remains unchanged. Smaller size has larger specific area, thus a larger initial burst could be obtained. Since the dominating factor for the release of etanidazole from discs is the degradation, no major difference should be observed in the in vitro release profiles.

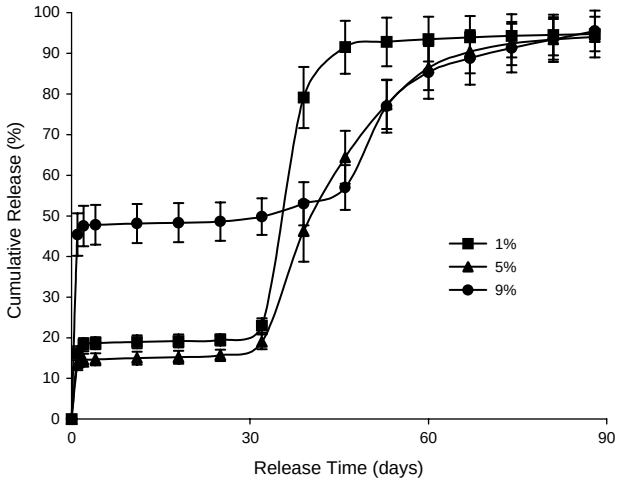

Fig. 5. Release profile of PLGA 65:35 discs of 0.5 mm thickness (diameter = 5 mm) for different drug loadings. Fabrication conditions of microspheres are given in Table 2. Discs compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

3.4. Thickness of discs

The effect of the thickness of discs on the release behavior of etanidazole has been studied, and the release curve for PLGA discs of 0.5 mm (diameter = 5 mm) is shown in Fig. 5. The initial burst of the three drug loadings has been greatly improved. For 1% and 5% drug loadings, the initial burst within the first day can reach 16%, however, at an even higher loading of 9%, the initial burst is increased dramatically, reaching more than 40% for the first day. This is not surprising since the mass transfer resistance is proportional to the diffusion distance, i.e. the disc thickness for the present case. After the initial burst, the subsequent release is very slow followed by the second burst due to the degradation. It seems that 9% drug loading has a smaller second burst and this burst is also delayed for 2 weeks. 5% drug loading discs emerge their second burst nearly at the same time as 1% drug loading discs, however, the second burst for 5% is smaller and lasts longer than 1%. Higher drug loading seems to delay the degradation rate of the polymer materials due to the drug-polymer interactions. Compared with the effect of changing the disc diameter, thickness of the discs plays a more important role in determining the release behavior.

3.5. Compression pressure

It is necessary to know the influences of the compression pressure on the fabrication of discs. Various pressure loads (1, 4, 7 and 10 ton/m^2) are applied and the PLGA discs formed are evaluated for their release behavior as shown in Fig. 6. It is clear that pressure load does not make significant difference on the release behavior. The only exception is, when the lowest pressure (1 ton/m^2) is applied, the initial burst is lower (6%). This result may be due to the cracking of the microspheres when higher pressure is loaded, while lower pressure can prevent this from happening.

3.6. PEG modulated discs

In previous sections, discs were fabricated and the influences of various preparation conditions were evaluated. It is found that by varying the fabrication parameters such as polymer type, disc thickness and diameter, drug loading and compression pressure, no significant improvement on the release profile is obtained. This is due to the fact that the release mechanism is degradation-controlled. In order to achieve a sustained and more uniform release, measures must be taken to modify the surface structure of the discs, otherwise there will be little practical use for these discs.

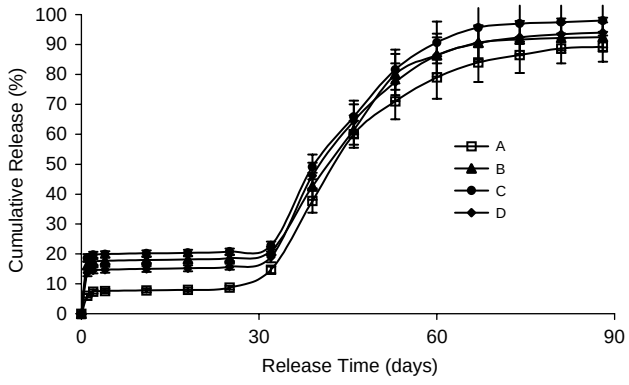

Fig. 6. Release profile of PLGA 65:35 discs prepared under different compression pressures (diameter 5 mm, thickness 0.5 mm) (A) 1 ton/m^2, (B) 4 ton/m^2, (C) 7 ton/m^2 and (D) 10 ton/m^2. Fabrication conditions of microspheres are given in Table 2, drug loading = 5%. Retention time for compression = 3 min. The figure represents geometric mean ± s.e. for all points.

PEG has attractive properties that result in its wide applications in the controlled release filed. Excellent solubility in aqueous medium, chain flexibility, and low toxicity are some of its typical characteristics. Although PEG is not biodegradable, it has no toxic effect on the human body. The more important factor is that PEG can readily be excreted from the body via kidneys [26]. PEG is also used as a coating material for the microparticles injected into the body since it can provide protection against interaction with blood components, and thus prolongs blood circulation times by reducing the particle clearance via the RES cells [27]. As an acceptable polymer, PEG is of significance for pharmaceutical applications. The use of PEG in the fabrication of microspheres via the solvent extraction and evaporation method has been reported [28]. However, only a small amount of PEG is able to retain in the microspheres due to the diffusion into the aqueous phase.

In this section, PEG is incorporated into the microspheres using a spray-drying technique. Its enhancement on the drug release characteristics is investigated. Eight samples are prepared and tested for different PEG types and loadings (Table 3). It seems that adding PEG (MW 8000) with a loading ranging from 1% to 5% into PLGA 65:35 systems could enhance the yield of the microspheres. However, the particle size distribution of the microspheres becomes worse. A 10% PEG loading produces an adverse effect of lower yield. When other PEG type [PEG (MW 3350)] is used, the yield of the microspheres is lower, no matter whether 5% or 10% PEG loading is employed. This seems to be associated with the molecular weight of PEG. Lower molecular weight achieves an enhanced plasticity effect, resulting in the difficulty in collecting the microspheres. Encapsulation efficiency is around 90% for most of the

Table 3
PLGA65: 35 microspheres with different PEG types and loadings

PEG concentration	Yield (%)	Size (μm)	Polydispersity	Encapsulation efficiency (%)
PEG, 0%	32.5	1.49 ± 0.10	0.23 ± 0.03	88.3 ± 2.0
PEG (MW 8000), 1%	43.3	1.91 ± 0.10	0.22 ± 0.08	96.8 ± 4.3
PEG (MW 8000), 3%	36.1	2.17 ± 0.0.08	0.33 ± 0.07	92.4 ± 1.3
PEG (MW 8000), 5%	43.1	2.95 ± 0.11	0.41 ± 0.10	96.6 ± 5.1
PEG (MW 8000), 10%	30.7	—	—	92.2 ± 6.4
PEG (MW 3350), 5%	28.4	—	—	92.4 ± 4.4
PEG (MW 3350), 10%	30.7	—	—	83.7 ± 4.5
PEG (MW 1145), 5%	30.2	—	—	88.8 ± 1.2

Fabrication conditions: inlet temperature 55°C, pump feed rate 20%, compressed air flow rate 700 Nl/h, aspirator ratio 100%, solvent DCM, drug loading 1%, polymer concentration 3% (g/ml).
— Particle aggregates are formed, no data on the particle size and polydispersity is reported.

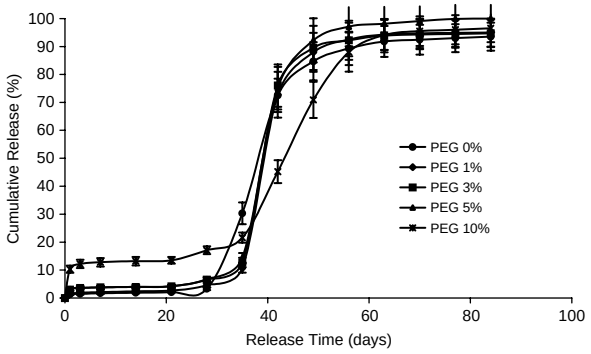

Fig. 7. Release profile of small PLGA 65:35 discs incorporated with PEG (MW 8000). Fabrication conditions of microspheres are given in Table 3. Disc diameter = 5 mm, thickness = 1 mm, compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

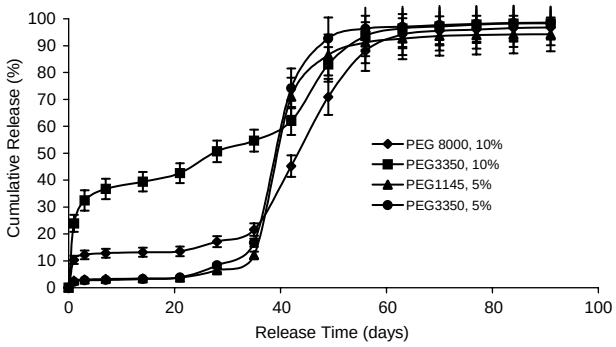

Fig. 8. Release profile of etanidazole from small PLGA 65:35 discs using different PEG types and loadings. Fabrication conditions of microspheres are given in Table 3. Disc diameter = 5 mm, thickness = 1 mm, compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

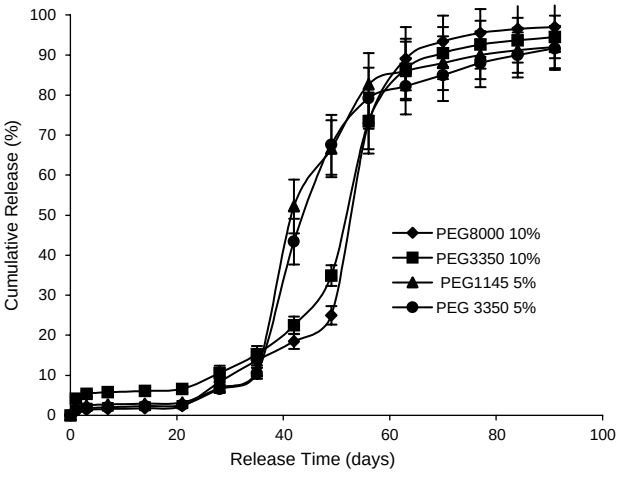

Fig. 9. Release profile of etanidazole from large PLGA 65:35 discs using different PEG types and loadings. Fabrication conditions of microspheres are given in Table 3. Disc diameter = 13 mm, thickness = 1 mm, compression pressure = 2 ton/m^2, retention time = 3 min. The figure represents geometric mean ± s.e. for all points.

samples, as commonly seen for the spray drying technique.

In order to investigate the geometrical effect of the discs and to optimize the design, discs of two different diameters are prepared by using different pellet dies. The small and large discs have a diameter of 5 and 13 mm, respectively. Since the thinner discs have a significant impact on the initial burst without improving the whole release behavior, we keep both the discs having the same thickness of 1 mm. The compression pressure used for the fabrication of discs is 2 ton/m^2 with a retention time of 3 min.

PEG (MW 8000) is first tested as the model additive with 1% to 10% PEG loading in the PLGA 65:35 system. Fig. 7 shows the release profile of etanidazole from the small discs (diameter = 5 mm). 1%, 3% and 5% PEG loadings have nearly no observable difference in the release curve. In contrast, a 10% PEG loading in the disc enhances the initial burst from 1% to more than 10%. In the meantime, the second burst is reduced and delayed by 1 or 2 weeks although the amount of drug released in the second burst is still very large.

Other types of PEG are also tested for their potential use to modulate the release behavior of the disc. Since only a higher PEG loading can have observable effect, only 5% and 10% loadings are evaluated. Figs. 8 and 9 show the release profile of the small and large discs after adding different PEG types and loadings, respectively. The molecular weight and PEG loadings in the microspheres have a significant effect on the release behavior. The addition of 10% PEG (MW 3350) has resulted in a sustained and uniform release, lasting for about 2 months or more. The only disadvantage is that the initial burst is a bit higher, nearly up to 30% for the first day. The PEG (MW 8000) 10% loading is unable to give a sustained release rate comparable to the PEG (MW 3500) 10% loading. However, its initial burst is higher than the 5% loading samples. In contrast, the corresponding second burst is reduced. This most likely results from the difference of the molecular weight of PEG. Low molecular weight PEG has a higher solubility in water; in contrast, high molecular weight PEG may not achieve the desired properties due to its limited solubility. The release behavior of 5% PEG (MW 3350) loaded disc is quite different from that of 10% PEG loading, proving that the PEG loading can make a big difference in the release characteristics. A 5% loading is not sufficient to make a uniform release. It is important that the molecular weight of PEG and its loadings should be jointly considered while developing a suitable PLGA disc.

The drug release from the disc incorporated with PEG depends very much on the type and loading of PEG. At the initial stage of release, PEG acts more as a precursor easily dissolved in the buffer solution to form channels

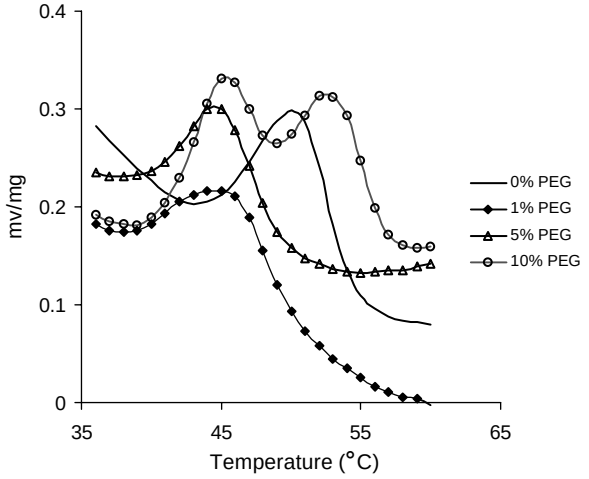

Fig. 10. DSC curves of PLGA 65:35 microspheres incorporated with different loadings of PEG (MW 8000). Fabrication conditions of microspheres are given in Table 3. Drug loading = 1%.

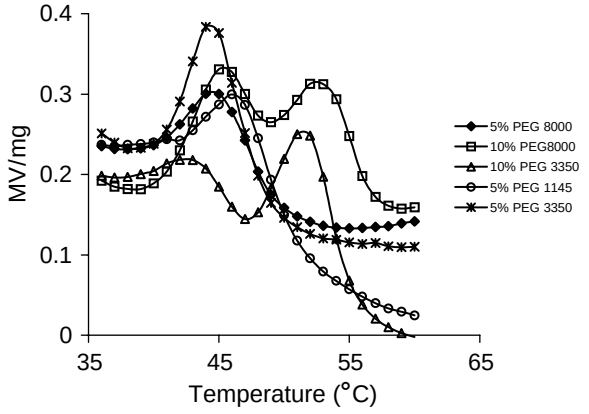

Fig. 11. DSC curves of PLGA 65:35 microspheres incorporated with different types and loadings of PEG. Fabrication conditions of microspheres are given in Table 3. Drug loading = 1%.

in the polymer matrix. Accordingly, etanidazole is released in a continuous way by diffusing through these channels. DSC data illustrated in Figs. 10 and 11 show that a 10% PEG loading produces two distinct peaks, the first peak should be attributed to the interaction among PLGA 65:35, the drug and PEG. Since some part of the PEG is still uniformly mixed with the polymer and the drug, the T_g temperature of the PLGA 65:35 is decreased; The second peak is the melting point for PEG. In contrast, a 5% or lower loading generates only one peak. The two peaks for 10% PEG loadings show that PEG and PLGA are no longer shown as one integrated phase at a higher PEG concentration. Some PEG remains as a separate phase, and thereby enhances the formation of channels in the course of PEG diffusion and dissolution. This favors a more sustained release of drugs from the polymer matrix. In the meantime, the addition of PEG can decrease the T_g temperature of the polymer, and therefore the release rate of etanidazole from the disc can also be enhanced due to the increased chain mobility.

Compared with small discs, the release of etanidazole from large discs shows a different trend. Although the initial burst of the disc increases with higher PEG loadings, the subsequent release of the drug is still very slow (until 20 days later) due to the difficulty for drug to diffuse through the larger disc. Larger discs have a poor mass transfer for etanidazole due to their reduced specific surface area, hence resulting in a small initial burst and a slow subsequent release. The second burst for the large discs appears at around 50 days after starting the release. The reason for the delayed second burst may also be due to the delay in losing the integrity of the disc. After adding a higher loading of PEG, the overall mechanical properties are changed in such a way that the discs become very soft and it is very hard for them to lose shape and break up into small pieces, ultimately leading to a lower and delayed second burst.

Fig. 12 illustrates the SEM pictures of the microspheres incorporated with different loadings of PEG (MW 8000). Microspheres have a spherical shape with pores on the surface when no PEG is used (panel A). The morphology of the microspheres is changed even if only 1% of PEG is added (panel B). They become more irregular and greatly shriveled. A 5% PEG loading also achieves a similar morphology (panel C). This morphology may be due to the softening and plasticity effect of the PEG. As shown in Fig. 10, when only 1% PEG is added into PLGA 65:35, the T_g temperature is decreased, after that, even the PEG content is increased to 10%, the T_g peak related to PLGA 65:35 polymer is hardly changed. This indicates that PEG has strong plasticity effect and can reduce the viscosity of the polymer solution. This may lead to the shriveled morphology of the microspheres. Another possible reason for this phenomenon is that PEG can stay between neighboring polymer molecules due to its small molecular weight and flexible structure. This in turn can hinder the solvent evaporation during the spray drying process, and lead to the final eruption and collapse of the microspheres. However, when a 10% PEG (MW 8000) loading is employed, there occurs a dramatic change in the shape of the microspheres (panel D). The products obtained are not spheres but appear like grains with irregular shapes. As shown in the DSC curves (Figs. 10 and 11), a 5% loading can produce only one peak, but a 10% loading of PEG (MW 8000) achieves two peaks: one for the PLGA 65:35/drug/PEG phase and the other for the separated PEG phase. Owing to the low T_g temperature of PEG and the phase separation between the polymer and PEG, particles are heavily aggregated and fused together, resulting in a slice like structure. Fig. 13 gives a series of SEM images of microspheres and discs before and after the release.

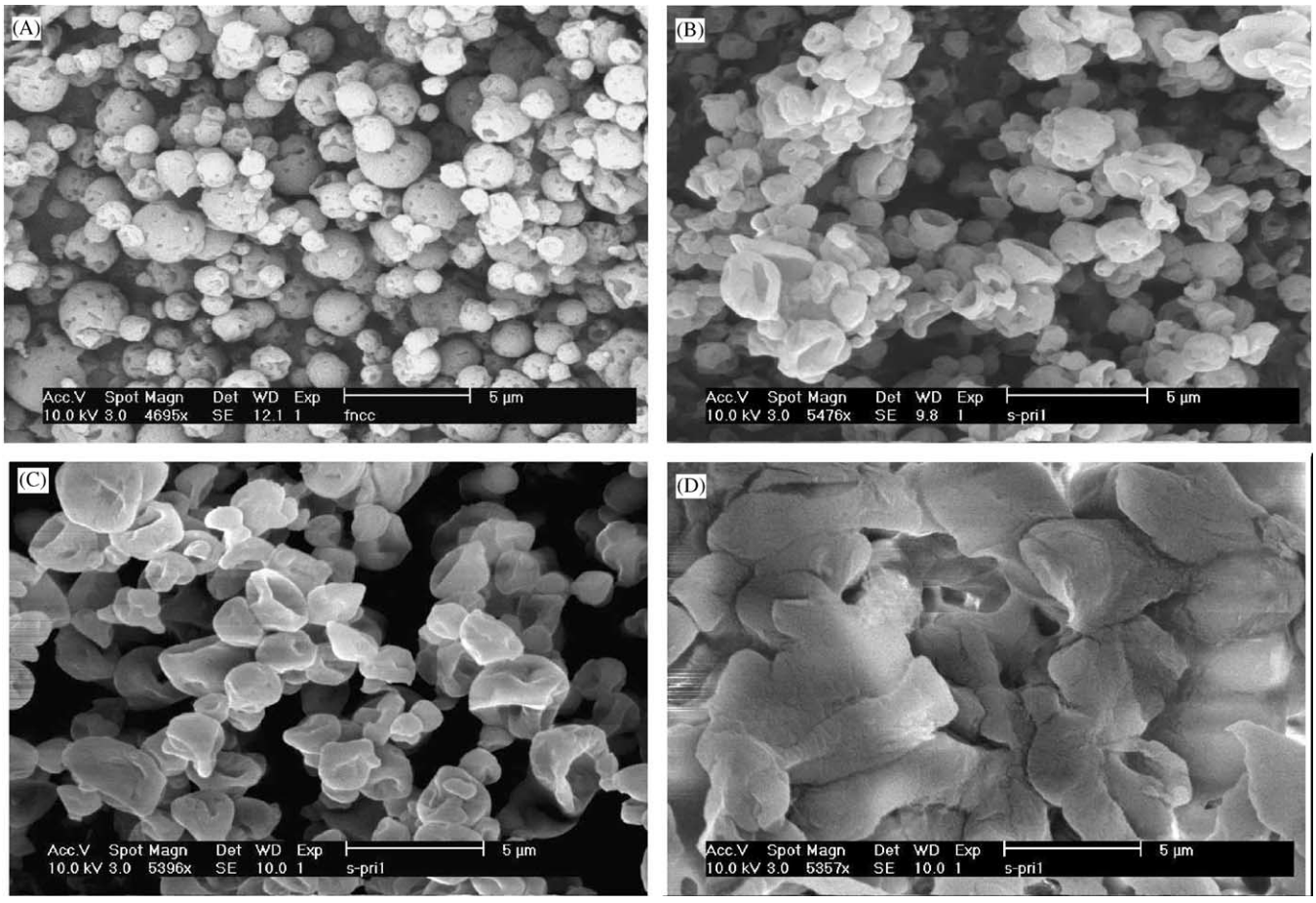

Fig. 12. Scanning electron microscopy pictures of PLGA 65:35 microspheres incorporated with different loadings of PEG (MW 8000) (A) 0% PEG, (B) 1% PEG, (C) 5% PEG and (D) 10% PEG. Fabrication conditions of microspheres are given in Table 3.

Even after being compressed by a hydraulic press, the surface of the disc remains mostly unchanged (panel B). After 2–3 weeks release, particles are hard to be identified. In addition, porous surface structure is found after 4 weeks due to the degradation of the disc. After 5 weeks, a transparent, soft and sticky film is produced due to the degradation and weight loss of the disc. At the end of 2 months, most of the disc gradually vanishes into the buffer solution.

3.7. Degradation of microspheres and discs

For the microspheres, even after 1 month's release, it is still likely to identify some particles although most of the microspheres have aggregated together and formed a dense film-like structure. After 2-month release, the polymer has not yet been dissolved into the phosphate buffer completely (data not shown). In contrast, after 1 month's release, the disc has already been turned into sticky and transparent film-like structure (Fig. 13). This comparison indicates that disc can have a faster release rate than that of microspheres. The drastic variation arises form the geometry difference. In the discs, the degradation products such as lactide and glycolide can keep up with a relatively high $[H^+]$ concentration which may catalyze the degradation process of discs. For the microspheres, due to the relatively large specific surface area, the degradation products can easily diffuse out into the aqueous medium, resulting in no marked influence on the polymer degradation rate. This result is similar with those obtained in the literature [29–31].

4. Conclusions

In this work, the release behaviors of discs compressed from spray-dried microspheres have been investigated. Due to the reduction of the specific surface area in the compressed discs, diffusion is no longer the dominant mode for the transport of etanidazole. Instead, the polymer degradation plays a key role in the release of etanidazole from discs, characterized by the second burst release followed by a very slow release rate towards the final stage. Although the drug loading and the disc thickness can have observable effect on the initial burst, no obvious effect on the release behavior of

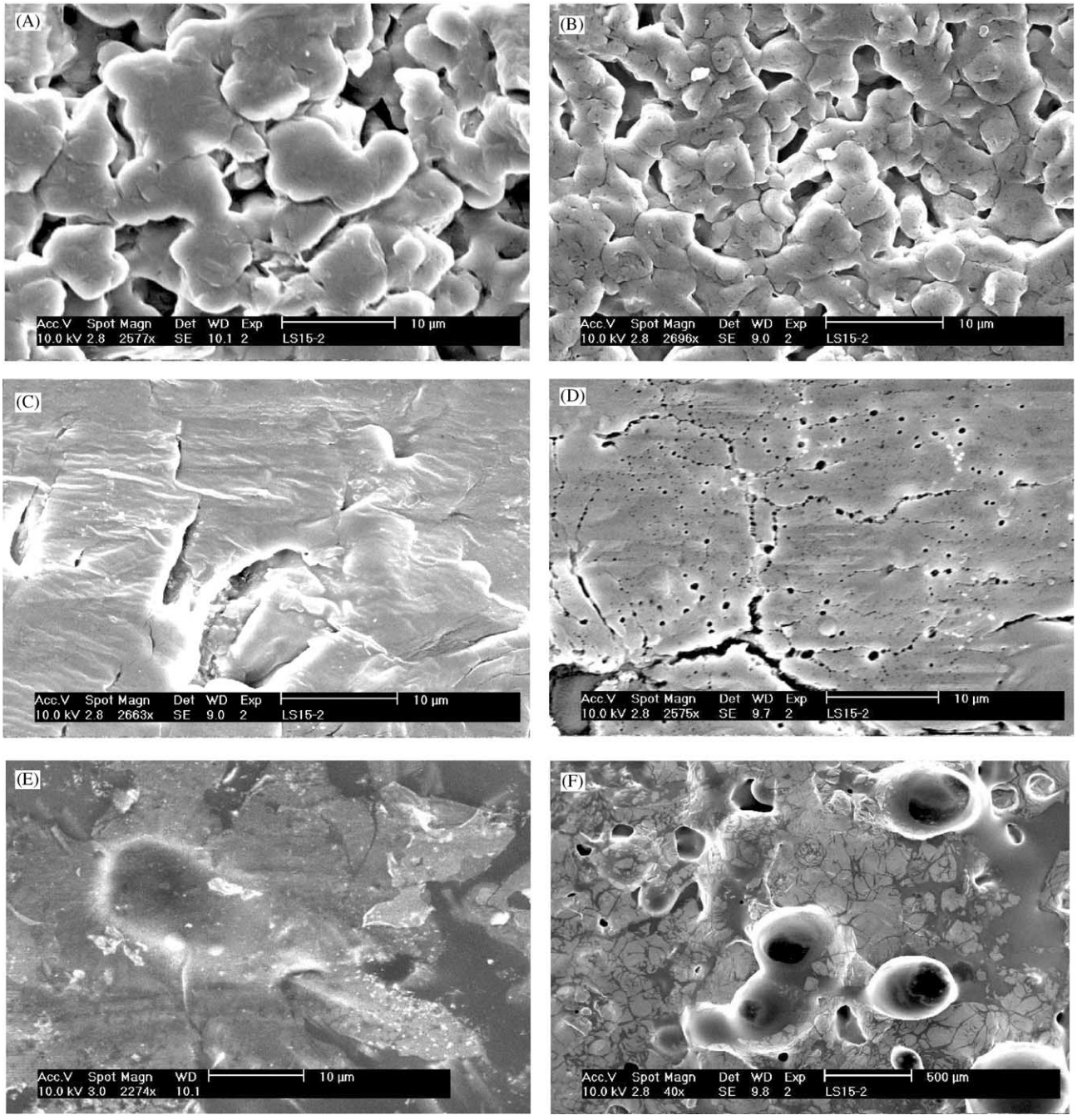

Fig. 13. Scanning electron microscopy pictures of discs fabricated by PLGA 65:35 microspheres with a 10% PEG (MW 3350) loading (A) microspheres, (B) discs before release, (C) after 2-week release, (D) after 4-week release, (E&F) after 5-week release (shown in the scales of 10 µm and 500 µm, respectively).

the discs is detected since polymer degradation is the dominant mechanism, and it depends weakly on the drug loadings and disc thickness. In general, no fabrication parameters mentioned above (drug loading, disc thickness, diameter, compression pressure) can significantly alter the release rate of the discs.

However, the addition of PEG into the microspheres can modify the release behavior of the compressed discs. PEG type, molecular weight and disc size are the three key parameters for determining the release behavior of discs. Lower PEG loading (5%) and higher molecular weight PEG (MW 8000) will have no significant effect on the release behavior of large discs. However, when smaller molecular weight PEG (MW 3350) and 10% loading are employed, a sustained release for about 2 months could be obtained with the small discs.

Although a sustained release system has been established by using PEG as the additive, the initial burst of the release is still a bit high, which may be dangerous in the clinical applications. Methods have to

be explored in order to develop a more suitable and useful system.

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