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Conjugation of drug to poly(D,L-lactic-co-glycolic acid) for controlled release from biodegradable microspheres

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Abstract

Poly(D,L-lactic-co-glycolic acid) (PLGA) was chemically conjugated to a model drug, *N*-(9-fluorenylmethoxycarbonyl-*N*-*tert*-butoxycarbonyl-L-tryptophan (Fmoc-Trp(Boc)) via an ester linkage. Various coupling reaction conditions were tested to optimize the conjugation process between a hydroxyl terminal group of PLGA and a carboxylic acid group of Fmoc-Trp(Boc). Two different lactic/glycolic acid compositions of PLGA (50/50 and 75/25) were used for the conjugation. The Fmoc-Trp(Boc)–PLGA conjugates were formulated into microspheres by a solvent evaporation technique for controlled release of Fmoc-Trp(Boc) over an one month period. A linear constant release of Fmoc-Trp(Boc) and its water-soluble PLGA oligomer conjugates was observed over an extended period without any initial burst effect, while unconjugated Fmoc-Trp(Boc) encapsulated within microspheres exhibited a rapid release profile. In addition, Fmoc-Trp(Boc) release rate solely depended on the PLGA composition that affected polymer degradation rate. The release rate of Fmoc-Trp(Boc) conjugated with fast degrading 50/50 PLGA was more rapid than that conjugated with relatively slow degrading 75/25 PLGA. This study demonstrates that PLGA–drug conjugation approach is a new and novel strategy to control the drug release rate from PLGA microspheres by utilizing the chemical degradation rate of PLGA backbone.

Keywords: Conjugation; PLGA; Microspheres; Zero-order release; Degradation-controlled

1. Introduction

Biodegradable PLGA microspheres have been widely used for an injectable depot formulation of various small molecular weight drugs, peptides, and proteins which required multiple administrations. It has been known that drug release kinetic rate from the microspheres is determined by diffusion and/or polymer erosion process [1]. For small diameter microspheres as an injectable dosage form, it has been difficult to predictably control the drug release kinetic rate over a desired period due to an initial burst effect combined with the process of relatively faster diffusion of the drug than the erosion of microspheres. This problem is particularly acute for hydrophilic drugs that are believed to exist in pre-formed microporous aqueous channels within the microspheres [2]. The most common method of preparing microspheres for hydrophilic drugs is a

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double emulsion solvent evaporation technique which adopts a two phase emulsion system composed of polymer dissolved organic phase containing primary aqueous emulsion droplets as a dispersed phase and water as a continuous phase [3]. This method inevitably generates porous morphology in the microspheres matrices, leading to burst and very fast release kinetics of the hydrophilic drugs through pre-existing macro- and micro-pores. For hydrophobic drugs, a single oil-in-water emulsion system has been employed to prepare drug loaded microspheres. In this case, drug release kinetic rate was mainly controlled initially by diffusion through existing pores and later by polymer erosion process, resulting in a triphasic release profile. Most of previous studies for controlled release of hydrophilic drug from PLGA microspheres, however, could not achieve a zero-order release profile over an extended period because of complicated nature of drug release mechanism, that is, a diffusion coupled polymer erosion process [4].

In this study, we attempted to obtain a zero-order release kinetic profile from PLGA microspheres by conjugating a model hydrophobic amino acid derivative, Fmoc-Trp(Boc), to a hydroxyl terminal end group of PLGA via a biodegradable ester bond linkage. The strategy is that when the conjugated PLGA chains are randomly hydrolyzed and water-soluble fractions are leached out [5], drug molecules bound to the terminal ends of the cleaved PLGA oligomers are released out concomitantly. A similar conjugation approach of a hydrophilic drug to PLGA was recently proposed for better controlled release from PLGA microspheres [6]. The drug release rate from the proposed system is expected to be proportional to mass erosion rate of the PLGA microspheres. After the release, PLGA oligomer chains conjugated to the drug moiety would be further degraded, eventually regenerating an intact free drug. Previously, we have chemically conjugated a carboxylic terminal group of PLGA to lysozyme molecule by a carbodiimide coupling process [7]. This yielded an amide linkage between primary amino groups in lysozyme and PLGA. The amide bond is not desirable for the drug conjugation since it is not easily cleavable. The main objective of this study is, *first* to conjugate the model drug, Fmoc-Trp(Boc), to relatively fast and slow degrading PLGA polymers via a cleavable ester bond, *second* to formulate the drug-PLGA conjugates into microspheres by a single O/W emulsion method, *third* to compare the drug release kinetics from the conjugated drug loaded microspheres with the conventional unconjugated drug loaded microspheres. To this end, various chemical synthetic routes for the conjugation were explored and the conjugation process was optimized. Fmoc-Trp(Boc) was conjugated to two different PLGA having 50/50 and 75/25 lactic/glycolic acid compositions. The microspheres containing PLGA conjugated and unconjugated Fmoc-Trp(Boc) were fabricated and their drug release kinetic behaviors were examined.

2. Materials and methods

2.1. Materials

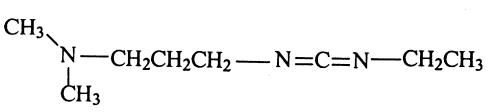
Poly(D,L-lactic-co-glycolic acid) (lactic acid/glycolic acid ratio, 75/25 and 50/50) were purchased from Wako Pure Chemical Industries (PLGA 5010 and 7510). The two PLGA polymers with 75/25 and 50/50 lactic/glycolic monomer composition ratios had weight average molecular weights (M_w) of 9800 and 8700, respectively, as determined by gel permeation chromatography. The PLGA polymer used in this study has an uncapped free carboxylic acid group at one terminal end and a hydroxyl group at the other end. Fmoc-Trp(Boc), 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *N*-hydroxybenzotriazole (HOBt), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), and bromo-tris-pyrrolidino-phosphonium hexafluorophosphate (pyBrop) were obtained from Novabiochem. Bis(2-oxo-3-oxazolidinyl)-phosphonic chloride (BOP-Cl) was purchased from Advanced Chemtech. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC), tetrahydrofuran (THF), methylene chloride (anhydrous), diethyl ether (anhydrous), triethylamine (TEA), polyvinylalcohol (PVA, 88% hydrolyzed, M_w 25 000) and 4-dimethylaminopyridine (DMAP) were obtained from Aldrich. All other reagents were analytical grade and used without further purification.

2.2. Coupling reaction of Fmoc-Trp(Boc) to PLGA

First, different coupling reactions were explored by employing various coupling agents for PLGA 50/50 to maximize the conjugation yield. The coupling agents were shown in Fig. 1. Typically, Fmoc-Trp(Boc) (80 mg, 1.5×10^{-4} mol) dissolved in CH_2Cl_2 (3 ml) under nitrogen atmosphere was mixed with various combinations of coupling reagents (1.5×10^{-4} mol) and bases (1.5×10^{-4} mol). PLGA with lactic/glycolic acid 50/50 ratio (0.43 g, 5×10^{-5} mol) dissolved in 7 ml of CH_2Cl_2 was then added to the solution. The coupling reaction was carried out at room temperature for 4–6 h. The resulting solution was diluted with the addition of 20 ml CH_2Cl_2 and washed with brine. An organic layer was separated, concentrated, and dried under vacuum. The conjugated Fmoc-Trp(Boc)–PLGA was further purified by the following precipitation procedure. The product mixture dissolved in 2 ml CH_2Cl_2 was precipitated by the addition of 30 ml diethyl ether. This precipitation was repeated three times. The separation of unconjugated free Fmoc-Trp(Boc) from a mixture of Fmoc-Trp(Boc)–PLGA conjugate and unreacted PLGA was confirmed by gel permeation chromatography (GPC). The conjugate was lyophilized and stored at −20°C until use. The optimized conjugation condition for the PLGA 50/50 was used for the conjugation of PLGA 75/25.

2.3. Characterization of Fmoc-Trp(Boc)–PLGA

The conjugates were characterized by GPC using Gilson 306 pump with UV detector. The GPC column was Shodex K-803 (300×7.8 mm, Phenomenex) and THF was used as a mobile phase with a flow rate of 1 ml/min. The eluted conjugate was monitored by UV at dual wavelengths (230 nm for



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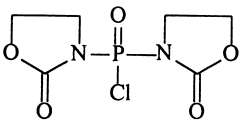

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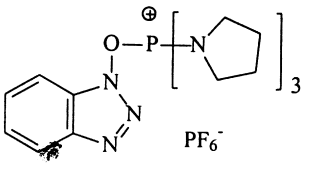
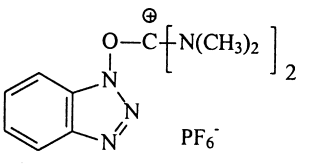
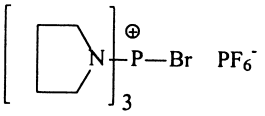


PyBOP



HBTU



PyBroP

Fig. 1. Various coupling agents used for conjugation of Fmoc-Trp(Boc) to PLGA.

PLGA and 267 nm for Fmoc group). Weight average molecular weight of the conjugate was calculated using a series of polystyrene standards (M_r: 114 200, 44 000, 13 700, and 3700). The conjugation was confirmed by measuring fluorescence of Fmoc-Trp(Boc)–PLGA (excitation at 295 nm and emission at 355 nm for Trp group and excitation at 260 nm and emission at 305 nm for Fmoc group). The conjugation percent of Fmoc-Trp(Boc) to PLGA was determined by measuring absorbance of Fmoc-Trp(Boc)–PLGA in THF at 267 nm using a series of Fmoc-Trp(Boc) concentrations as standards. The conjugation percent was calculated as a relative molar ratio of Fmoc-Trp(Boc) conjugated PLGA compared to the total PLGA amount in the sample.

2.4. Microsphere preparation

PLGA microspheres containing Fmoc-Trp(Boc)–PLGA conjugates were prepared by an oil-in-water (O/W) single emulsion technique. Four different formulations were prepared as listed in Table 2. One hundred mg of Fmoc-Trp(Boc)–PLGA conjugates (PLGA 50/50 (formulation A), a 50:50 mixture of PLGA 50/50 and 75/25 (formulation B), and PLGA 75/25 (formulation C)) and 400 mg of PLGA 50/50 were dissolved in a co-solvent mixture of 2.5 ml of DMSO and 2.5 ml of methylene chloride. As control microspheres (formulation D), 10 mg of free Fmoc-Trp(Boc) and 490 mg of PLGA 50/50 were used for the formulation. The resulting solutions were emulsified in 500 ml of 0.3% (w/v) PVA/phosphate-buffered saline (PBS) solution for 20 min by homogenization at 6000 rpm using a PowerGen 700 (Fisher Scientific) and subsequently stirred magnetically for 3 h at room temperature to extract DMSO and evaporate methylene chloride. The hardened microspheres were collected by centrifugation at 8000 rpm for 20 min, washed twice with deionized water, and then lyophilized.

2.5. Scanning electron microscopy and particle size distribution

Microsphere shape and surface morphology were estimated using a scanning electron microscopy (SEM, Philips 535M). Freeze-dried microspheres were mounted on an aluminum stub covered with a carbon adhesive tape and then coated with gold. Size average and distribution of microspheres were determined by a Coulter Counter (CASY^{TM}, Scharfe System, Germany). Microspheres suspended in isotonic saline solution were forced to flow through a capillary. The resistance change by a particle was electronically scaled and counted.

2.6. In vitro release study

One hundred mg of freeze-dried microspheres were suspended in 30 ml of 0.033 M PBS, pH 7.0, containing 0.01% sodium azide. They were incubated in a polypropylene tube at 37°C under static condition. At predetermined intervals, the supernatant from each tube was collected by centrifugation at 8000 rpm for 30 min and replaced with an equal volume of fresh buffer medium. The supernatant was frozen and stored at −20°C until analyzed.

2.7. Determination of Fmoc-Trp(Boc) concentration in release medium

The Fmoc-Trp(Boc) concentration in the release medium was determined by measuring fluorescence intensity with a spectrofluorometer (RF-5301PC, Shimadzu, Japan) with an excitation at 295 nm and an emission at 314 nm. A standard calibration curve was constructed by dissolving 10 mg of free Fmoc-Trp(Boc) in 2 ml of acetonitrile and diluting it with excessive PBS buffer solution (pH 8). For the evaluation of cumulative release percent, the microspheres after incubation for 31 days were freeze-dried and the amount of unreleased Fmoc-Trp(Boc) in the microspheres was determined. Ten mg of dry microspheres dissolved in 1 ml of 0.1 N NaOH solution were incubated at 37°C for 2 days. The remaining amount of Fmoc-Trp(Boc) was then determined. Osmolality build-up in the release media was measured by a freezing point osmometer (model A0300, Knauser, Germany).

2.8. Identification of released products from microsphere-containing Fmoc-Trp(Boc)–PLGA conjugates

Released products from the microspheres encapsulated with Fmoc-Trp(Boc)–PLGA conjugate and free

Fmoc-Trp(Boc) were separated by a C_{18} reversed-phase column by high-performance liquid chromatography (HPLC) and the elution was monitored by fluorescence (excitation 260 nm, emission 305 nm). The released Fmoc-Trp(Boc)–PLGA oligomer conjugates were further incubated in 0.033 M sodium phosphate buffer (pH 9) at 37°C for additional 3 days to see whether authentic free molecule of Fmoc-Trp(Boc) was regenerated. This was performed by fractional separation of the conjugates in HPLC followed by taking its mass spectrum. The mass spectrometer (Platform II, Micromass, UK) was operated in a positive ion mode. Ion spray mass spectra were recorded on a simple quadrapole mass spectrometer API equipped with an ion spray nebulizer assisted with electrospray. Samples dissolved in 50% acetonitrile/water containing 0.1% formic acid were introduced to a source with HPLC pump (PU-980, Jasco, Japan). The instrument was scanned in a multi-channel analyzer over a mass range from m/z 200 to 700 with 4-s scan time. The potential of the capillary was held at 3.53 kV and cone voltage was maintained at 30 eV.

3. Results and discussion

Uncapped hydrophilic PLGA has two functional groups, a hydroxyl group and a carboxylic acid group, at its terminal ends. Both of them can be utilized for the chemical conjugation of a drug. The carboxylic acid terminal end in PLGA is easily conjugated to amine groups in drug molecules via a carbodiimide derivative to form an amide linkage. However, one drawback in the above conjugation approach is that the amide linkage is not readily cleaved when water-soluble oligomer PLGA–drug conjugate molecules are liberated from microspheres [8]. The released drug molecules are expected to have uncleaved lactic or glycolic acid monomer residues left at the conjugation site although the degradation of conjugated PLGA oligomer chain further proceeds. To introduce a cleavable bond in the conjugation site, an ester linkage between the hydroxyl group of PLGA and the carboxylic acid group present in Fmoc-Trp(Boc) was synthesized by using various coupling agents.

Various coupling agents and bases in different combinations were used for the activation of the carboxylic acid group present in Fmoc-Trp(Boc) (Table 1). As shown in Fig. 2, an active ester form of Fmoc-Trp(Boc) was first reacted with a hydroxyl group of PLGA 50/50 in methylene chloride. The conjugation percent was dependent on the kind of coupling agents. Among the reaction conditions tested, PyBroP/TEA combination resulted in the greatest conjugation percent (63%). Thus, the PyBroP/TEA reaction was also used for the conjugation of PLGA 75/25. The conjugation percentage of Fmoc-Trp(Boc) to PLGA 75/25 was 46%. The lowered conjugation percent for the PLGA 75/25 is likely to be caused by the fact that it has a greater probability of lactic acid termination in the polymer chain end than the PLGA 50/50. The hydroxyl group in lactic acid is less reactive than that in glycolic acid because it is a secondary alcohol. Fig. 3 shows GPC profiles of PLGA 50/50 and its conjugate with Fmoc-Trp(Boc). It can be seen that the conjugate was eluted slightly earlier than the unconjugated PLGA due to the additional presence of

Table 1
Synthesis of Fmoc-Trp(Boc)–PLGA (50/50)

Mole ratio of PLGA:Fmoc-Trp (Boc)	Coupling reagent	Base or additive	Reaction time (h)	Conjugation (%)
1:3	BOP-Cl	TEA	4	No reaction
1:10	BOP-Cl	TEA	6	<10
1:3	EDC	HOBt	4	<10
1:3	EDC	HOBt	6	<10
1:3	PyBOP	TEA	4	37
1:3	HBTU	TEA	4	20
1:3	PyBroP	TEA	4	60
1:3	PyBroP	TEA	6	63
1:3	PyBroP	DMAP	4	63

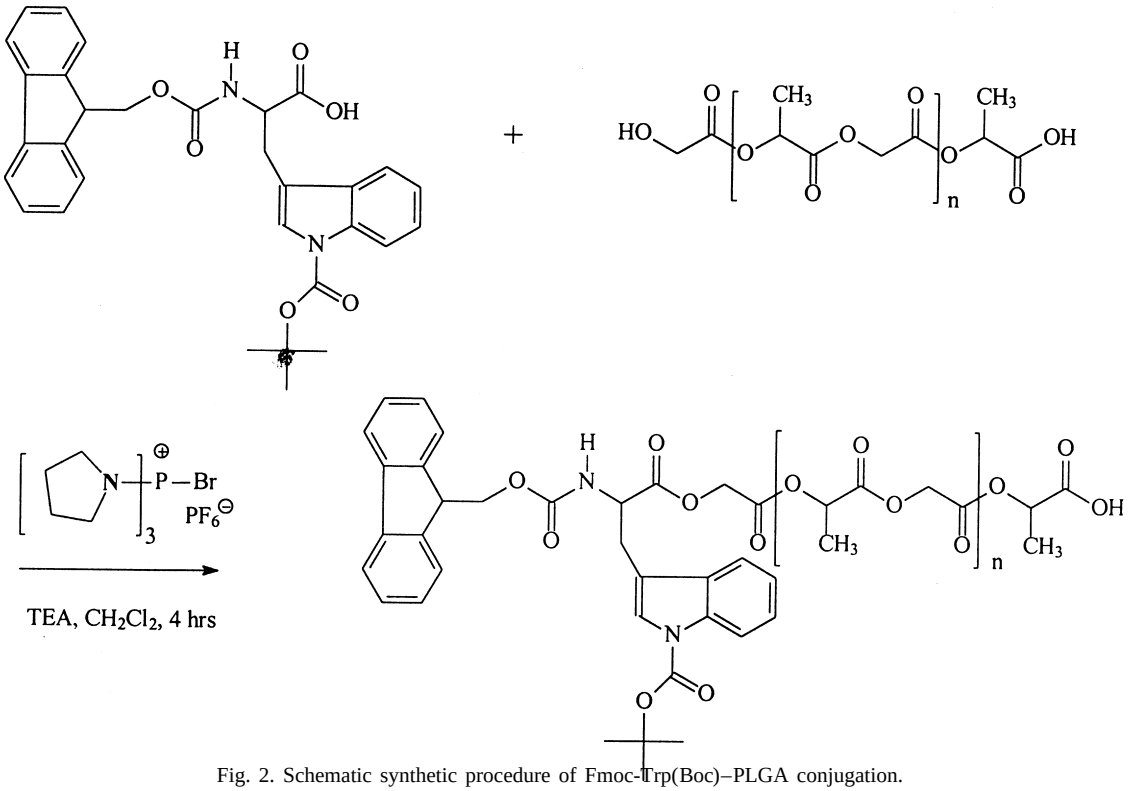


Fig. 2. Schematic synthetic procedure of Fmoc-Trp(Boc)–PLGA conjugation.

Fmoc-Trp(Boc) group in one end terminal of PLGA chain. Since the two GPC profiles, monitored at dual UV wavelengths of 230 and 267 nm for PLGA and Fmoc-Trp(Boc), respectively, were almost overlapped, it was confirmed that the 1:1 conjugate was successfully prepared. It should be mentioned that the elution peak of the conjugate monitored at 267 nm does not reflect a fraction of unconjugated PLGA that was not detected at that wavelength. Elemental analysis data (calculated C 47.3%, H 4.73%, N 0.19%, and O 47.8%; measured C 47.0%, H 4.6%, N 0.2%, and O 48.2%) support the conjugation percent of 63%. It should be noted that 100% conjugation of Fmoc-Trp(Boc) to PLGA is not necessary for our specific research goal, because the unreacted PLGA polymer would be eventually constituted in the matrix material of PLGA microspheres to be formulated.

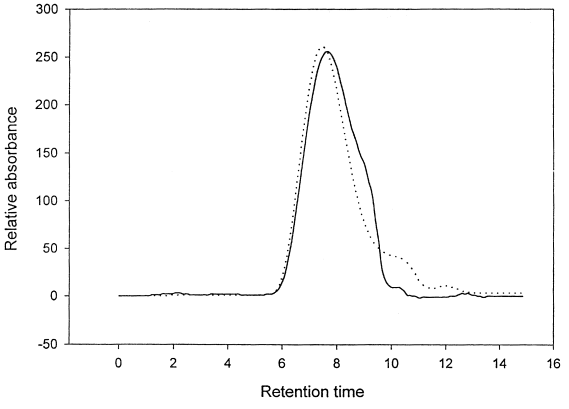


Fig. 3. GPC profiles of PLGA 50/50 and after its conjugation with Fmoc-Trp(Boc). Solid line, PLGA 50/50 before conjugation monitored at 230 nm; dotted line, Fmoc-Trp(Boc)–PLGA 50/50 conjugate monitored at 267 nm.

The Fmoc-Trp(Boc)–PLGA conjugates synthesized with different copolymer compositions of 50/50 and 75/25 were incorporated into PLGA 50/50 matrix microspheres using a single oil-in-water emulsion method as shown in Table 2. The microspheres (formulations A, B, and C) containing the Fmoc-Trp(Boc)–PLGA conjugates exhibit almost 100% encapsulation efficiencies owing to their lim-

Table 2
Formulation of microspheres

Formulation	PLGA 50/50 weight (mg)	Fmoc-Trp(Boc)–PLGA, weight (mg)	Encapsulation efficiency (%)	Average size (μm)
A	400	100[a]	100	8.76 (1.2)
B	400	100[b]	100	8.33 (0.9)
C	400	100[c]	100	8.52 (1.7)
D	490	10[d]	20.1	8.62 (1.6)

[a]Fmoc-Trp(Boc)–PLGA 50/50 conjugate.
[b]Fmoc-Trp(Boc)–PLGA 75/25 conjugate.
[c]A 1:1 mixture of Fmoc-Trp(Boc)–PLGA 50/50 conjugate and Fmoc-Trp(Boc)–PLGA 75/25 conjugate.
[d]Free Fmoc-Trp(Boc).

ited solubility in water, whereas conventionally prepared microspheres (formulation D) encapsulated with unconjugated Fmoc-Trp(Boc) show only 20.1% encapsulation efficiency due to the diffusion of moderately water-soluble Fmoc-Trp(Boc) into the aqueous phase during the solvent evaporation procedure. All the microspheres have their average size in diameter between 8.33 and 8.76 μm. SEM pictures indicate spherical shaped microspheres having a very smooth surface morphology. There were no apparent discrepancies in morphologies between conjugated and unconjugated Fmoc-Trp(Boc) containing microspheres.

Fig. 4 shows release profiles of Fmoc-Trp(Boc)–PLGA oligomer conjugates in the incubation medium from various microsphere formulations. Previously, it was established that a critical molecular weight of PLGA oligomers to be solubilized in water is around 1000–1100; that is, 12–13 units of lactic or glycolic acid [9]. A mixture of the water-soluble Fmoc-Trp(Boc)–PLGA oligomers are likely to be released in a similar diffusion rate because of their small difference in molecular size. It can be seen that the microspheres conjugated with Fmoc-Trp(Boc) exhibit constant release profiles over 20 days with an initial, short lag time period. On the other hand, the microspheres containing unconjugated Fmoc-Trp(Boc) show a rapid release in the initial incubation stage, resulting in the early termination of release within 5 days. This is a typical type of release kinetic pattern from PLGA microspheres encapsulated with moderately hydrophilic drugs, which is mainly caused by diffusion of encapsulated free drug molecules through aqueous inter-connecting channels in the microspheres generated upon hydration [10]. The microspheres (formulation A) containing Fmoc-Trp(Boc) conjugated with relatively fast degrading PLGA 50/50 polymer release their degradation products, Fmoc-Trp(Boc)–PLGA oligomer conjugates, faster than those (formulation B) conjugated with slowly degrading PLGA 75/25. Although their release rates do not differ greatly due to the relative small incorporation percentage of the conjugates in microspheres, this finding reveals that release kinetic rates of the drug conjugated with PLGA, indeed, can be judiciously controlled by appropriately selecting various PLGA polymers that degrade in different rates. Therefore, molecular weight and/or copolymer composition of PLGA to

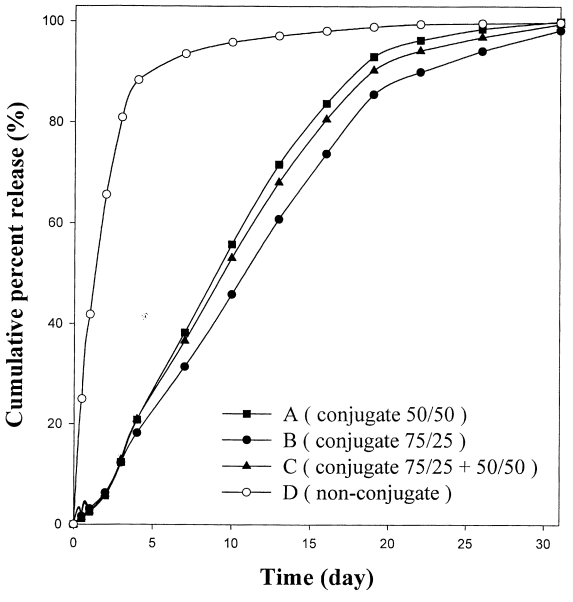


Fig. 4. Release profiles of water-soluble Fmoc-Trp(Boc)–PLGA oligomer conjugates in the medium as expressed in cumulative release percent.

be conjugated would be an important variable to control the release rate in the present system. The formulation C having a 50:50 blend mixture composition of PLGA 50/50 and PLGA 75/25 in the Fmoc-Trp(Boc) conjugation demonstrates an intermediate release kinetic rate between the two release rates of formulations A and B, supporting our hypothesis that the chemical degradation rate of conjugated PLGA chains modulates the liberation rate of water solubilizable Fmoc-Trp(Boc)–PLGA oligomer fraction into the incubation medium as mentioned earlier. Since the two PLGA polymers used in this study have similar molecular weights, their chemical degradation rates solely depend on the composition of polymer chains [11]. Thus, the above results indicates that different hydrolytic scission rates of polymer backbone composed of PLGA 50/50 and 75/25 are mainly responsible for the controlled liberation of Fmoc-Trp(Boc)–PLGA oligomer conjugates.

It is of particular interest to note short lag time periods in the early stage of incubation for the microspheres containing conjugated Fmoc-Trp(Boc). This is most likely to be caused by the fact that a latent period is needed for the solubilization of PLGA chains in the aqueous medium. Hydrolyzed PLGA chain segments are required to reach a critical molecular weight of about 1000–1100 to gain sufficient water solubility [8]. A lag time was generally observed in the mass erosion profile of PLGA microspheres, while the molecular weight of PLGA chains decreases continuously without exhibiting any lag time [5]. Thus, it can be said that drug release kinetic rate in the present PLGA–drug conjugate system is determined by the mass erosion rate of PLGA polymer matrices.

Fig. 5 shows pH and cumulative osmolality changes in the incubation medium as a function of time. Since the incubation medium was completely replaced by fresh buffer solution at every sampling period, there are insignificant pH variations during the release period, while the continuous build-up of osmolality by the accumulation of water-soluble PLGA degradation products in the medium was observed. This suggests that the release of degradation products from the PLGA 50/50 microspheres is a linear function of time [12]. Initially increased osmolality upon incubation can be attributed to the

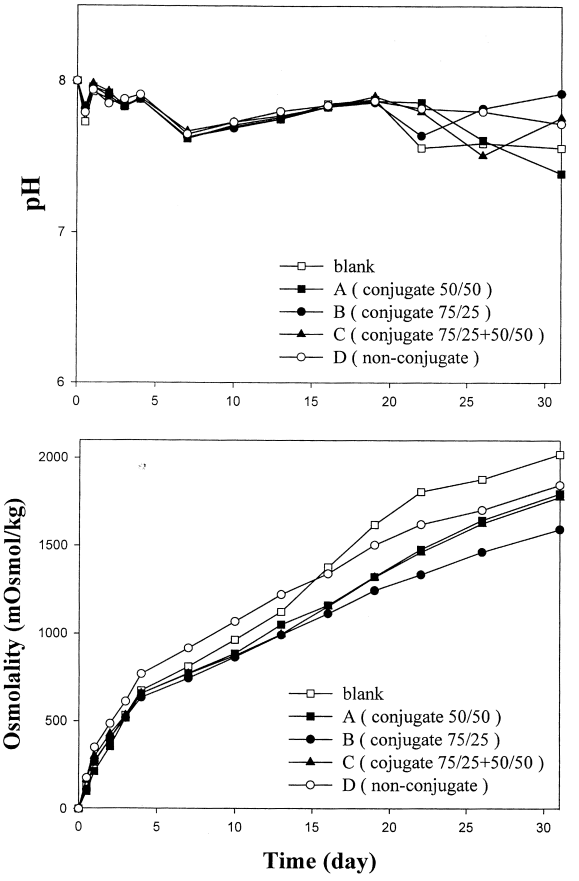


Fig. 5. pH (top) and osmolarity (bottom) change in the release medium as a function of incubation time.

preferential diffusion of pre-existing small molecular weight PLGA oligomer fragments generated during the microsphere formulation process. It was reported that water-soluble PLGA fragments were produced to a large extent within microspheres during the microsphere formulation by the solvent evaporation method [9]. Because the slower release of Fmoc-Trp(Boc)–PLGA oligomer conjugates from the microspheres during the same initial period was observed in Fig. 4, it is conceivable that the initially released water-soluble PLGA fragments were mostly from PLGA 50/50 polymer base matrix which was constituted in an excess amount for the microsphere formulation. It can also be seen that the degradation rates of PLGA polymer chains in the microspheres, as judged from the osmolarity build-up difference, are not so different for various formulations. This is

due to the fact that PLGA 50/50 was used as a major matrix constituent (90%, w/w) for the microsphere formulation.

Fig. 6 shows the reversed-phase HPLC results obtained from the released Fmoc-Trp(Boc)–PLGA conjugates in the medium from the formulation A microspheres at day 7. The released conjugates are expected to have a mixture of molecular species composed of Fmoc-Trp(Boc) linked with different chain lengths of water-soluble PLGA oligomers resulting from the random cleavage of PLGA chain. The HPLC result, however, demonstrates that the released Fmoc-Trp(Boc)–PLGA oligomer conjugates in the buffer medium were eluted earlier as a major single peak than the authentic Fmoc-Trp(Boc), although many other small unidentified peaks followed can also be seen. This may be caused by an insufficient resolution problem of the current reversed-phase HPLC lacking the separation capability of the conjugate species. Because the released conju-

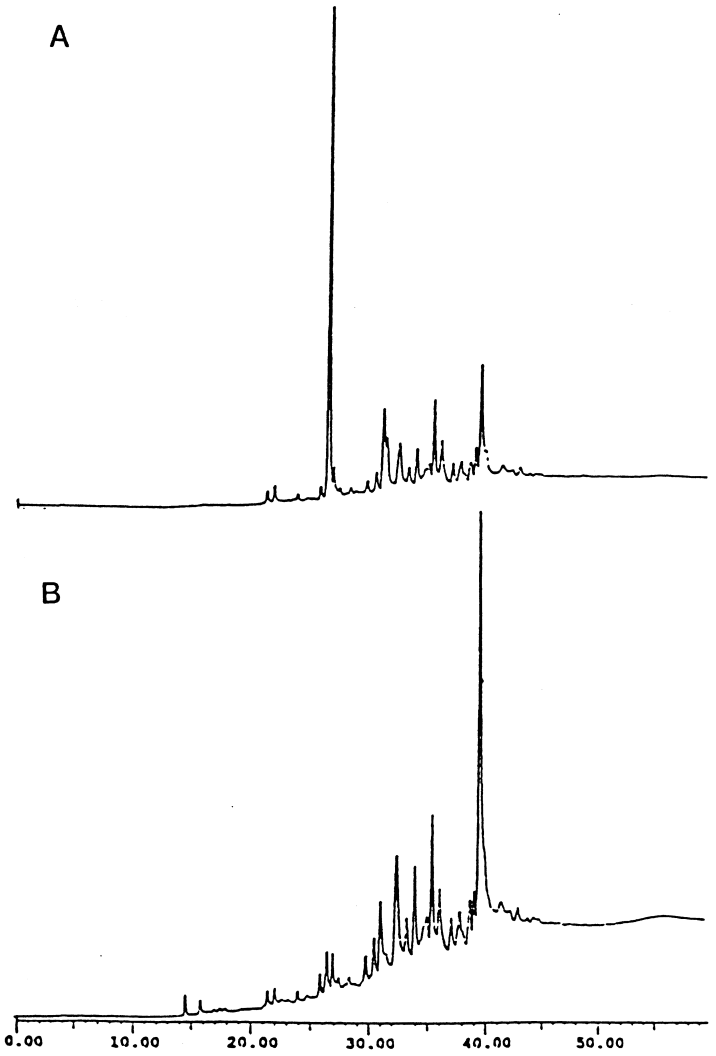


Fig. 6. Reversed-phase HPLC chromatogram of released Fmoc-Trp(Boc)–PLGA conjugates from the formulation A microspheres at day 7 (A) and further degraded products after the incubation of the released Fmoc-Trp(Boc)–PLGA conjugates at pH 9 buffer solution for the additional 3 days (B). Note that the elution peak appearing at 39.5 min is authentic Fmoc-Trp(Boc).

gates have a terminal carboxylic acid group at their chain end, they eluted earlier than the authentic Fmoc-Trp(Boc) peak in C_{18} reversed-phase HPLC chromatogram. Further incubation of the released Fmoc-Trp(Boc)–PLGA oligomer conjugates in the buffer medium permitted the regeneration of intact Fmoc-Trp(Boc) peak as a result of chain scission of PLGA oligomers attached to Fmoc-Trp(Boc). This was additionally confirmed by mass spectroscopy. As shown in Fig. 7, the major product from the additional incubation of the released Fmoc-Trp(Boc)–PLGA oligomers was identified as Fmoc-Trp(Boc) by its mass spectrum which shows $[M+K]^+$ and $[M+H+K]^{2+}$ ion peak at $m/z=563.38$ (calculated 565) and $m/z=282.19$ (calculated 283). Comparison of mass spectrum relative to that of intact Fmoc-Trp(Boc) indicates that the major components released from the microspheres were Fmoc-Trp(Boc)–PLGA oligomer conjugates and intact molecule of Fmoc-Trp(Boc) could be regenerated by further degradation. In the mass spectrum obtained from the release and further degradation experiment, a molecular ion peak appearing at 509.25 seems to be the dehydrated fragment product of Fmoc-Trp(Boc) (M_w 526) generated in the process of incubation. This needs further careful characterization of a mixture of the released conjugates. In a separate experiment, it was confirmed that Fmoc and Boc protecting groups for Trp have a sufficient stability in the experimental condition adopted in this study.

It has been demonstrated that drug molecules could be delivered in a zero-order fashion from biodegradable microspheres in a desired time span by the conjugation approach of a drug to PLGA chain end via a cleavable ester linkage. A schematic illustration of the proposed drug-PLGA conjugate system is depicted in Fig. 8. This strategy can be potentially applied to a wide range of hydrophilic drugs like antibiotics to improve their therapeutic effectiveness which require an extended duration of release at the injection site in vivo. However, it

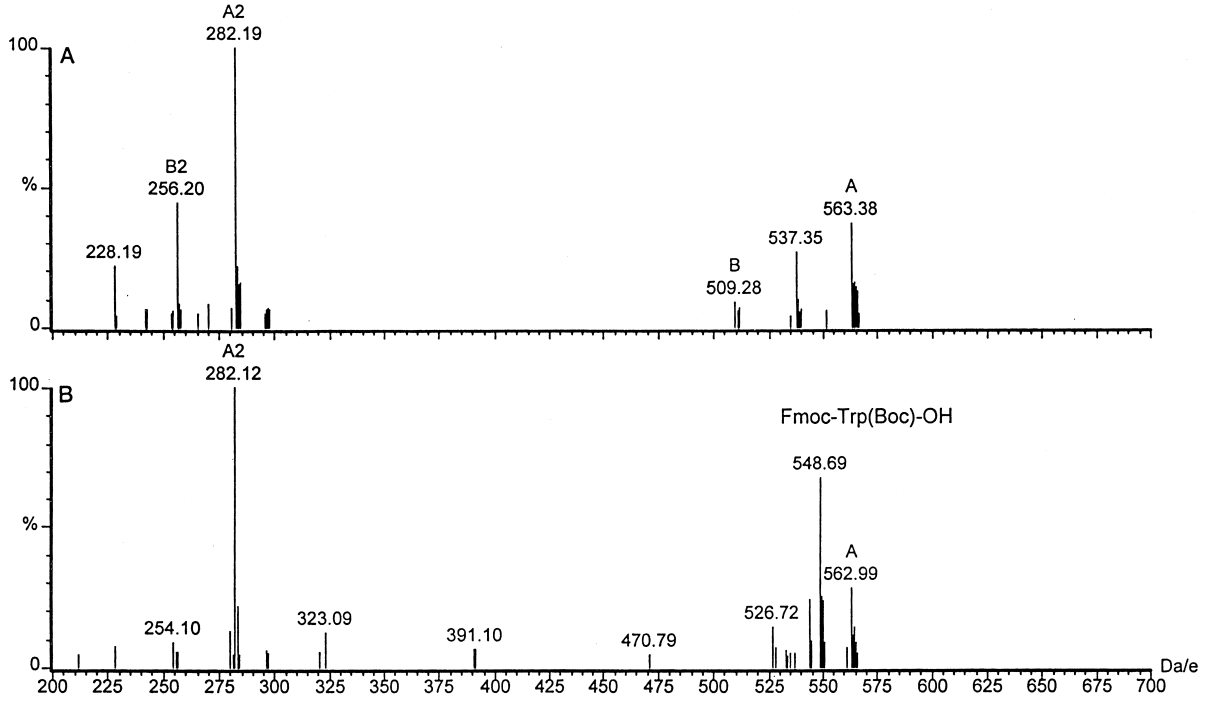


Fig. 7. Mass spectra of hydrolyzed products obtained from Fmoc-Trp(Boc)–PLGA oligomer conjugates released in the incubation medium (A) and authentic Fmoc-Trp(Boc) (B).

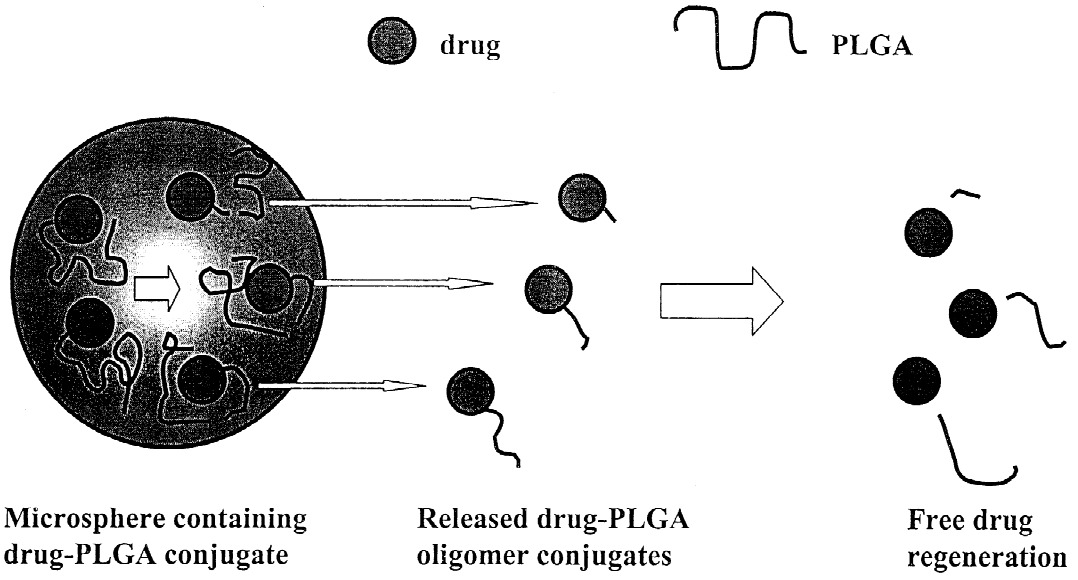


Fig. 8. Schematic illustration of drug–PLGA conjugation system.

remains to see whether the regeneration rate of released drug–PLGA oligomer conjugates to native drug molecules is faster than their elimination rate in the body. Otherwise, the insufficiently degraded drug–PLGA oligomer conjugates that are released in the body would have lower biological activity compared to that of free drug molecules. In vivo experiments based on the conjugates of PLGA and a therapeutic drug are in progress to address that issue.

Acknowledgements

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