Preparation and Characterization of Gelatin Surface Modified PLGA Microspheres

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ABSTRACT This study optimized conditions for preparing and characterizing gelatin surface modified poly (lactic-co-glycolic acid) (PLGA) copolymer microspheres and determined this system's interaction with fibronectin. Some gelatin microspheres have an affinity for fibronectin-bearing surfaces; these microspheres exploit the interaction between gelatin and fibronectin. PLGA copolymer microspheres were selected because they have reproducible and slow-release characteristics *in vivo*. The PLGA microspheres were surface modified with gelatin to impart fibronectin recognition. Dexamethasone was incorporated into these microspheres because dexamethasone is beneficial in chronic human diseases associated with extra fibronectin expression (eg, cardiovascular disease, inflammatory disorders, rheumatoid arthritis). The gelatin surface modified PLGA microspheres (prepared by adsorption, conjugation, and spray coating) were investigated and characterized by encapsulation efficiency, particle size, *in vitro* release, and affinity for fibronectin. The gelatin-coated PLGA microspheres had higher interaction with fibronectin compared with the other gelatin surface modified PLGA microspheres (adsorption and conjugation). Dexamethasone was released slowly (over 21 days) from gelatin surface modified PLGA microspheres.

Key Words: Microspheres, Surface Modification, Gelatin, Fibronectin, PLGA, Dexamethasone

INTRODUCTION

Biodegradable microspheres are used to control drug release rates and to target drugs to specific sites in the body, thereby optimizing their therapeutic response, decreasing toxic side effects, and eliminating the inconvenience of repeated injections. Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal. Poly (lactic-co-glycolic acid) (PLGA) copolymer is one of the synthetic

biodegradable and biocompatible polymers that has reproducible and slow-release characteristics *in vivo* (1-3). An advantage of PLGA copolymers is that their degradation rate ranges from months to years and is a function of the polymer molecular weight and the ratio of polylactic acid to polyglycolic acid residues (2). Several products using PLGA for parenteral applications are currently on the market (1,4), including Lupron Depot and Zoladex in the United States and Enantone Depot, Decapeptil, and Pariodel LA in Europe (2).

Microspheres have large surface areas on which ligands can be attached for delivery to a specific local area (5). Modifications to microsphere surfaces with specific ligands have been attempted to use these microspheres as targeted drug delivery systems (1,6). In this study, PLGA microspheres were surface modified with gelatin to invoke interaction with fibronectin. Gelatin has a specific interaction with fibronectin (7,8). Gelatin microspheres have been investigated and demonstrated to target S-180 mouse sarcoma cells (pathological tissue), which are known to express extra fibronectin on their surface. This results in the cell line's extra fibronectin creating an affinity between cancer cells and gelatin microspheres (8). Excess fibronectin in the local tissues is associated with a number of disease states, such as inflammatory disorders, cardiovascular disease, rheumatoid arthritis, and cancer (7-12). A disadvantage of gelatin microspheres is that drug release rates are usually rapid; therefore, these microspheres are not useful for long-term controlled release (13). Consequently, gelatin surface modified PLGA microspheres may be useful as a targetable controlled-release microsphere system. These microspheres are intended for localized delivery to fibronectin-enriched pathological tissues.

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Dexamethasone was selected as a model drug because it is useful for most chronic human diseases associated with fibronectin-cardiovascular disease, inflammation, and rheumatoid arthritis (14-16). An oil/water emulsion/solvent evaporation method was used to prepare PLGA microspheres (17). Three processing methods were used to modify the surface of the PLGA microspheres: gelatin coating, adsorption, and conjugation at the PLGA microsphere surface. Any effect of surface modification on gelatin-fibronectin interaction was evaluated using a direct binding method. The developed formulations with particle size distributions in the range of 1 μ m to 7 μ m are intended for localized delivery to angioplasty areas during balloon angioplasty or stenting *in vivo* for preventing restenosis.

EXPERIMENTAL

Materials

Phosphatidic acid: dimyristoyl-sn-glycero-3-phosphate (DMPA.Na) was a gift from Genzyme (Cambridge, MA). Type B (alkali-processed) gelatin (MW 57 kd, Bloom No. 250, isoelectric, pH 4.7) was a gift from Gelatin Products Ltd (Sutton Weaver, Cheshire, UK). PLGA (poly (dl-lactide-co-glycolide), 50:50; Resomer RG 503, MW 40-75 kd) was a gift from Boehringer Ingelheim (Ingelheim, Germany). Dexamethasone, PVA (polyvinyl alcohol) (MW 30-70 kd), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), human

plasma fibronectin, dexamethasone, 3-(N-morpholino) propanesulfonic acid (MOPS) buffer, and Tween 80 were obtained from Sigma Chemical Company (St. Louis, MO). Hydrochloric acid, sodium hydroxide (NaOH), methylene chloride (CH_2Cl_2), acetone (CH_3COCH_3), isopropanol, potassium monophosphate acid, and hydrochloride acid (HCl, 1N) were purchased from Fisher Scientific (Springfield, NJ). Bicinchoninic acid (BCA) protein assay kit was purchased from

Pierce (Rockford, IL). Single-distilled deionized water, obtained from a NANOpure ultrapure water system (D4700, Barnstead, Dubuque, IA), was used in all studies. Phosphate buffered saline (PBS) at pH 7.4 and MOPS buffer at pH 6.5 were used.

METHODS

Formulation of Microspheres

PLGA microspheres were prepared by an oil/water emulsion/solvent evaporation method. The aqueous phase consisted of 100 mL, 1% wt/vol PVA, and the oil phase consisted of 10 mL of various ratios of organic solvents: CH_2Cl_2 , chloroform, and methanol, containing 500 mg PLGA. DMPA.Na (1-5 mg/mL) was added to the oil phase when necessary. Where appropriate, 10% wt/wt dexamethasone to polymer was incorporated into the organic phase. Briefly, 10 mL of 5% (wt/vol) PLGA in CH_2Cl_2 and 100 mL of PVA solution (1% wt/vol) were emulsified using a homogenizer (16 000 rpm for 20 seconds) (Omni-mixer, Ivan Sorvall Inc, CT). The resulting emulsions were stirred using a magnetic stirrer for 12 hours to allow the CH_2Cl_2 to evaporate. The microspheres were collected by centrifugation at 4000 rpm, 2000g (Beckman) for 15 minutes at 10°C. The precipitates were resuspended and washed with a 2% (vol/vol) isopropanol solution and water (500 mL) to remove PVA and unencapsulated dexamethasone. The concentrated microsphere suspensions were collected by freeze drying or were treated to achieve gelatin modification (Flow Chart 1). Because the stability of dexamethasone is affected by light, care was taken to minimize exposure to light during preparation and characterization (18).

Gelatin Surface Modified PLGA Microspheres

Gelatin surface modified PLGA microspheres were prepared by the following methods (Figure 1).

Gelatin-Adsorbed PLGA Microspheres: PLGA microspheres were dispersed in gelatin solution (1 mg/mL) for 4 hours at room temperature. The microspheres were collected by centrifugation at 4000 rpm for 15 minutes at 25°C, washed with 500 mL of water, and freeze dried.

Gelatin-Conjugated PLGA Microspheres:

The amine groups of gelatin molecules were chemically attached to the carboxyl groups on the surface of the microparticles.

PLGA microspheres were dispersed into a gelatin/MOPS buffer (1 mg/mL), to which EDC (0.5 mg/mL) was added.

Flow Chart 1. Processing of surface modified PLGA microspheres.

Figure 1. Gelatin surface modified PLGA microspheres were developed using the following methods.

A. Adsorption: Gelatin is bound ionically onto the negatively charged surface of PLGA microspheres

B. Conjugation: Conjugation using the carboxyl group of PLGA and the amine-group of gelatin.

C. Spray coating: Gelatin-coated PLGA microspheres using a mini spray dryer.

After 4 hours at room temperature, the microspheres were collected by centrifugation at 4000 rpm for 15 minutes at 25°C, washed twice with 500 mL of water, and freeze dried.

Gelatin-Coated PLGA Microsphere using Spray Dryer:

Gelatin coating was achieved by dispersing the microspheres in 100 mL of gelatin (1 mg/mL) for 4 hours at room temperature, followed by spray drying. A BUCHI 190 mini spray dryer (Brinkmann Instrument, Inc, Westbury, NY) was used at the following operating conditions: pump intensity, 2; aspiration intensity, 18; inlet temp, 58°C to 60°C; outlet temp, 36°C to -37°C; and flow rate, 4.5 mL/min.

Surface Charge

Electrophoretic mobility was measured using a ZetaPlus, Zeta Potential Analyzer (Brookhaven Instruments, Holtsville, NY). The microspheres were suspended in aqueous solution at constant ionic strength (1 mM) at pH 3.14 using NaCl, HCl, and NaOH solutions to adjust the pH. The electrophoretic mobility was measured at least 15 times (19).

Particle Size

The mean particle size was measured using an Accusizer (Model 770, Particle Sizing Systems, Inc, Santa Barbara, CA). Microsphere suspensions (0.5 mL) were dispersed into 50 mL of deionized distilled water. The Accusizer operates on the light-blockage principle, detecting particles in the range of 0.5 µm to 500 µm. All particle size measurements were repeated 3 times per sample and each sample was prepared in triplicate. The average values and standard deviations were calculated.

Morphology

Microsphere morphology was observed by optical microscopy and a Philips 2020 environmental scanning electron microscope (ESEM). The microspheres were coated with platinum for the ESEM studies. The microspheres were suspended in water and air-dried onto aluminum stubs and sputter coated with platinum under vacuum.

Encapsulation Efficiency for Dexamethasone (EE)

The microspheres were dissolved in acetonitrile, homogenized and analyzed by reversed-phase high-

performance liquid chromatography (HPLC) for dexamethasone. The HPLC system consisted of: an integrator (Chromjet Integrator, Spectra-Physics, Mountain View, CA); a UV Detector (Bio-Dimension UV/Vis HPLC detector, Bio-Rad Laboratories, Hercules, CA); a sampling system (Model AS-100 HR:C automatic sampling system (Bio-Rad, Hercules, CA); a solvent delivery system (constaMetric 3500 solvent delivery system); and a vacuum membrane degasser (LDC Analytical, Riviera Beach, FL). The HPLC conditions for analysis of dexamethasone were stationary reversed phase micro-Bondapak C-18 column (4.9 µm × 18 µm, particle size 4.9 µm); mobile phase (pH 4.8 acetic buffer:acetonitrile, [60:40]; flow rate, 1.5 mL/min; injection volume, 20 µL; and a UV detector set at 246 nm (20).

Determination of Gelatin Surface Modification

Total protein bound to the microspheres was measured by a BCA assay. A known amount of microspheres was suspended in 100 µL of water in microcentrifuge tubes. Next, 1 mL BCA working reagent was added to the tubes, and the samples were incubated at 37°C for 40 minutes. Microsphere suspensions were centrifuged for 2 minutes at 4°C using a microcentrifuge (14 000 rpm; 16 000g) (Fisher Scientific, Springfield, NJ). The supernatants were pipetted into microwell plates at 4°C and measured spectrophotometrically at 570 nm. The amount of protein bound per milligram of microspheres was calculated using a standard curve prepared with gelatin.

Interaction Study between Microspheres and Fibronectin

A direct binding assay was used to evaluate the amount of fibronectin bound to the microspheres and to gelatin alone. The microspheres (0.5 mg) were suspended in 0.3 mL of PBS and added into 1.5-mL micro test tubes. A touch stirrer was used to achieve homogeneous suspensions. Fibronectin in PBS (0.2 mL, 200 µg/mL)

was added to the suspensions. The micro test tubes were incubated at 37°C for 1 hour and centrifuged, then the supernatants were collected and analyzed for total protein to determine free fibronectin. Microspheres suspended in PBS without fibronectin were used as a reference (13).

Release Studies

All release studies were conducted under sink conditions. The dexamethasone-loaded microspheres were placed in 100 mL of phosphate buffered saline

(pH 7.4, 37°C) and stirred at 100 rpm. At preset time intervals, 5-mL samples were withdrawn, filtered using 0.45 µm microfilters, and the filtrate was refluxed back using 5 mL of PBS (37°C). Dexamethasone in the release media was analyzed by reversed-phase HPLC for dexamethasone (19).

Data Analysis

All data were expressed as mean $\pm$ standard deviation. The number of experiments (n) used to calculate a

mean value was at least 3. An analysis of variance was used to compare sample means and to determine statistical significance. A Tukey test was used to evaluate all pairwise comparisons. All the results were considered statistically significant if $P < 0.05$.

RESULTS AND DISCUSSION

PLGA Microspheres Formulation with DMPA.Na

To facilitate gelatin adsorption onto PLGA microspheres, an effort was made to invoke electrostatic interaction. PLGA microspheres were prepared in the presence of phosphatidic acid DMPA.Na to achieve a negative charge. Different ratios of solvent systems were investigated to select a

Table 1. Screening for DMPA.Na/PLGA Microsphere Formation

A. The Solubility of DMPA.Na in Different Solvent Ratios

Solvent Ratio (vol:vol)	Solvent System	
	CH ₂ Cl ₂ :MeOH	CHCl ₃ :MeOH
4:1	Not soluble	Not soluble
3:2	Partly soluble	Partly soluble
1:1	Soluble	Soluble
2:3	Partly soluble	Partly soluble

B. DMPA.Na Solubility in Different Ratios of DMPA.Na in Cosolvent and CH₂Cl₂ (volume ratio)

DMPA.Na Conc (mg/mL)	Different Ratios of DMPA.Na in Cosolvent and CH ₂ Cl ₂ (vol/vol)					
	DMPA.Na Cosolvent in (CH ₂ Cl ₂ : MeOH=1:1)			DMPA.Na Cosolvent in (CHCl ₃ : MeOH=1:1)		
	3:7	2:8	1:9	3:7	2:8	1:9
5	X	X	X	X	X	X
4	√	X	X	√	X	X
3	√	√	X	√	√	X
2	√	√	X	√	√	X
1	√	√	√	√	√	√

X: not soluble; √: soluble

solvent system suitable for dissolving both PLGA and DMPA.Na (Table 1).

A combination of carbon tetrachloride and methanol ($\text{CHCl}_3/\text{MeOH}$) is reportedly a good solvent system for DMPA.Na, and a combination of CH_2Cl_2 and CH_3COCH_3 has been reported as a good solvent system when PLGA is used to prepare microspheres (17). Good solvents for DMPA.Na are 1:1 ratios of both $\text{CH}_2\text{Cl}_2/\text{MeOH}$ and $\text{CHCl}_3/\text{MeOH}$ (Table 1). The maximum solubility of DMPA.Na in the solvent systems investigated was 5 mg/mL. Different ratios of CH_2Cl_2 were added to DMPA.Na in these 2 solvent systems to determine whether the DMPA.Na would remain dissolved when added to the PLGA oil phase (CH_2Cl_2) (Table 1). The higher the ratio of the DMPA.Na solvent system to CH_2Cl_2 , the more soluble the DMPA.Na. The concentration of DMPA.Na was reduced until complete solubility was achieved at all solvent ratios investigated. According to these data, 1 mg/mL (DMPA.Na) was selected for preparing microspheres.

Effect of Cosolvent on the Particle Size and Surface Charge of the Microspheres

The different solvent systems and the amount of DMPA.Na incorporated did not affect microsphere particle size (Table 2). The particle size of PLGA

microspheres following gelatin coating increased slightly. Microspheres particle size was between 3 μm and 5 μm . The particle size of PLGA microspheres is affected by the mixing conditions (stirring rate) and the concentration of PLGA. Because DMPA.Na is a small molecule and is added at a relatively low concentration during processing, it is unlikely to affect particle size. However, gelatin is added after microsphere preparation; therefore, gelatin slightly increases the microsphere particle size.

Microsphere surface charge increased with increasing concentrations of DMPA.Na (Table 2). The surface charge of the PLGA microspheres changed from negative to positive on adsorption of gelatin (Table 2). There appeared to be no concentration dependency in the DMPA.Na concentration range studied. At pH 3.14 (ionic strength 1 mM), gelatin B has a higher positive charge (19). At increasing pH values, it would be difficult to determine whether gelatin was adsorbed on the surface of microspheres since the charge of gelatin B and PLGA microspheres are similar and negatively charged (13).

Effect of DMPA.Na on Microsphere Dexamethasone Encapsulation

Dexamethasone encapsulation increased slightly with the addition of a cosolvent (MeOH) (Table 3).

Table 2. Effect of coSolvent and DMPA.Na on the Particle Size and Electrophoretic Mobility of PLGA Microspheres before and after Gelatin Coating Using Spray Dryer

Ratio of CH_2Cl_2 to DMPA.Na solvent (mL:mL)	Ratio of CH_2Cl_2 to MeOH	Mean Diameter $\pm$ SD (μm), n = 9		Electrophoretic Mobility $\pm$ SD ($\mu\text{m/s/V/cm}$), n = 9	
		DMPA.Na Microspheres	Gelatin-Coated Microspheres	DMPA.Na Microspheres	Gelatin-Coated Microspheres
5:0	10:0	3.19 $\pm$ 0.36	4.34 $\pm$ 0.18	-0.62 $\pm$ 0.31	0.62 $\pm$ 0.24
4.5:0.5	19:1	3.29 $\pm$ 0.05	3.59 $\pm$ 0.22	-0.76 $\pm$ 0.16	0.55 $\pm$ 0.40
4:1	18:2	3.50 $\pm$ 0.06	4.97 $\pm$ 0.44	-1.23 $\pm$ 0.30	0.71 $\pm$ 0.19
3.5:1.5	17:3	3.56 $\pm$ 0.03	3.63 $\pm$ 0.06	-2.1 $\pm$ 0.03	0.83 $\pm$ 0.13

SD = standard deviation

Table 3. Effect of Cosolvent and DMPA.Na on the Dexamethasone Encapsulation Efficiency of Microspheres

Ratio of CH ₂ Cl ₂ to DMPA.Na Solvent (mL:mL)	Ratio of CH ₂ Cl ₂ to MeOH	Mean E.E. $\pm$ SD (%), n = 3
5:0	10:0	72.22 $\pm$ 1.28
4.5:0.5	19:1	75.47 $\pm$ 2.33
4:1	18:2	78.50 $\pm$ 2.42
3.5:1.5	17:3	79.56 $\pm$ 0.03

EE = encapsulation efficiency; SD = standard deviation

Dexamethasone is a synthetic hydrophobic glucocorticoid with a particle size of 3 μ m to 4 μ m (13). It is soluble in MeOH and insoluble in CH₂Cl₂ and water. The relatively low encapsulation efficiency (approximately 78%) (Table 3) is considered to be a result of dexamethasone's low solubility in CH₂Cl₂ and consequent partitioning into the aqueous phase during the oil/water solvent-evaporation process. Once in the aqueous phase dexamethasone precipitated out and mixed with PLGA microspheres; this was confirmed by polarized light microscopy because unencapsulated dexamethasone crystals and precipitates were observed in the microsphere samples. Thus, it was necessary to wash unencapsulated dexamethasone from the surface of the PLGA microspheres using 2% vol/vol isopropanol in water.

Effect of DMPA.Na and Cosolvent on Amount of Gelatin Absorbed onto the PLGA Microspheres

There was no significant difference in the amount of gelatin adsorbed with or without incorporated

DMPA.Na; this did not change during the DMPA.Na concentration range studied (Table 4).

In addition, the amounts of gelatin incorporated onto the PLGA microspheres using the adsorption or conjugation methods were essentially the same (Table 4). However, the spray-dried, gelatin-coated microspheres had a much higher amount of gelatin incorporated on their surface. The increased amount of gelatin on these microspheres is probably a consequence of the preparation method, which involves drying droplets of gelatin containing the PLGA microspheres rather than surface adsorption from a gelatin solution.

Adsorption of proteins to PLGA microspheres has been reported by Rouzes et al (21-23). PLGA microspheres have a high adsorption capacity for protein and peptides; consequently, proteins or peptides are not completely released during in vitro studies. This absorption phenomenon depends on both the protein and PLGA microsphere concentrations. At low protein

Table 4. Effect of DMPA.Na on the Amount of Gelatin in PLGA Microspheres

Ratio of CH ₂ Cl ₂ to DMPA.Na Solvent (mL:mL)	Ratio of CH ₂ Cl ₂ to MeOH	Amount of Gelatin Detected (μ g/mg) Mean $\pm$ SD, n = 3		
		Gelatin Adsorbed	Gelatin Coated	Covalent Bond
5:0	10:0	107.29 $\pm$ 16.67	734.31 $\pm$ 46.04	116.71 $\pm$ 10.12
4.5:0.5	19:1	135.42 $\pm$ 10.76	743.74 $\pm$ 44.21	135.81 $\pm$ 9.24
4:1	18:2	120.40 $\pm$ 13.47	713.40 $\pm$ 40.16	154.22 $\pm$ 10.21
3.5:1.5	17:3	129.05 $\pm$ 11.69	842.55 $\pm$ 44.04	153.81 $\pm$ 10.24

SD = standard deviation

concentrations, protein-PLGA interactions favor monolayer adsorption; whereas at high protein concentrations, protein-PLGA interactions favor multilayer adsorption layer resulting from self-association of the protein. Both the adsorption and conjugation methods involve washing and centrifugation to remove unadsorbed gelatin from the aqueous phase. The heat involved in the spray-drying process ensures that the gelatin is bound to the surface of the microspheres.

Interaction Study Between Microspheres and Fibronectin

Interaction between the microspheres and fibronectin was measured using the BCA assay method. Surface modified PLGA microspheres had a higher affinity for fibronectin compared to unmodified PLGA microspheres. The fibronectin affinity of the surface-modified PLGA microspheres prepared by the adsorption and covalent conjugation methods were not significantly different (Figure 2).

This is in agreement with the amount of gelatin incorporated by these 2 methods, which was not significantly different. On the other hand, the spray-dried, gelatin-coated PLGA microspheres, which had a higher amount of gelatin incorporated, had a higher interaction with fibronectin.

Dexamethasone Release Profiles

The dexamethasone release profiles from the gelatin-surface modified PLGA microspheres were slow initially and then increased rapidly around day 10 (Figure 3). This is in agreement with other studies in our laboratory that have shown that degradation of PLGA microspheres is slow initially and accelerates around day 10 (24). There was no initial burst release of dexamethasone from the microsphere surfaces because unencapsulated and surface-associated dexamethasone were washed from the microspheres during preparation.

Surface Morphology

The PLGA microspheres were spherical with smooth surfaces. There was no change in the appearance of the microspheres on addition of DMPA.Na or gelatin and following spray drying (Figure 4).

Figure 2. Effect of DMPA.Na and surface modification process on PLGA microsphere interaction with fibronectin (pH 7.4, PBS, 37°C, n = 3).

Figure 3. Dexamethasone release profile (PBS, at pH 7.4, 37°C, stirred at 100 rpm, gelatin-coated PLGA microspheres, n = 3).

A. Before gelatin coating.

B. After gelatin coating using spray-drying.

Figure 4. The morphology of the microspheres using ESEM.

CONCLUSIONS

Small-sized, gelatin, surface-modified PLGA microspheres were prepared. A cosolvent system (MeOH and CH₂Cl₂) was used to incorporate phospholipid (DMPA.Na) into the PLGA microspheres, which resulted in a negative surface charge. Surface treatment with gelatin changed the PLGA microsphere surface charge to positive. An MeOH and CH₂Cl₂ cosolvent system increased dexamethasone encapsulation. The gelatin adsorption and conjugation methods resulted in low and similar amounts of gelatin being incorporated onto the surfaces of PLGA microspheres. The spray-drying method resulted in a thick coating of gelatin; consequently, these microspheres had a more favorable interaction with fibronectin compared to the microspheres that were surface treated using the gelatin adsorption and conjugation methods. Gelatin-coated PLGA microspheres containing dexamethasone had a slow release profile over 21 days. The developed formulations had particle size distribution ranges from 1 µm to 7 µm and were intended for localized delivery to affected blood vessels during balloon angioplasty or stenting to help prevent restenosis of the blood vessels at the affected sites.

REFERENCES

- Perrin DA, English JP. Polyglycolide and polylactide. In: Domb AJ, Kost J, Wiseman DM, eds. *Handbook of Biodegradable Polymers*. Amsterdam: Harwood Academic Publishers;1997:3-25.
- Watts PJ, Davies MC, Melia CD. Microencapsulation using emulsification/solvent evaporation: an overview of techniques and applications. *Crit Rev Ther Drug Carrier Syst*. 1990;7:235-259.
- Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv Drug Deliv Rev*. 1997;28:5-24.
- Leach KJ. Cancer, drug delivery to treat- local & systemic. In: Mathiowitz E. ed. *Encyclopedia of Controlled Drug Delivery*. Vol 1. New York: John Wiley & Sons;1999:119-142.
- Scholes PD, Coombes AGA, Davies MC, Illum I, Davis SS. Particle engineering of biodegradable colloids for site-specific drug delivery. In: Park K, ed. *Controlled Drug Delivery: Challenges and Strategies*. Washington, DC: American Chemical Society;1997:73-106.
- Stolnik S, Dunn SE, Gamett MC, et al. Surface modification of poly(lactide-co-glycolide) nanospheres by biodegradable poly(lactide)-poly(ethylene glycol) copolymers. *Pharm Res*. 1994; 11:1800-1808.
- Groves MJ. Parenteral drug delivery systems. In: Mathiowitz E. ed. *Encyclopedia of Controlled Drug Delivery*. Vol 1. New York: John Wiley & Sons;1999:743-777.
- Lou Y, Olson WP, Tian XX, Klegerman ME, Groves MJ. Interaction between fibronectin-bearing surfaces and *Bacillus Calmette-Guerin* (BCG) or gelatin microparticles. *J Pharm Pharmacol*. 1995;47:177-181.
- Colvin RB. Fibronectin in wound healing. In: Mosher DF, ed. *Fibronectin*. San Diego: Academic Press;1989:201-218.
- Carsons S, Lavietes BB, Diamond HS. Role of fibronectin in rheumatic diseases. In: Mosher DF, ed. *Fibronectin*. San Diego: Academic Press;1989:218-232.
- Hynes RO. *Fibronectins*. New York: Springer-Verlag;1990:113-145.
- Thompson PN, Cho E, Blumenstock FA, Shah DM, Saba TM. Rebound elevation of fibronectin after tissue injury and ischemia: role of fibronectin synthesis. *Am J Physiol*. 1992;263(4 Pt 1):G437-G445.
- Tsung M, Burgess DJ. Preparation and characterization of heparin/gelatin microspheres. Submitted to *J Pharm Pharmacol*, 2001.
- Dev V, Eigler N, Fishbein MC, et al. Sustained local drug delivery to the arterial wall via biodegradable microspheres. *Cathet Cardiovasc Diagn*. 1997;41:324-332.
- Labhasetwar V, Song C, Levy RJ. Nanoparticle drug delivery system for restenosis. *Adv Drug Deliv Reviews*. 1997;24:63-85.
- Humphreya WR, Erickson LA, Simmons CA, et al. The effect of intramural delivery of polymeric nanoparticles loaded with the antiproliferative 2-aminochromone U-86983 on neointimal hyperplasia development in balloon-injured porcine coronary arteries. *Adv Drug Deliv Rev*. 1997;24:87-108.
- Bodmeier R, McGinity JW. Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method. *Int J Pharm*. 1988;43:179-186.
- Cohen EM. Dexamethasone [9 -fluoro-11 ,17 ,21-trihydroxy-16 -methyl pregna-1,4-diene-3,20-dione]. *Anal Profiles Drug Subst*. 1973;2:163-197.
- Tsung M, Burgess DJ. Preparation and stabilization of heparin/gelatin complex coacervate microcapsules. *J Pharm Sci*. 1997;86:603-607.
- Lamielle D, Vistelle R, Millart H, et al. High-performance liquid chromatographic determination of dexamethasone in human plasma. *J Chromatogr*. 1986;378:486-491.
- Calis S, Jeyanthi R, Tsai T , Mehta RC, DeLuca PP. Adsorption of salmon calcitonin to PLGA microspheres. *Pharm Res*. 1995;12:1072-1076.
- Rouzes C, Gref R, Leonard M, Delgado AD, Dellacherie E. Surface modification of poly(lactic acid) nanospheres using hydrophobically modified dextrans as stabilizers in an o/w emulsion/evaporation technique. *J Biomed Materials Res*. 2000;50:557-565.
- Nam YS, Song SH, Choi JY, Park TA. Lysozyme microencapsulation within biodegradable PLGA microspheres: urea effect on protein release and stability. *Biotechnol Bioengineering*. 2000;70:270-277.
- Kim TA, Burgess DJ. Formulation and release characteristics of poly(lactic-co-glycolic acid) microspheres containing chemically modified protein. *J Pharm Pharmacol*. 2001;53:23-31.