Preparation and evaluation of biodegradable films containing the potent osteogenic compound BFB0261 for localized delivery

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ABSTRACT

To achieve sustained release of 3-ethyl-4-(4-methylisoxazol-5-yl)-5-(methylthio) thiophene-2-carboxamide (BFB0261), a new potent osteogenic compound for treating bone disorders, we prepared film formulations containing BFB0261 and the following newly synthesized biodegradable polymers by a solvent casting technique: poly(D,L-lactic acid) (PLA), poly(D,L-lactic acid-*co*-glycolic acid) (PLGA), poly(D,L-lactic acid)-*block*-poly(ethylene glycol) (PLA–PEG), and poly(D,L-lactic acid-*co*-trimethylene carbonate) (PLA–TMC) polymers or copolymers. Powder X-ray diffractometry (PXRD), differential thermal analysis (DTA), scanning electron microscopy (SEM), and tensile testing were performed to examine the physicochemical properties of these films. Almost all the films exhibited a smooth and homogeneous surface, as observed by SEM. In addition, PXRD and DTA revealed that BFB0261 existed in an amorphous state in the films. The *in vitro* release of BFB0261 from PLA100 (M_w: 251 kDa), PLAPEG9604H (PLA/PEG ratio: 96:4; M_w: 181 kDa), PLAPEG8515H (PLA/PEG ratio: 85:15; M_w: 51.5 kDa), or PLAPEG8020 (PLA/PEG ratio: 80:20; M_w: 33.7 kDa) films followed zero-order kinetics with slow release up to 12 weeks following incubation. Although release of BFB0261 from PLA–TMC films followed first-order kinetics, sustained release of BFB0261 for 12 weeks was still observed for PLATMC8416 (PLA/TMC ratio: 84:16; M_w: 170 kDa) films. Furthermore, when the BFB0261-loaded films constructed from various polymers were implanted subcutaneously on rat backs, the PLAPEG8515H and PLATMC8416 films were capable of achieving sustained release of BFB0261 at the administrated site for 12 weeks. Therefore, the present data indicate that films constructed from PLAPEG8515H or PLATMC8416 may be applicable to bone or tissue engineering.

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1. Introduction

Drug delivery systems (DDSs) have been developed for the past two decades using engineered polymers that have a variety of unique properties. These polymers have various specific functions that allow for formulations such as time-controlled release (delayed release, immediate release, and pulsed release), pH sensitivity, temperature sensitivity, biomolecule sensitivity, receptor specificity, and biocompatibility (Kim et al., 2009).

Biocompatibility, an important factor for polymers, has been utilized for developing implantable controlled release systems for applications such as ocular disease treatment, contraception treatment, dental treatment, immunotherapy, anti-coagulation treatment, cancer therapy, narcotic antagonism, and insulin delivery (Langer, 1983). Among the most promising materials for implantable controlled release systems are poly-α-hydroxyacids such as poly(lactic acid) (PLA) and poly(lactic-*co*-glycolic acid) (PLGA), owing to their excellent biocompatibility and controllable biodegradability through natural pathways. In addition, poly-α-hydroxyacids can easily be processed to obtain several types of devices, namely, micro- and nano-spheres, scaffolds, and microfibers (Sokolsky-Papkov et al., 2007). Therefore, these implantable functional polymers play a key role in formulations for regenerative medicine, which aims to regenerate, replace, repair, or enhance the biological function of damaged cells or tissues.

Abbreviations: BFB0261, 3-ethyl-4-(4-methylisoxazol-5-yl)-5-(methylthio) thiophene-2-carboxamide; PLA, poly(D,L-lactic acid); PGA, poly(glycolic acid); PLGA, poly(D,L-lactic acid-*co*-glycolic acid); PLAPEG, poly(D,L-lactic acid)-*block*-poly(ethylene glycol); PLATMC, poly(D,L-lactic acid-*co*-trimethylene carbonate).
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In general, to enhance the efficacy of regenerative medicine, invariable shapes serving as templates are required. To date, the three-dimensional (3D) scaffold has attracted attention because it serves as both a temporary substrate for defect sites and a delivery carrier for controlled release of regenerative medicine. Macro porosity, pore size, and open structures of an artificially designed 3D scaffold are crucial factors to control the release of regenerative medicine. However, the difficulty of obtaining the desired macro/micro porosity and structures of the 3D scaffolds is matter of concern (Kang et al., 2005; Wei and Ma, 2008). Therefore, we focused on the formulation of a biodegradable film (Hadlock et al., 1998), which is placed around the defect site without affecting tissue regeneration. An advantage of film formulations over 3D scaffolds is that the effects of film porosity and structure do not require consideration. In addition, biodegradable film has flexibility of design because it can be freely adjusted to various shapes and sizes simply by cutting it to fit. This property enables films to control the release of medicine. Accordingly, film formulations can potentially be applied extensively in regenerative medicine, including bone regeneration.

Recently, we developed BFB0261, a novel compound that enhances bone formation. BFB0261 elevates the activities of alkaline phosphatase, an index of bone differentiation, in a concentration-dependent manner in human osteoblastic cells. Moreover, treatment with BFB0261 also increased cell calcification (Harada et al., 2000). More recent study has demonstrated that using new synthetic biodegradable polymers has achieved sustained release of BFB0261 from microspheres (Umeki et al., 2010). In the present study, we synthesized several new synthetic biodegradable polymers, namely, one PLA polymer (PLA100), two PLA–PGA (PLGA) copolymers of various molecular weights with various PLA/PGA molar ratios (PLGA7723 and PLGA6139), five PLA–poly(ethylene glycol) (PEG) block copolymers of various molecular weights with various PLA/PEG molar ratios (PLAPEG9604H, PLAPEG8515H, PLAPEG8119, PLAPEG7525, and PLAPEG6337), and three PLA–trimethylene carbonate (TMC) copolymers of various molecular weights with various PLA/TMC molar ratios (PLATMC8416, PLATMC6436, and PLATMC5248). We also prepared film formulations containing the various polymers with BFB0261 as an active ingredient, to achieve three-month sustained release. Surface morphology of the films was assessed by scanning electron microscopy (SEM). Physicochemical properties such as crystalline form and tensile strength were measured by differential thermal analysis (DTA), powder X-ray diffractometry (PXRD), and rheometry. We then determined *in vitro* and *in vivo* release patterns of BFB0261 from the films constructed from the various polymers. Finally, the effects of the various polymers on the release performance of the films were also evaluated.

2. Materials and methods

2.1. Materials

New synthetic polymers such as poly(D,L-lactic acid) (PLA) polymer: PLA100 (weight-average molecular weight (M_w): 251 kDa); poly(D,L-lactic acid-*co*-glycolic acid) (PLGA) polymers: PLGA7723 (PLA/PGA ratio: 77:23; M_w: 217 kDa), and PLGA6139 (PLA/PGA ratio: 61:39; M_w: 128 kDa); and poly(D,L-lactic acid)-*block*-poly(ethylene glycol) (PLAPEG) polymers: PLAPEG9604H (PLA/PEG ratio: 96:4; M_w: 181 kDa), PLAPEG8515H (PLA/PEG ratio: 85:15; M_w: 51.1 kDa), PLAPEG8020 (PLA/PEG ratio: 80:20; M_w: 33.7 kDa), PLAPEG7525 (PLA/PEG ratio: 75:25; M_w: 25.5 kDa), and PLAPEG6337 (PLA/PEG ratio: 63:37; M_w: 17.3 kDa); poly(D,L-lactic acid-*co*-trimethylene carbonate) (PLATMC) polymers: PLATMC8416 (PLA/TMC ratio: 84:16; M_w: 170 kDa), PLATMC6436 (PLA/TMC ratio: 64:36; M_w: 220 kDa), and PLATMC5248 (PLA/TMC ratio: 52:48; M_w: 220 kDa) we designed and ordered were supplied by Taki Chemical Co. Ltd. (Hyogo, Japan). The molecular characteristics of the polymers are summarized in Table 1.

BFB0261 was synthesized at Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co. Ltd. (Saitama, Japan) (Harada et al., 2000). BFB0261 is a crystalline neutral compound with pH-independent solubility, and the solubility is found to be 100 μg/mL at all pH conditions. In addition, the melting point of BFB0261 is found to be 93.5–94.5 °C (Harada et al., 2000). PLA0020 (M_w: 19.9 kDa) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan), and alpha-minimum essential medium (α-MEM) was acquired from Irvine Scientific (CA, USA). All reagents were of the highest grade available from commercial sources.

2.2. Preparation of biodegradable film

The biodegradable films constructed from various PLA, PLGA, PLAPEG, or PLATMC polymers in the presence or absence of BFB0261 were prepared by a casting method (Perugini et al., 2003). Briefly, 12.8 mg of BFB0261 and 1011.2 mg of each polymer were dissolved in 10 mL of trichloromethane (CHCl$_3$) and the solution was degassed by ultrasonication. The solution was poured into a Teflon dish (diameter: 90 mm), dried at room temperature for 2 days in air, and then placed under vacuum for 3 days. Finally, the biodegradable films were peeled off the Teflon dish and stored at 5 °C until used. In addition, the BFB0261 films were sterilized

Table 1
Polymer composition and physical properties.

Polymer	Polymer molar ratio (%)				M_w[a] (kDa)	M_n[b] (kDa)	M_w/M_n
	PLA	PGA	PEG	TMC			
PLA0020	100	–	–	–	19.9	11.1	1.81
PLA100	100	–	–	–	251	150	1.67
PLGA7723	77	23	–	–	217	127	1.71
PLGA6139	61	39	–	–	128	72	1.78
PLAPEG9604H	96	–	4	–	181	129	1.4
PLAPEG8515H	85	–	15	–	51.1	36.8	1.39
PLAPEG8020	80	–	20	–	33.7	27.2	1.24
PLAPEG7525	75	–	25	–	25.5	20.9	1.22
PLAPEG6337	63	–	37	–	17.3	15	1.15
PLATMC8416	84	–	–	16	170	110	1.55
PLATMC6436	64	–	–	36	220	140	1.57
PLATMC5248	52	–	–	48	220	130	1.69

[a] M_w, weight-average molecular weight.
[b] M_n, number-average molecular weight.

with a gamma-ray burst (10 kGy) before being used *in vitro* or *in vivo*.

2.3. Evaluation of the physicochemical properties of the BFB0261-containing films

2.3.1. Scanning electron microscopy (SEM)

The surface morphology of the films was assessed by SEM (S-2500, Hitachi, Tokyo, Japan). Samples were placed on double-sided adhesive tape, which had previously been applied to an aluminum stub, then sputter coated with platinum/palladium under argon gas prior to imaging.

2.3.2. Powder X-ray diffractometry (PXRD)

PXRD was performed on a RINT 2200 Ultima/PC system (Rigaku, Tokyo, Japan). The films or powder were placed on the glass sample folder, and measurements were performed using a copper target at 40 mA with a scanning speed of $10°/min$ and a 2θ range of $3–40°$.

2.3.3. Differential thermal analysis (DTA)

Thermal behavior of the BFB0261, BFB0261-loaded PLAPEG film, PLAPEG film, and PLAPEG polymer was investigated by DTA (TG8120, Rigaku, Tokyo, Japan) under nitrogen at a flow rate of 20 mL/min. The samples were heated from 30 to 300 °C at a rate of 3 °C/min.

2.3.4. Tensile testing

Thickness of the films was measured with a micrometer (MDQ-30M, Mitutoyo, Tokyo, Japan). Strips of BFB0261-loaded or non-loaded film measuring 10 mm × 40 mm were prepared for tensile testing. Measurements were performed on an Eztest rheometer (Shimadzu, Kyoto, Japan) with a crosshead speed of 10 mm/min. The initial gauge length was 10 mm. The following equations were used to calculate the tensile strength (TS), elongation at break (EB), and elastic modulus (EM) of the films:

$$TS(MPa) = \frac{Force\ at\ break(N)}{Initial\ cross\ section(mm^2)} \times \frac{1}{M_w}$$

$$EB(\%/mm^2) = \frac{Increase\ in\ length(mm)}{Original\ length(mm)}$$
$$\times \frac{1}{Cross\ sectional\ area(mm^2)} \times \frac{1}{M_w}$$

$$EM(MPa) = \frac{Force\ at\ corresponding\ strain(N)}{Initial\ cross\ section(mm^2)}$$
$$\times \frac{1}{Corresponding\ strain(N)} \times \frac{1}{M_w}$$

$$Strain = \frac{TS}{EM}$$

2.3.5. Determination of BFB0261 concentration in the films

The concentration of BFB0261 in the films was determined on an HPLC system (Hitachi, Tokyo, Japan) consisting of a pump and a UV detector set at 253 nm. The analytical column was a reversed-phase C18 column (CAPCELL PAK C18 UG250, 5 μm, 4.6 mm × 150 mm, Shiseido, Tokyo, Japan), and column temperature was maintained at 40 °C. The mobile phase was a mixture of acetonitrile and 0.01 M ammonium acetate (70:30, v/v), and the flow rate was 1.0 mL/min. Next, 10 mg of film was dissolved by ultrasonication in 5 mL of acetonitrile for 10 min and the resulting solution was centrifuged for 10 min at 15,000 rpm. Next, 10-μL aliquots of the supernatant were injected into the HPLC system and the concentration was determined from a calibration curve.

2.4. In vitro drug release study

In vitro BFB0261 release studies were performed using a 12-well chamber without agitation. BFB0261 films (8 mg; 5 mm × 5 mm) were placed in the well chamber, and 1000 μL of α-MEM containing 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES, pH 7.0) buffer was added and incubated at 37 ± 1 °C. Following incubation for the indicated durations, the well chamber was centrifuged at 3000 rpm for 10 min and the supernatant was collected as a test sample. Fresh release medium (1000 μL) was added to the well chamber to continue the study. Next, a 500-μL aliquot of the supernatant was centrifuged at 15,000 rpm for 10 min. Thereafter, a 40-μL aliquot of the supernatant was added and mixed with 160 μL of acetonitrile. The mixture was centrifuged at 15,000 rpm for 10 min. The concentration of BFB0261 in the final supernatant was measured on the HPLC system, as noted above (Section 2.3.5). All *in vitro* release tests were performed in triplicate.

2.5. In vivo drug release studies

2.5.1. Animals

Six-week-old female Wister rats were purchased from Charles River Japan Inc. (Atsugi, Japan). The animals were maintained under conventional housing (23 ± 3 °C; 50 ± 20% relative humidity) and lighting (lights on: 7:00–19:00) conditions, and were used after at least 5 days of acclimation. All animal experiments were reviewed and approved by the Taisho Pharmaceutical Animal Care Committee and conformed to the Japanese Experimental Animal Research Association Standards defined in the Guidelines for Animal Experiments (1987).

2.5.2. Experimental methods

Release of BFB0261 from the films *in vivo* was evaluated by determining the residual concentration of BFB0261 under the skin. Films constructed from PLGA7723, PLAPEG8515H, and PLATMC8416 (10 mm × 25 mm) were implanted (under anesthesia) beneath the dorsal dermal layer of rats. At the indicated times, the rats were sacrificed with a lethal dose of ether. The remaining films were resected from the implanted site and frozen. The films were soaked and dissolved in 5 mL of acetonitrile then sonicated for 10 min. Thereafter, the dissolved solutions were centrifuged at 15,000 rpm for 10 min. The amount of BFB0261 in the supernatant was measured on the HPLC system as noted above (Section 2.3.5). All *in vivo* release tests were performed four times.

2.6. Statistics analysis

Statistical analysis was performed using the Student's *t*-test. A probability value of *P* < 0.05 was considered to indicate statistical significance.

3. Results and discussion

3.1. Preparation of BFB0261 films constructed from various synthetic polymers and evaluation of their physicochemical properties

The composition and physical properties of the various types of PLA, PLGA, PLA–PEG, and PLA–TMC polymers used in this study are listed in Table 1. Most of the preparations, with the exception of PLA0020 (M_w: 20 kDa), formed films with an approximately uniform thickness ranging from 100 to 130 μm (Table 2) and appeared

Table 2
Mechanical properties of various polymers and BFB0261 films (mean ± SD; n = 3).

Name	Film thickness (μm)	Tensile strength[a] ($\times 10^{-5}$ MPa)	Elastic modulus[a] ($\times 10^{-7}$ MPa)	Elongation at break[a] ($\times 10^{-2}$%/mm^2)
PLA100	114.0 ± 5.8	2.22 ± 0.16	2.26 ± 0.06	0.29 ± 0.03
PLGA7723	111.7 ± 7.1	3.14 ± 0.09	3.72 ± 0.41	0.34 ± 0.02
PLGA6139	106.2 ± 6.0	3.95 ± 0.37	4.36 ± 0.49	0.61 ± 0.02
PLAPEG9604H	132.7 ± 11.2	2.79 ± 0.19	2.73 ± 0.28	0.25 ± 0.01
PLAPEG8515H	126.4 ± 5.7	14.81 ± 0.57	19.41 ± 1.40	0.70 ± 0.05
PLAPEG8020	100.3 ± 0.2	19.90 ± 0.30	22.04 ± 4.16	1.64 ± 0.21
PLATMC8416	100.8 ± 1.3	3.96 ± 0.08	3.79 ± 0.13	0.54 ± 0.01
PLAPEG8515H[b]	100.0 ± 6.7	24.94 ± 1.28	31.74 ± 3.62	0.95 ± 0.05

[a] Values divided by M_w.
[b] Non-loaded BFB0261 film.

visually transparent. On the other hand, the PLA0020 preparation did not form a film and remained in a powdered state. When the molecular weight of the PLA was greater than 250 kDa as in the case of PLA100, a uniform film could be obtained. This finding suggests that careful consideration of the molecular weight of PLA polymer is required for preparing uniform films.

Fig. 1 shows the SEM images of PLA100, PLGA7723 (PLA/PGA ratio: 77:23), and PLGA6139 (PLA/PGA ratio: 61:39) films containing BFB0261. These films had a smooth surface devoid of crystals and cracks. However, in the right-hand side of each SEM image (magnification: 10,000×), morphological changes at the surface can be seen in these formulations. In particular, the PLA100 film has slight surface asperity (Fig. 1a), whereas PLGA6139 film has an undulated surface which might be formed by fused microasperity (Fig. 1c). Therefore, we determined that the smoothness of a film's surface correlated with an increase in the glycolic acid (GA) ratio of the polymers.

Figs. 2 and 3 show SEM images of films consisting of PLAPEG and PLATMC polymers, respectively. These films also have a smooth surface, devoid of crystals or cracks. However, at high magnification (10,000×), the PLAPEG9604H film exhibits a few surface asperities similar to those of PLA100 (Fig. 2a). PLAPEG9604H consisted of 96 mol% PLA and 4 mol% PEG suggesting that the similarity was probably due to the high proportion of PLA. In addition, it was found that an increase in the proportion of PEG or TMC resulted in a smoother film surface (Figs. 2 and 3). These results suggest that film smoothness can be attained by using a high ratio of PEG and TMC in the polymer. In other words, copolymers or block polymers such as PEG and TMC could be used to prepare films with a smoother surface than those constructed from PLA.

As measured by PXRD, the diffraction patterns of BFB0261, PLAPEG8515H, and PLAPEG8515H films containing BFB0261 are shown in Fig. 4. The diffraction pattern of BFB0261 exhibits many diffraction peaks, revealing its crystalline state (Fig. 4a). Whereas the diffraction patterns of both PLAPEG8515H and PLAPEG8515H films containing BFB0261 featured halos (Fig. 4b and c). The BFB0261 content in the PLAPEG8515H films was only 1.25%; therefore, the PXRD peaks of BFB0261 were possibly not detected because of its low content. However, BFB0261-loaded PLAPEG8515H films appeared visually transparent and crystal formation was not observed by SEM (Fig. 2b). This suggests that BFB0261 was transformed from a crystalline state to an amorphous state during film preparation.

Fig. 5 shows the DTA thermograms of BFB0261 (at content levels of 9.726 mg and 0.173 mg), and PLAPEG8515H and PLAPEG8515H films containing 0.173 mg BFB0261. The DTA thermogram of BFB0261 (9.726 mg) shows an endothermic peak at around 95 °C, derived from its fusion (Fig. 5a). In addition, PLAPEG8515H films show a broad endothermic peak at around 280 °C as a result of polymer degradation (Fig. 5b). On the other hand, although PLAPEG8515H films containing BFB0261 exhibit no peak even when the temperature is increased to 300 °C (Fig. 5c), the thermograms of 0.173 mg of free BFB0261 (i.e., not in a film), which is an

amount equivalent to the BFB0261 contained in each film, show an endothermic peak at around 95 °C (Fig. 5d). The results from PXRD and DTA, respectively, confirm the amorphous state of BFB0261 in the film.

3.2. Mechanical properties of BFB0261 films constructed from various synthetic polymers

The mechanical properties of various types of polymer films containing BFB0261 as well as PLAPEG8515H film without BFB0261 are listed in Table 2. As indicators of mechanical properties, the following parameters were examined: tensile strength, elastic modulus, and elongation at break. To reveal the effects of polymer composition on these parameters, each parameter was divided by the molecular weight of the polymer. An increase in the molecular ratio of GA in PLGA gradually increased tensile strength, which was 2.22×10^{-5}, 3.14×10^{-5}, and 3.95×10^{-5} MPa for PLA, PLGA7723, and PLGA6139, respectively. Similarly, an increase in the molecular ratio of PEG resulted in a clear increase in tensile strength, which was 2.79×10^{-5} MPa, 14.8×10^{-5} MPa, and 19.0×10^{-5} MPa for PLAPEG9604H, PLAPEG8515H, and PLAPEG8020, respectively. In PLATMC polymers, the tensile strength was gradually increased in comparison with that of PLA, and was found to be 3.96×10^{-5} MPa. In addition, the elastic modulus and elongation at break exhibited the same trend as the tensile strength. Therefore, by using PLGA, PLAPEG, and PLATMC polymers, films could be prepared that were tougher and more flexible than those prepared with PLA polymers. In particular, a formulation using PLAPEG8020 exhibited the strongest and the most flexible properties with tensile strength, elastic modulus and elongation at break values of 19.0×10^{-5} MPa, 22.0×10^{-7} MPa and 0.016%, respectively.

The mechanical properties of BFB0261-free PLAPEG8515H films were also compared with BFB0261-loaded PLAPEG8515H films. Although slight differences in elongation at break between these films were observed (0.95×10^{-2}%/mm^2 vs. $0.70\% \times 10^{-2}$%/mm^2), the tensile strength and tensile modulus significantly decreased to 24.9×10^{-5} MPa and 31.7×10^{-7} MPa for PLAPEG8515H films without BFB0261 vs. 14.8×10^{-5} MPa and 19.4×10^{-7} MPa for BFB0261-loaded PLAPEG8515H films, respectively. It has been reported that drugs may act as the plasticizer to increase the flexibility of films (Peh and Wong, 1999; Dhanikula and Panchagnula, 2004; El-Kamel et al., 2007; Dong et al., 2008). However, our results indicate that BFB0261 did not increase the flexibility of the films. This phenomenon could be explained by many factors, such as the differences in polymer composition or film composition. We speculate that the dispersal of BFB0261 molecules in the polymer networks resulted in a reduction of the mechanical strength and flexibility of the films.
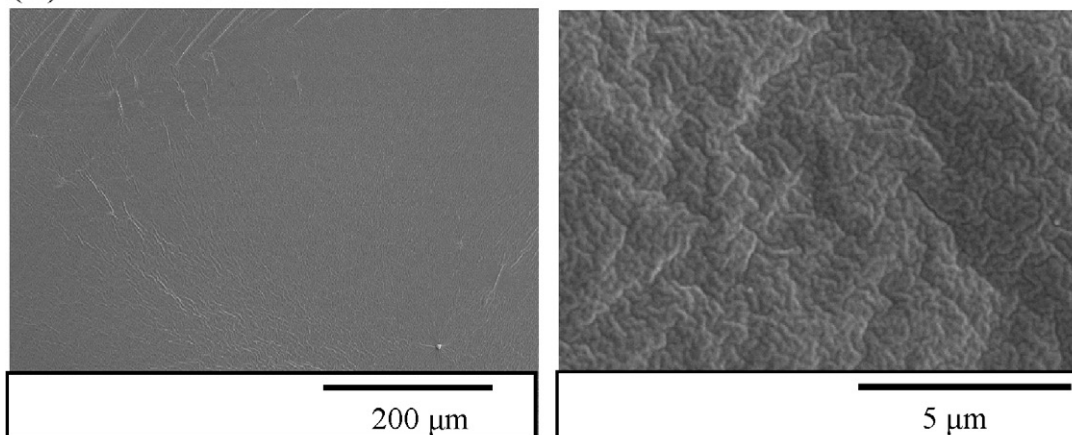
3.3. In vitro release of BFB0261 from the films

Fig. 6 shows the *in vitro* release of BFB0261 from films constructed from PLA100 or PLGA polymers. Release of BFB0261 from
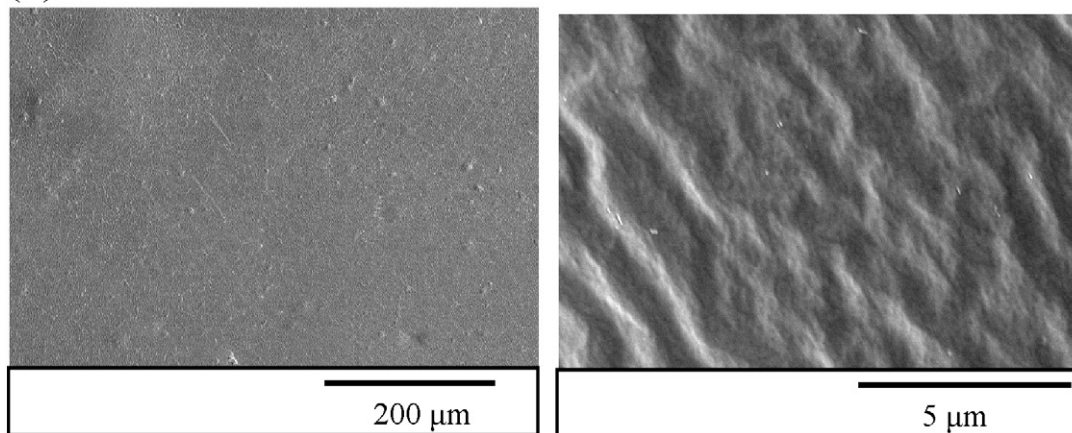
## (a) PLA100
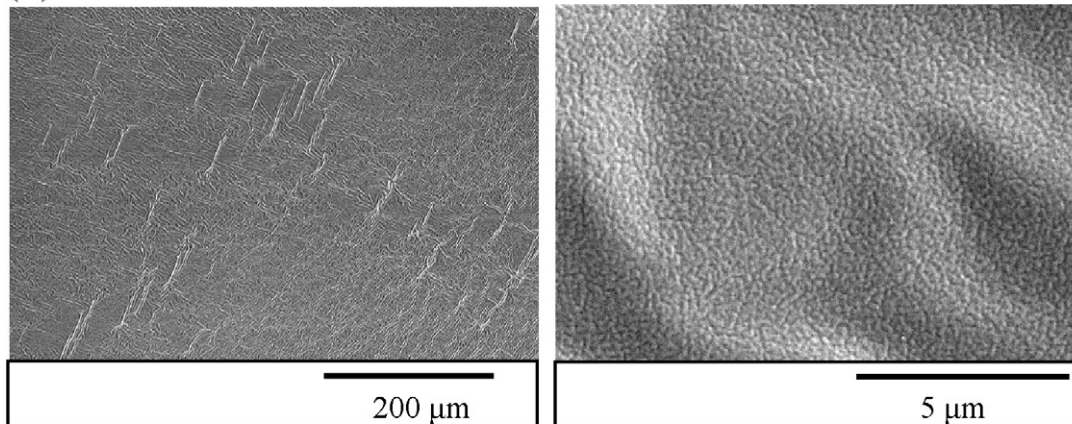
(b) PLGA7723

(c) PLGA6139

Fig. 1. Scanning electron microscope photographs of BFB0261 films consisting of PLA and PLGA polymers. (a) PLA100, (b) PLGA7723 (molar ratio: 77:23), (c) PLGA6139 (molar ratio: 61:39). Magnification: 200× (left), 10,000× (right).

PLA100 films followed zero-order kinetics with approximately 87% of the residual drug remaining in the PLA film after 12 weeks. On the other hand, the release pattern of PLGA7723 and PLGA6139 films exhibited sigmoidal kinetics and two phases of release were observed. As the molar proportion of GA decreased, the initial release of BFB0261 was more suppressed. The release rate of BFB0261 from PLGA7723 film changed after 8 weeks by which time only about 20% of BFB0261 had been released, while the residual of about 80% of BFB0261 was completely released from the PLGA7723

film by 12 weeks. The release rate of BFB0261 from PLGA6139 increased at 3 weeks, and the residual of about 70% of BFB0261 was completely released from PLGA6139 film at 7 weeks. To date, it has been reported that acidic by-products of the hydrolysis of PLGA polymers accumulate in the interior or vicinity of the films and subsequently promote its degradation (Fu et al., 2000; Houchin et al., 2007; Ding and Schwendeman, 2008). Therefore, we speculate that even if the molecular weight or molar proportion of PLGA in new synthetic polymers is altered, their decomposition would
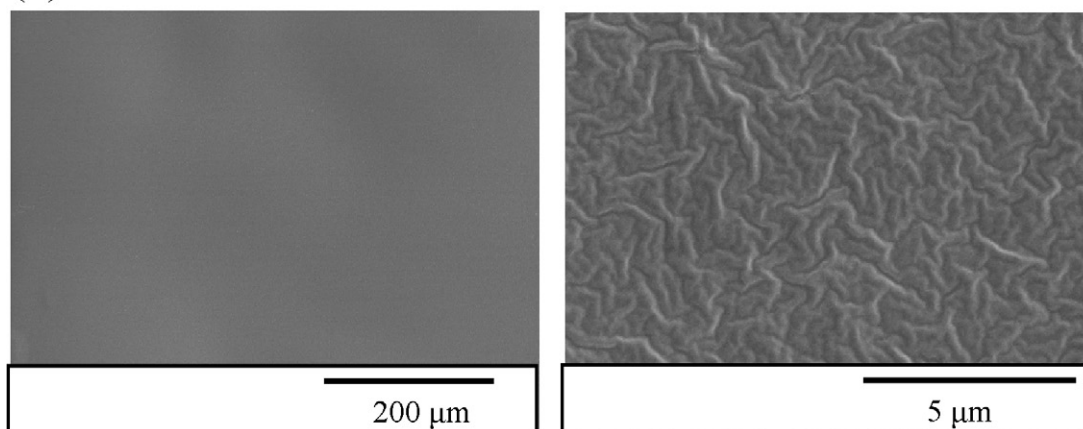
(a) PLAPEG9604H
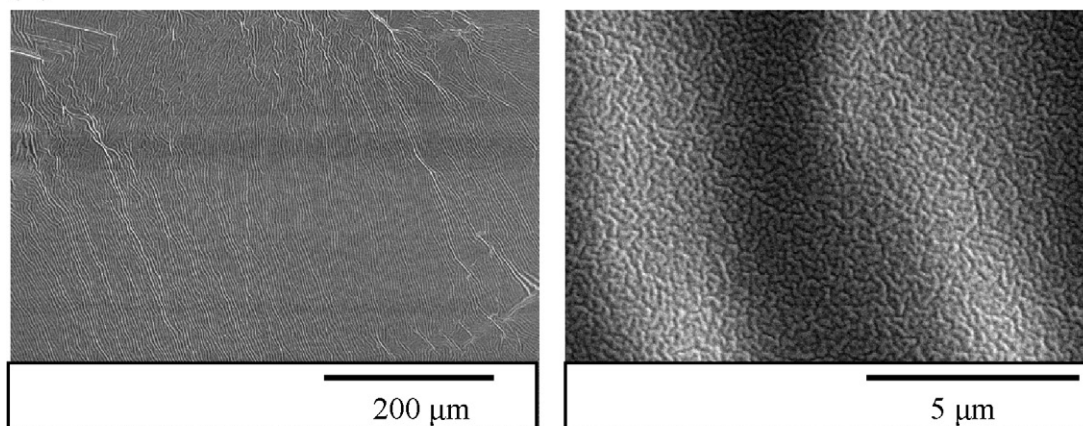
(b) PLAPEG8515H
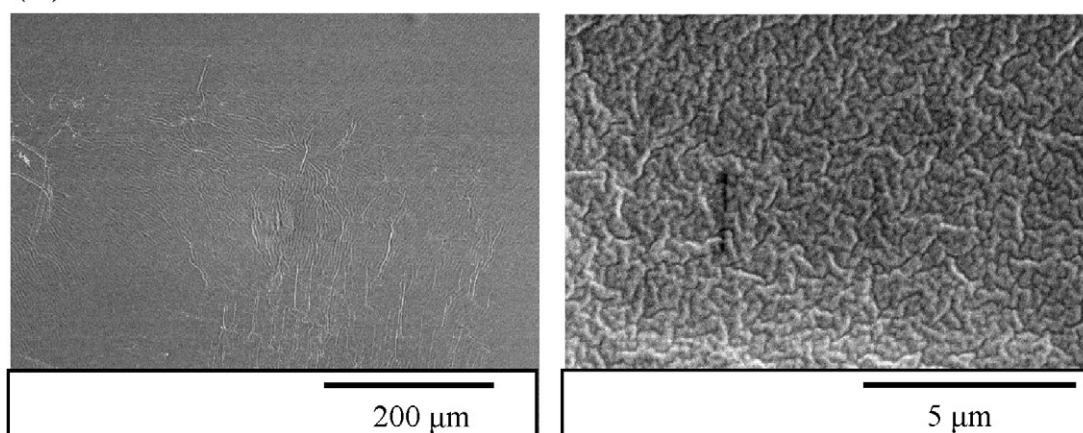
(c) PLAPEG8020

Fig. 2. Scanning electron microscope photographs of BFB0261 films consisting of PLAPEG polymers. (a) PLAPEG9604H (molar ratio: 96:4), (b) PLAPEG8515H (molar ratio: 85:15), (c) PLAPEG8020 (molar ratio: 80:20). Magnification: 200× (left), 10,000× (right).

be promoted by the acidic by-products in the interior of the films, which would consequently result in a change in the release rate of BFB0261.

Fig. 7 shows the *in vitro* release of BFB0261 from films consisting of PLAPEG polymers. A sustained release of BFB0261 for 12 weeks was observed for PLAPEG9604H, PLAPEG8515H, and PLAPEG8020 films. The proportion of the residual drug remaining in the PLAPEG9604H films at 12 weeks was approximately 95%. The release of BFB0261 from PLAPEG8515H and PLAPEG8020 films fol-

lowed zero-order kinetics, and the proportion of the residual drug remaining in the films at 12 weeks was approximately 20% and 5%, respectively. However, BFB0261 was rapidly released (more than 80% in a week) from the PLAPEG7525 and PLAPEG6040 films.

Fig. 8 shows the *in vitro* release of BFB0261 from PLATMC films. The proportion of the residual drug remaining in the PLATMC8416 films at 12 weeks was approximately 30%. However, BFB0261 was rapidly released (more than 60% in a week) from the PLATMC6436 and PLATMC5248 films. Taken together, the release of BFB0261
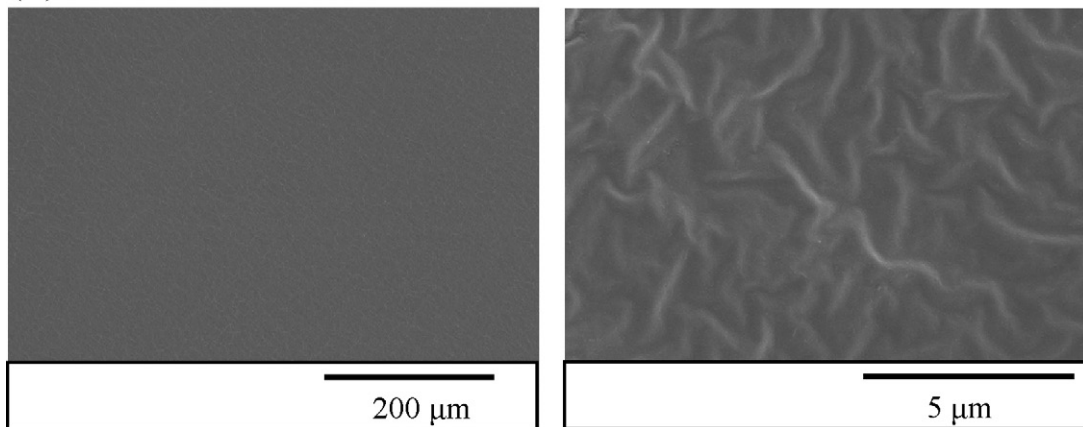
(a) PLATMC8416
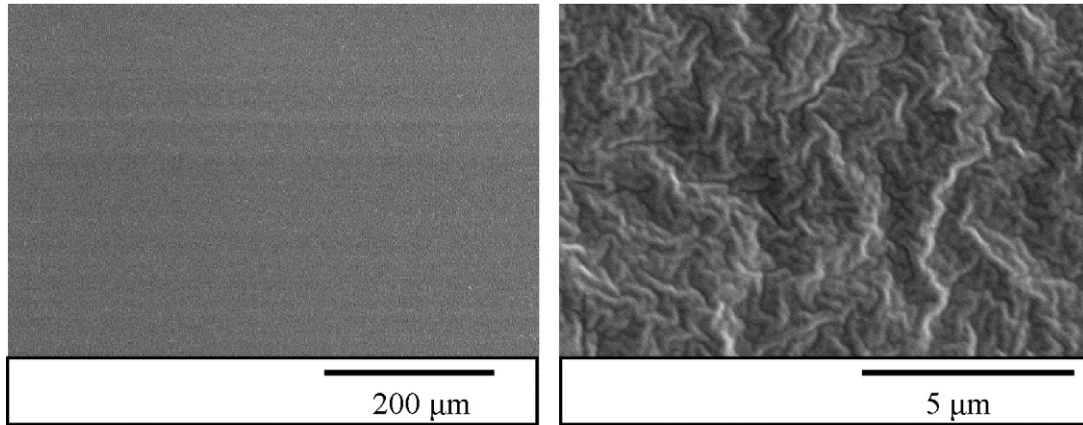
## (b) PLATMC6436
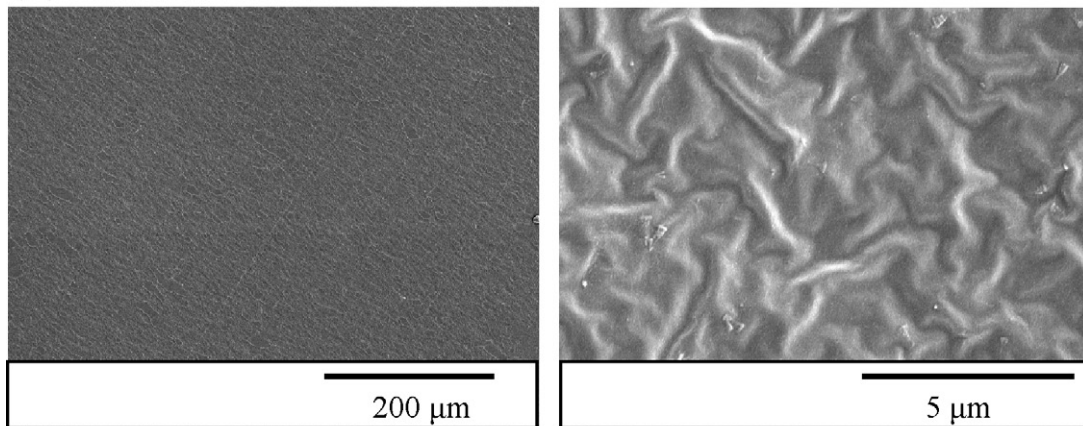
(c) PLATMC5248

Fig. 3. Scanning electron microscope photographs of BFB0261 films consisting of PLATMC polymers. (a) PLATMC8416 (molar ratio: 84:16), (b) PLATMC6436 (molar ratio: 64:36), (c) PLATMC5248 (molar ratio: 52:48). Magnification: 200× (left), 10,000× (right).

from PLATMC films follows first-order kinetics and an increase of the molar proportion of TMC results in an increase in the BFB0261 release rate.

3.4. In vivo BFB0261 release properties of the films

Fig. 9 shows the *in vivo* BFB0261 release patterns following subcutaneous implantation of BFB0261 films constructed from various polymers such as PLGA7723, PLAPEG8515H, and PLATMC8416 on rat backs. The films consisting of PLGA7723, a PLGA deriva-

tive, completely released BFB0261 within 10 weeks. Although the *in vitro* release rate of PLGA7723 films increased after 8 weeks (Fig. 6), it was determined that PLGA7723 cannot fully control sustained *in vivo* release of BFB0261. However, for the films constructed from PLAPEG8515H and PLATMC8416, slow release of BFB0261 was observed, and the portion of the residual drug remaining in both PLAPEG and PLATMC films at 12 weeks was shown to be approximately 10%. The proportion of the residual BFB0261 remaining in the films at 6 weeks was shown to be approximately 70% and 50% for PLAPEG8515H and PLATMC8416, respectively. In
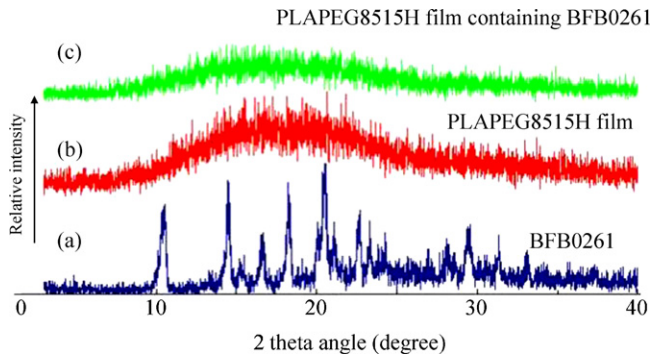
Fig. 4. Powder X-ray diffraction patterns of (a) BFB0261, (b) PLAPEG8515H film, and (c) PLAPEG8515H with BFB0261 film.
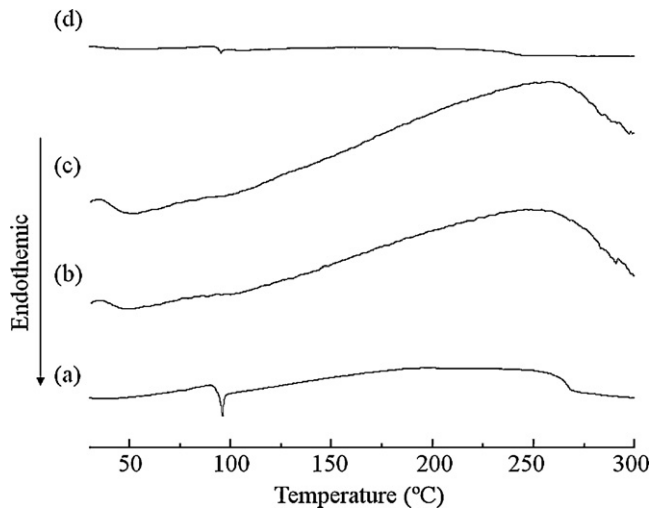

Fig. 5. DTA thermograms of (a) BFB0261, (b) PLAPEG8515H film, (c) PLAPEG8515H with BFB0261 film, and (d) an equivalent amount of BFB0261 in film.

the *in vitro* experiment (Figs. 7 and 8), the release of BFB0261 from PLAPEG8515H film followed zero-order kinetics and approximately 70% of the drug remained in the films at 6 weeks. The PLATMC8416 film showed a first-order release and approximately 40% of the drug remained in the films at 6 weeks. Therefore, the *in vitro*
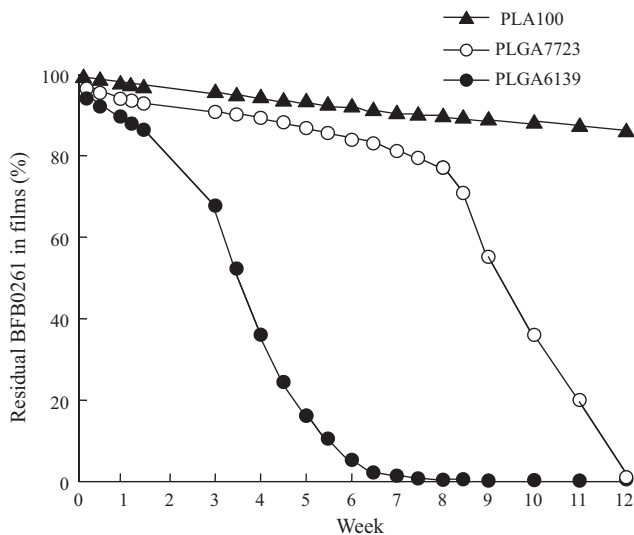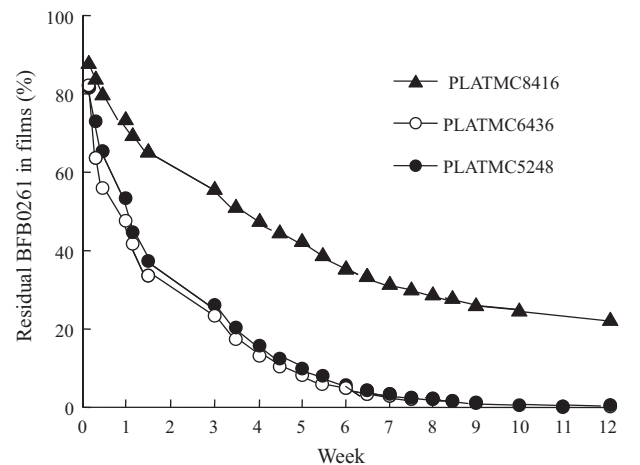

Fig. 6. Effect of PLA and PLGA polymer compositions on the *in vitro* release of BFB0261 from PLA and PLGA films. Each point represents mean ± SD (*n* = 3).
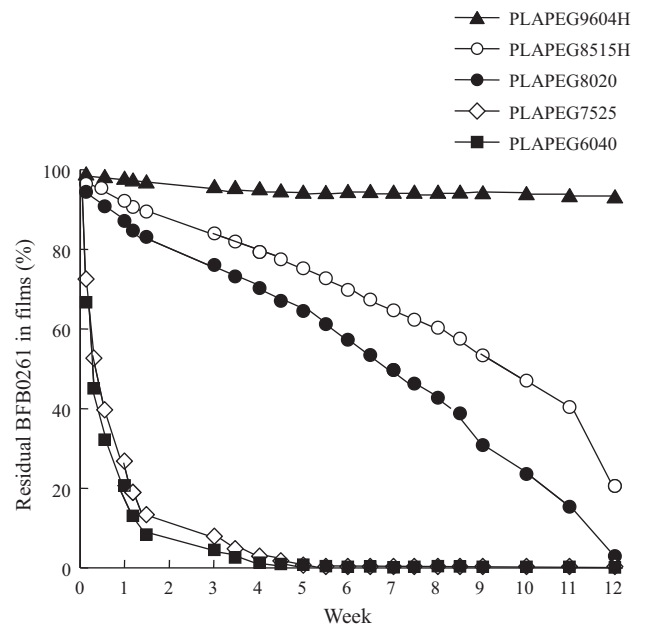
Fig. 7. Effect of PLAPEG polymer composition on *in vitro* release of BFB0261 from PLAPEG films. Each point represents mean ± SD (*n* = 3).

results reflect the results of the *in vivo* experiments. Unfortunately, although we did not measure the release behavior of BFB0261 from the PLA100, PLAPEG9604H, and PLAPEG8020 films, we speculate that these polymers could also achieve sustained release of BFB0261 similar to that of PLAPEG8515H and PLATMC8416 films.

There are two possible driving forces behind BFB0261 release from films. The first is the regulation of diffusion by new synthetic polymers, and the second involves the concentration gradient between the films and the outer phase (Batycky et al., 1997; Zhou et al., 2007; Siepmann and Siepmann, 2008; Fredenberg et al., 2009). However, it remains unclear which driving force was involved in the slow drug releases from films in the present study. Therefore, we examined the effect of varying the drug load in the films to clarify the concentration gradient between the films and the outer phase. Fig. 10 shows the *in vivo* release of BFB0261 following subcutaneous implantation of 0.2 mg, 1 mg, or 5 mg BFB0261 per 80 mg PLAPEG8515H film (dimensions: 10 mm × 25 mm) on rat backs. All BFB0261-containing films regardless of concentration or drug content showed similar release patterns. Therefore, we spec-


Fig. 8. Effect of PLATMC polymer composition on the *in vitro* release of BFB0261 from PLATMC films. Each point represents mean ± SD (*n* = 3).
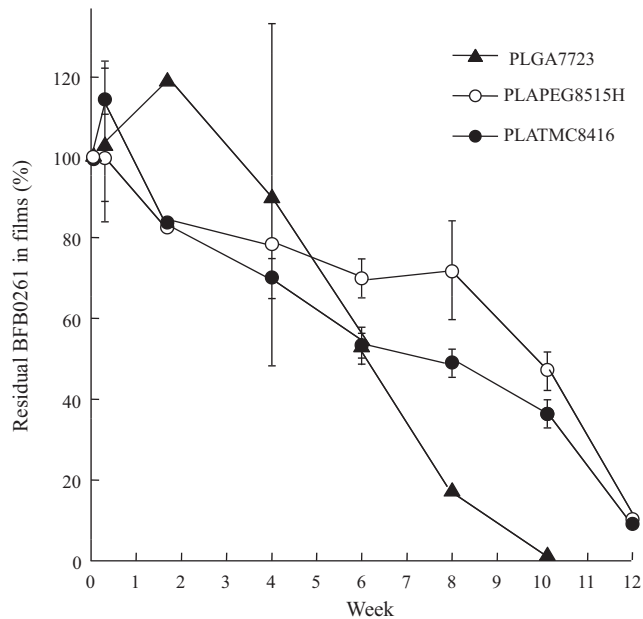
Fig. 9. *In vivo* BFB0261 release profiles of films following subcutaneous implantation on rat backs. Film dimension was 10 mm × 25 mm. Films containing 1 mg of BFB0261 were constructed from various polymers such as PLGA7723, PLAPEG8515H, and PLATMC8416. Each point represents the mean ± SE (n = 4).
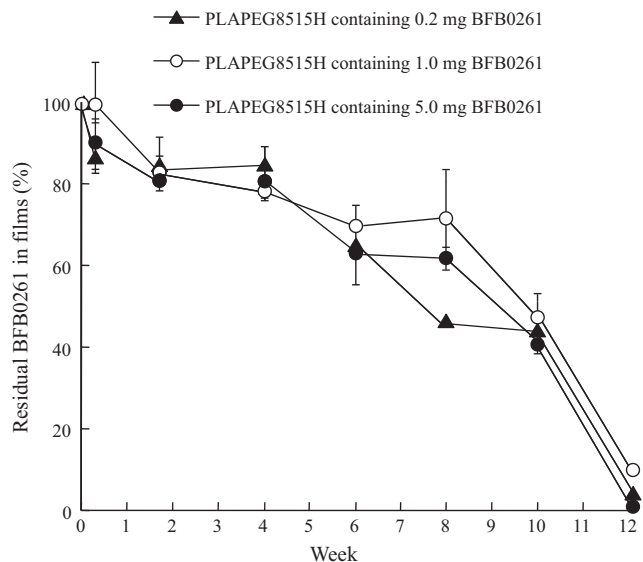
Fig. 10. *In vivo* BFB0261 release profiles of microspheres following subcutaneous administration on rat backs. Films dimension was 10 mm × 25 mm. Films containing 0.2 mg, 1 mg, or 5 mg of BFB0261 were constructed from PLAPEG8515H. Each point represents the mean ± SD (n = 4).

ulate that these new synthetic polymers, such as PLAPEG8515H or PLATMC8416, facilitate the slow drug diffusion from films in which they are present.

4. Conclusions

The treatment of bone disorders such as osteoporosis-derived comminuted fractures as well as bone defects caused by extirpa-

tion of maxilla with sarcoma requires a new drug formulation of active ingredients combined with biodegradable and biocompatible polymers to sustain the slow release of active ingredients at the damaged site. In the present study, we prepared films containing BFB0261, a new potent osteogenic compound, and various other biodegradable PLA, PLGA, PLA–PEG, and PLA–TMC polymers. Each film constructed from PLGA, PLA–PEG, or PLA–TMC polymers appeared to be tougher and more elastic than the PLA films. In the *in vitro* release study, the release profiles of BFB0261 from films constructed from PLA100, PLAPEG9604H, PLAPEG8515H, or PLAPEG8020 exhibited zero-order kinetics with slow release up to 12 weeks following incubation. On the other hand, the release profile of BFB0261 from PLATMC8416 films followed first-order kinetics with a slow release at 12 weeks. Furthermore, films constructed from PLAPEG8515H or PLATMC8416 were capable of achieving a sustained release of BFB0261 for 12 weeks *in vivo*. Therefore, it was found that films constructed from PLAPEG8515H or PLATMC8416 are feasible for regenerative medicine.

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