PLG Microsphere Size Controls Drug Release Rate through Several Competing Factors

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Purpose. Although the rate of drug release from poly(D,L-lactide–co-glycolide) (PLG) microspheres is often modulated by changing fabrication conditions or materials, the specific factors directly controlling the release profiles are often unclear. We have fabricated uniform rhodamine- and piroxicam-containing microspheres, 10 to 100 μm in diameter, to better understand how microsphere size controls drug release.

Methods. Drug distribution within the microspheres was examined using confocal fluorescence microscopy. The rate of polymer degradation was determined as the change in molecular weight, measured by gel permeation chromatography, during *in vitro* degradation experiments. Further, changes in the surface and interior morphology of the particles during *in vitro* degradation were investigated by scanning electron microscopy.

Results. Microsphere size greatly affected drug distribution. Small (~10-μm) microspheres showed an essentially uniform drug distribution. Larger (~100-μm) microspheres showed redistribution of drug to specific regions of the microspheres. Rhodamine partitioned to the surface and piroxicam partitioned to the interior of large PLG microspheres. Further, the rate of polymer degradation increased with microsphere size, possibly the result of a more acidic interior caused by increased accumulation of hydrolyzed polymer products in larger particles. Finally, larger microspheres developed a more porous interior structure during the drug release.

Conclusions. Microsphere size affects drug release not only through changes in diffusion rates but also through secondary effects including drug distribution in the particle, polymer degradation rate, and microsphere erosion rates.

KEY WORDS: controlled release; zero-order release; uniform microspheres; poly(lactide–co-glycolide); piroxicam.

INTRODUCTION

Poly(D,L-lactide–co-glycolide) (PLG) microspheres have been widely investigated as delivery devices for a variety of therapeutics because they have several important advantages over conventional dosage forms. For example, biodegradable polymer microspheres that can deliver a therapeutic at a constant rate over a prolonged time following a single administration can avoid peak-related side effects, aid patient comfort and compliance, provide localized drug delivery and high local drug concentrations, and potentially optimize efficacy. More importantly, the rate at which a drug is delivered can impact its efficacy as much as the identity of the drug molecule itself. The rate of drug release from biodegradable polymer devices can be controlled by the size of the device (1–5), the degradation kinetics of the polymer (in turn a function of polymer composition, tacticity, molecular weight, etc.) (5–7), and by variation of several of the important parameters used in the formulation process (8–10). Existing devices can release drug over periods from days to months, and the delivery rate can be adequately controlled for many applications. However, there still exists a need for microsphere systems that can easily and reproducibly provide control of delivery rates including "zero-order" and "pulsatile" release kinetics.

The size of biodegradable polymer microspheres is well known to be a primary determinant of polymer degradation and drug release rates. PLG degradation occurs by hydrolysis of the ester bonds and can be autocatalyzed by the accumulation of acidic degradation products (11). As particle size increases, surface area:volume ratio decreases, which decreases both buffer penetration and release of degradation products. Thus, larger particles exhibit a more acidic intrapolymer pH microenvironment (12) and degrade more rapidly (13). Because the mechanism of drug release is typically diffusion through the polymer phase or through aqueous-filled pores in the polymer matrix, the decrease in surface area:volume ratio with increasing particle diameter translates into a decrease in drug release rate. Furthermore, the size of the particles can impact the kinetics of the fabrication process. For example, in particles formed by solvent extraction, smaller particles are expected to harden faster (because of their larger surface area:volume ratio), which may impact the structure of the polymer matrix and the distribution of drug within the particle. Although a number of studies of the effects of microsphere size on release rates have appeared in recent years (1–5), these various competing effects have been difficult to discern because of the nonuniformity in the size of microspheres prepared by most existing fabrication methods.

We have reported a novel methodology for fabrication of highly uniform polymer microparticles (14). With this process, we have generated monodisperse PLG microspheres from ~1 to >500 μm in diameter; in particular, we reported microspheres with diameters of 5–80 μm wherein the diameters of 95% of the particles were within 1.0–1.5 μm of the average. When small-molecule drug mimics were encapsulated in and released from these uniform microspheres, we found that the particle size impacted the release rates as expected, but also the size had a large effect on the shape of the release rate profiles (15). Release profiles (cumulative amount released vs. time) from microspheres smaller than ~20 μm were concave downward, typical of diffusion-controlled release. However, release profiles from microspheres larger than ~40 μm were sigmoidal. Such a shape cannot be explained by diffusion alone. We hypothesized that a time-dependent increase in the effective drug diffusivity, caused by degradation of the polymer chains, could account for the sigmoidal shape. In fact, we have shown that a simple model of Fickian diffusion of drug from the particles incorporating an exponentially increasing diffusivity [$D_{eff}(t) = D_{eff}(0)\cdot\exp(kt)$, where k is a constant characterizing the polymer degradation rate] provides an accurate fit for release of piroxicam from 10-, 50-, and 100-μm diameter PLG microspheres (16). However, several other confounding factors

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could result in the change from concave-downward to sigmoidal release rate profiles.

In the present work, we examine several of the mechanisms by which microsphere size can impact drug release rate for the two model compounds examined previously, rhodamine and piroxicam (15). Our ability to fabricate monodisperse particles allows us to investigate these effects without interference from the broad size distributions typical in previous studies. We have examined the effects of particle size on polymer degradation rate and investigated the erosion of the various-sized microspheres by SEM. In addition, we noted a distinct difference in the distribution of drug within the microsphere as a function of both sphere size and the type of drug molecule encapsulated.

MATERIALS AND METHODS

Materials

Rhodamine B chloride was purchased from Sigma. Piroxicam was a gift from Dongwha Pharmaceuticals (Seoul, Korea). Poly(D,L-lactide–co-glycolide) copolymer (50:50 lactic acid:glycolic acid; i.v. = 0.20–24 dL/g corresponding to M_w ~15,000) was purchased from Birmingham Polymers. We purchased 88% hydrolyzed poly(vinyl alcohol) (PVA) from Polysciences, Inc. HPLC grade dichloromethane (DCM), dimethylsulfoxide, and sodium hydroxide were purchased from Fisher Scientific.

Microsphere Preparation

We fabricated uniform PLG microspheres using technology previously reported (14). Briefly, PLG (5% w/v) was codissolved with rhodamine B or piroxicam in DCM at various theoretical drug loadings as indicated. Drug-loaded polymer solutions were sprayed through a small hypodermic needle. The ejected polymer was acoustically excited using an ultrasonic transducer (Branson Ultrasonics) controlled by a frequency generator (Hewlett Packard model 3325A) resulting in regular jet instabilities, which broke the stream into uniform polymer/drug/solvent droplets. A coaxial nozzle produced an annular carrier stream (~1% w/v PVA in distilled water), which surrounded the emerging PLG jet. The coaxial streams flowed into a beaker containing approximately 500-mL of 1% PVA. Nonuniform microspheres were fabricated using a traditional stirred emulsion method wherein 2 mL of polymer solution was added to 50 mL of 1% PVA and stirred for 30 s using a stir bar and Corning stir plate. To facilitate solvent extraction, an additional 50 mL of PVA solution was then added. In all cases, nascent PLG drops were stirred for approximately 3 h, filtered, and rinsed with an equal volume of distilled water to remove residual PVA. Finally, microspheres were freeze dried (Labconco benchtop model) for 2 days and stored at –20°C under desiccant.

Drug Loading

Rhodamine B loading was determined by dissolving a known mass (~2–5 mg) of microspheres in 50 μL dimethylsulfoxide. PBS (500 μL) was added, and precipitated polymer was removed by centrifugation at 12,000 rpm for 10 min. The concentration of rhodamine in the supernatant was determined by measuring the absorbance at 550 nm in a multiwell plate spectrophotometer (Molecular Devices Spectra Max 340PC).

Piroxicam loading was also determined by dissolving a known mass (~5 mg) of microspheres in 1 mL of 0.25 M sodium hydroxide at room temperature for 5 min. Blank (piroxicam-free) microspheres of the same size were treated identically. The concentration of piroxicam in the resulting solution was determined by measuring the absorbance at 276 nm (Varian Cary 50) in a quartz cuvette then subtracting absorbance values for the blank microspheres.

In Vitro Drug Release

In vitro release of rhodamine was determined by resuspending ~5 mg of microspheres encapsulating rhodamine B in 2 mL of phosphate-buffered saline (PBS, pH 7.4) containing 0.5% Tween. Microsphere suspensions were continuously inverted at ~10 revolutions per minute in a 37°C incubator. Samples were centrifuged, the supernatant was removed, and the spheres were resuspended in fresh PBS at various time points. Rhodamine B concentration was determined by measuring the supernatant absorbance at 550 nm as described above. Rhodamine release was summed with the amounts at each previous time point, and the total divided by the amount of rhodamine in the microspheres (experimental loading times mass of microspheres), to arrive at the "cumulative percent released."

In addition, piroxicam release was determined by resuspending ~5 mg of microspheres in 1.3 mL of PBS containing 0.5% Tween. Release conditions were analogous to those described above for rhodamine-containing microspheres. After removal of the supernatant, the concentration of piroxicam was determined by measuring the absorbance at 276 nm. The addition of 0.5% Tween increased piroxicam solubility to ~1 mg/mL and helped to maintain adequate sink conditions. The average absorbance of the supernatant from tubes containing blank microspheres treated identically was subtracted from all piroxicam measurements.

Microsphere Degradation Study

The effect of microsphere size, drug loading, and drug type on polymer microsphere morphology was determined by examining microspheres at various time points during drug release. Microsphere samples for tracking PLG molecular weight were prepared by adding ~5 mg of blank, 15% (w/v) piroxicam, or 3% (w/v) rhodamine-loaded microspheres of various sizes to 1.3 mL of PBS containing 0.5% Tween to mimic *in vitro* release conditions. The suspensions were continuously agitated by inversion (at ~10 revolutions per minute) in a 37°C incubator. Supernatant was removed and discarded at regular time intervals during the duration of the study. At the appropriate time (0, 3, 6, 9, or 12 days), the ~5 mg microsphere suspensions were removed from the incubator and placed in a –20°C freezer. Once the study was completed, the microsphere suspensions were thawed. Microspheres were centrifuged, the supernatant was discarded, and samples were freeze dried (Labconco benchtop model).

Polymer molecular weight was determined by Triple Detector Size Exclusion Chromatography (TriSEC). TriSEC measurements were performed with a Waters 515 HPLC pump, Spectraseries AS100 autosampler, Viscotek model 300

triple detector array, and a series of three Polymer Laboratories Plgel, 10-μm pore size, mixed bed, light scattering (7.8 × 300 mm) columns. Molecular weight data were determined using Viscotek's TriSEC software. The light scattering (670 nm, RALLS), mass, and viscosity constants were determined from a single 90 kDa narrow polystyrene standard and checked against other known polystyrene standards for accuracy. TriSEC data were obtained in chloroform at 30°C and a flow rate of 1.0 mL/min.

Confocal Microscopy

Drug distribution within PLG microspheres was determined by confocal microscopy before *in vitro* release. A small amount (~1 mg) of each microsphere size and drug type was suspended in distilled water, placed on Petri dishes, and dried overnight. Microspheres were then imaged using an Olympus Fluoview FV300 Laser Scanning Biologic Microscope. Rhodamine was excited with a krypton laser, and piroxicam was exited with a helium/neon laser. Optical cross-sections were taken at various depths for each microsphere size and drug type to determine drug distribution at the center of the microsphere.

Scanning Electron Microscopy

Scanning electron microscopy (Hitachi S-4700) was used to image the surface and interior morphology of the microspheres. A droplet of an aqueous microsphere suspension was placed directly onto a scanning electron microscopy sample holder. The samples were freeze dried overnight (Labconco benchtop model) and then chopped with a razor blade immediately on removal. Samples were sputter coated with gold before imaging at 2–10 eV.

Particle Size Distribution

A Coulter Multisizer 3 (Beckman Coulter Inc.) equipped with a 100-μm or 280-μm aperture was used to determine the size distribution of the various sphere preparations. Lyophilized particles were resuspended in Isoton electrolyte with a dispersant to prevent aggregation when necessary. A minimum of 5000 microspheres was analyzed for each distribution.

RESULTS

Preparation of Uniform Microspheres

Microspheres having a uniform diameter were produced according to previously reported methods (14). To investigate the influence of microsphere size on factors affecting drug release kinetics, we fabricated microspheres of different diameters over a size range applicable to subcutaneous or intramuscular administration (~5-150 μm). Uniform 10-, 20-, 40-, 50-, 65-, and 100-μm diameter microspheres encapsulated rhodamine at 1, 3, and 5% (w/w). In addition, 10-, 50-, and 100-μm diameter microspheres encapsulated piroxicam at 5, 10, and 15% (w/w). The microspheres exhibited a very tight size distribution as compared to conventional production techniques (Fig. 1). Nonuniform microspheres, exhibiting a mean diameter of 50 μm, were also fabricated for comparison.

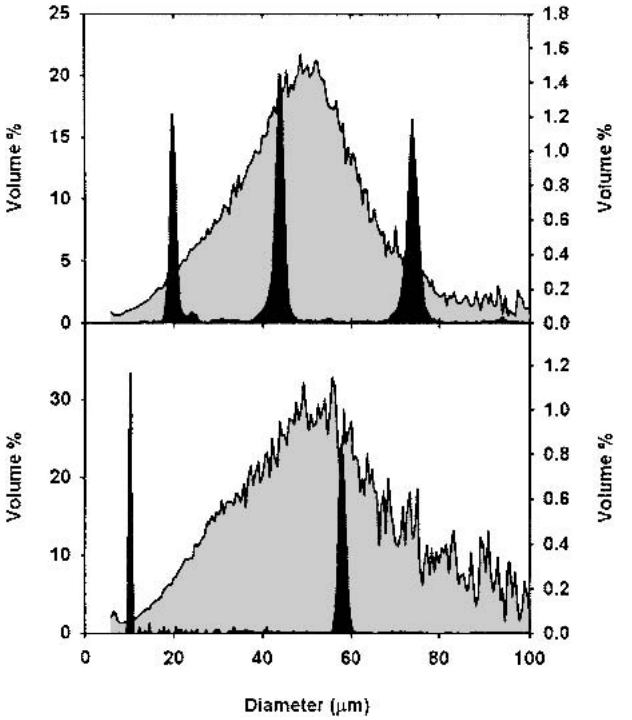

Fig. 1. Representative Coulter size distributions of microspheres loaded with rhodamine (A) and piroxicam (B) prepared using precision particle fabrication compared to microspheres fabricated using conventional methods. Left axis indicates volume percent for uniform microspheres (black), and right axis indicates volume percent for nonuniform microspheres (gray).

In Vitro Release of Rhodamine and Piroxicam from Uniform and Nonuniform Microspheres

In vitro studies were conducted to measure the amount of drug released from microspheres of various sizes encapsulating various amounts of either rhodamine or piroxicam. Theoretical drug loading and encapsulation efficiency for each drug type and microsphere size are summarized in Table I. Release kinetics were shown to depend strongly on microsphere size (Fig. 2). Small microspheres exhibited a rapid initial release (Fig. 2A,E), and increasing microsphere size caused a transition toward sigmoidal drug release profiles (Fig. 2C,G) for microspheres encapsulating both rhodamine and piroxicam. When like microsphere sizes were compared, the rate of drug release increased with increasing drug loading for microspheres encapsulating rhodamine. However, the rate of drug release decreased as drug loading increased for microspheres encapsulating piroxicam.

Regardless of the drug encapsulated, release from uniform microspheres followed a smoother and more regular release profile than nonuniform microspheres. Rhodamine release from nonuniform particles showed multiple phases of increasing and decreasing rates. In addition, drug release from nonuniform microspheres showed a burst of approximately 10–20% in the first several hours, whereas release from uniform microspheres showed no sign of initial drug burst.

Effect of Particle Size on Polymer Degradation Rates

To investigate the difference in the drug release profiles of different diameter microspheres, we first looked at the

Table I. Encapsulation Efficiency of Rhodamine and Piroxicam in Uniform PLG Microspheres of Different Sizes

Loaded drug	Microsphere size (μm)	Theoretical loading	Encapsulation efficiency
Rhodamine B	20	1%	63%
		3%	60%
		5%	50%
	40	1%	37%
		3%	35%
		5%	35%
	65	1%	61%
		3%	43%
		5%	60%
Piroxicam	10	5%	59%
		10%	46%
		15%	37%
	50	5%	19%
		10%	10%
		15%	10%
	100	5%	20%
		10%	31%
		15%	20%

effect of microsphere size and drug loading on polymer degradation. Ten-, 50-, and 100-μm rhodamine-loaded microspheres and 10-, 50-, and 100-μm piroxicam-loaded microspheres underwent *in vitro* degradation, analogous to conditions reported for drug release experiments, and polymer molecular weight was analyzed using TriSEC (see Materials and Methods). The degree of molecular weight loss varied according to the type of drug encapsulated and the microsphere size (Fig. 3). Blank (drug-free) and piroxicam-loaded microspheres exhibited a negligible loss of molecular weight after 2 weeks. The molecular weight loss of rhodamine-loaded microspheres was significantly faster. Microsphere size also affected the rate of polymer degradation. Larger, 100-μm microspheres showed a more rapid loss of molecular weight than smaller, 10-μm microspheres, which was especially evident in the case of rhodamine-loaded particles.

Effect of Particle Size on Drug Distribution

Drug distribution before *in vitro* drug release was determined via confocal fluorescence microscopy. Optical cross sections confirmed the presence of each drug inside the polymer matrix. Drug distributions were determined at the particle center for each microsphere size and drug load (Fig. 4). Rhodamine was found to preferentially distribute to the surface, and the effect became more prominent as microsphere size increased. Also, the drug seemed to remain finely distributed, showing no evidence of large rhodamine crystals, in the final hardened microsphere. Piroxicam behaved in the opposite manner, with the drug partitioning preferentially to the interior in increasing amounts as microsphere size increased. In the 100-μm microspheres, piroxicam tended to form large crystals visible under transmitted light microscopy. Both drugs were most evenly distributed in the smallest microspheres.

Visual Evidence of Polymer Degradation and Erosion

Scanning electron micrographs of representative microspheres for each size/drug type combination reveal the change in microsphere surface and interior morphology over the duration of *in vitro* drug release for each formulation. No noticeable difference in microsphere appearance was noted for different loadings of piroxicam or rhodamine over the loading range used for each drug in this experiment; therefore, differences in drug loading were not considered (data not shown). The images presented in Figs. 5 and 6 include, 20-, 40-, and 65-μm rhodamine-loaded microspheres and 10-, 50-, and 100-μm piroxicam-loaded microspheres, respectively. For each sample, it is important to note the time at which drug release is completed because termination of drug release could result in a change in microsphere morphology. Such changes were especially apparent in the rhodamine-loaded, 20-μm spheres, which develop large indentations and pores initially (Fig. 5, day 3) and then appear to revert to a smooth morphology after rhodamine release is complete (Fig. 5, day 6) (17).

Microsphere size had only small effects on surface and interior morphology of the microspheres over the time frame studied. When similar time points are compared, it appears that large microspheres may erode more quickly than smaller microspheres as the TriSEC data indicate (Fig. 5, 40- and 65-μm particles, day 6; and Fig. 6, 50- and 100-μm particles, day 12). Comparing the interior morphologies of these microspheres reveals a more porous interior in the larger microspheres, although the microsphere surface morphologies seem to be similar.

The effect of drug type on microsphere morphology was more pronounced. Rhodamine-loaded microspheres tended to show large, smooth indentions on their surface, whereas piroxicam-loaded microspheres exhibit a coarse, rippled surface (compare Fig. 5, 40 μm, and Fig. 6, 50 μm). Piroxicam-loaded microspheres gradually develop small, interior pores, whereas rhodamine-loaded microspheres exhibit some relatively large pores, which sometimes appear to collapse after drug release is complete (compare Fig. 5, 20 μm, and Fig. 6, 100 μm). Finally, in rhodamine-loaded microspheres, erosion of the polymer matrix in the interior of the microsphere is evident after only 3 days for all three microsphere sizes (Fig. 5). However, microspheres containing piroxicam show little or no development of interior pores until day 6 (Fig. 6).

DISCUSSION

Our ability to produce uniform PLG microspheres encapsulating two very different drugs has provided an opportunity to more precisely study the effect of microsphere size on variables controlling drug release such as drug distribution, polymer degradation, and microsphere erosion. A number of factors can contribute to the release of drug from bulk eroding polymer microspheres including diffusion of drug through the polymer matrix (17,18), desorption of drug adsorbed to the microsphere surface (19), and erosion of the polymer matrix releasing drug for subsequent diffusion from the surface or through developing pores (20,21). After our investigation of the effect of microsphere size on drug release, however, it appeared that the large differences in release profiles were in part caused by additional factors, such as polymer degradation kinetics and drug distribution, that are also affected by microsphere size.

Nonuniform microspheres were used for comparison to uniform microspheres to demonstrate how the fabrication of

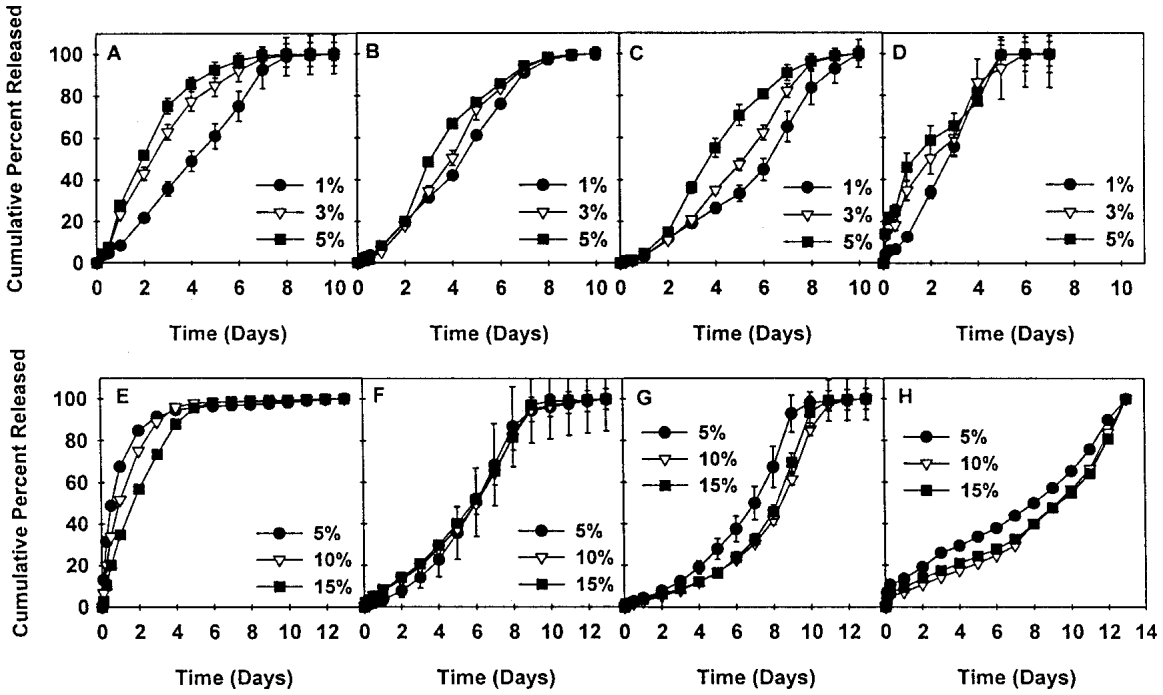

Fig. 2. *In vitro* release profiles for (A) 20-, (B) 40-, (C) 65-μm, and (D) nonuniform rhodamine-loaded microspheres and (E) 10-, (F) 50-, (G) 100-μm, and (H) nonuniform piroxicam-loaded microspheres. Theoretical drug loading (wt drug/wt polymer) is indicated in each legend.

uniform microspheres impacts drug release kinetics. Nonuniform microspheres often release drug in two distinct phases, an initial drug "burst" followed by a period of more regular, sustained release of drug (17,18). Drug release from uniform microspheres does not show a burst but rather follows smooth and regular drug release profiles, the shapes of which are dictated by microsphere size. Irregular release from nonuniform microspheres could be explained as a weighted combination of the release profiles resulting from the various microsphere sizes comprising the nonuniform samples (Fig. 1). Traditional fabrication methods often produce a substantial fraction of microspheres less than ~5–10 μm in diameter. One possible explanation for two-phase drug release from nonuniform microspheres is that these small microspheres, which release drug more rapidly as diameter decreases, are encapsulating a sufficient amount of drug to cause this drug "burst" (Fig. 2). Larger microspheres (>10 μm) are then primarily responsible for drug release beyond the first few days for such formulations. Other researchers have reported drug release profiles with a reduced or eliminated drug "burst" as a result of removing the smaller microspheres (1–5).

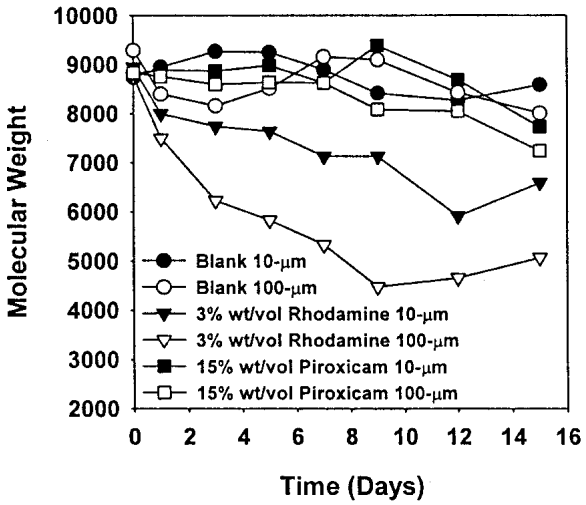

Fig. 3. *In vitro* PLG degradation profiles of blank, 3% rhodamine-, and 15% piroxicam-loaded (wt drug/wt polymer) microspheres.

To study the effect of microsphere size on polymer degradation, we fabricated 10- and 100-μm blank, rhodamine-loaded, and piroxicam-loaded microspheres and tracked the molecular weight loss of PLG with time during *in vitro* incubation at 37°C (Fig. 3). The most obvious result from this study was the higher rate of polymer degradation for rhodamine-loaded microspheres compared to the other formulations. Rhodamine and piroxicam exhibit very different solubility in water: ~8 mg/mL and <100 μg/mL, respectively, at neutral pH (22). The increased rate of PLG degradation observed for rhodamine-loaded microspheres may be the result of increased water penetration facilitated by the highly water-soluble rhodamine. On the contrary, piroxicam-loaded microspheres degrade more slowly, as do unloaded microspheres, apparently because of decreased water penetration.

Microsphere size also affected the rate of PLG degradation. PLG molecular weight decreased from 9 kDa to around 6.5 kDa for 10-μm microspheres and to approximately 4.5 kDa for 100-μm microspheres encapsulating 3% rhodamine after 10 to 14 days. Previous literature has reported a decreasing pH over time for PLG microspheres as a result of the buildup of lactic and glycolic acids (11–13). Smaller microspheres would offer a high surface area:volume ratio and shorter diffusion distance, allowing more rapid release of these acids. However, large 100-μm microspheres may accumulate the acids, increasing the rate of autocatalytic polymer degradation.

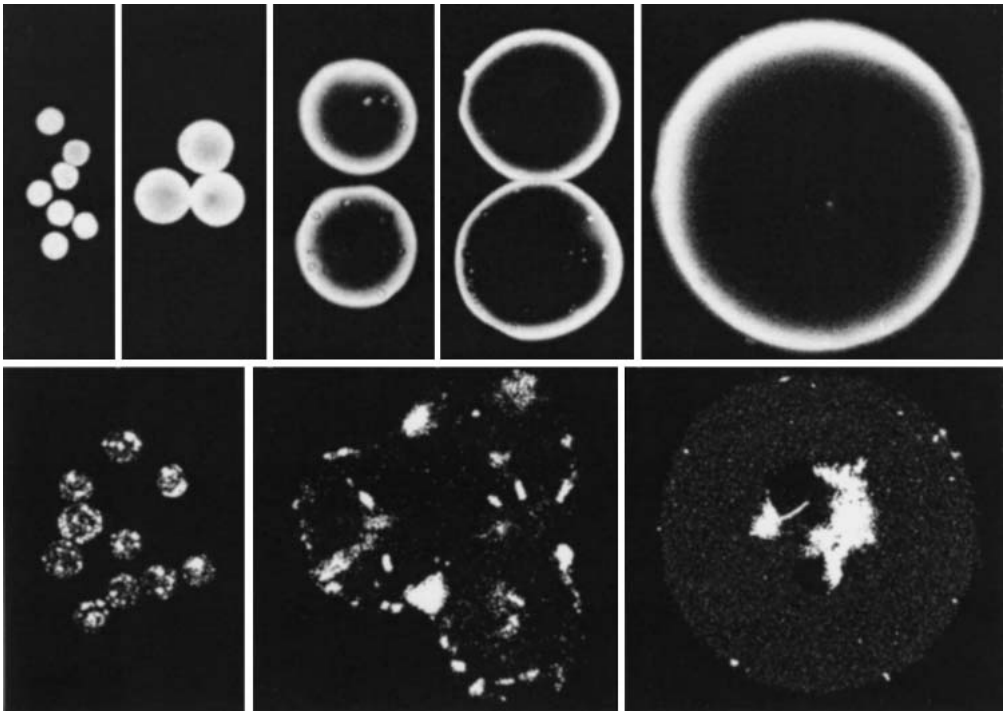

Fig. 4. Laser scanning confocal microscopy cross sections through the midline of 10-, 20-, 40-, 65-, and 100-μm rhodamine-loaded microspheres (top row), revealing increasing surface distribution of rhodamine as microsphere diameter increases. Similar cross sections of 10-, 50-, and 100-μm, piroxicam-loaded microspheres (bottom row) reveal increasing amounts of piroxicam in the microsphere interior as diameter increases.

Interestingly, microsphere size directly affected drug distribution within the polymer matrix as well (Fig. 4). Confocal microscopy verifies drug locale by taking a fluorescent cross-section at the midline of each microsphere size. As microsphere size increased, rhodamine tended to be preferentially located at the microsphere surface, whereas piroxicam shifted deeper to the microsphere interior. In addition, smaller 10-μm microspheres tended to exhibit higher drug encapsulation efficiency (Table I). These results suggest that microsphere size is a primary determinant of polymer phase-inversion rate. Faster polymer phase-inversion rate in 10-μm microspheres allows entrapment of a larger fraction of the drug, resulting in a more even drug distribution. Larger microspheres, 50 to 100 μm, which phase invert more slowly, allow time for drug to diffuse out of the particle and for redistribution of codissolved drug according to the drug's affinity for the aqueous phase.

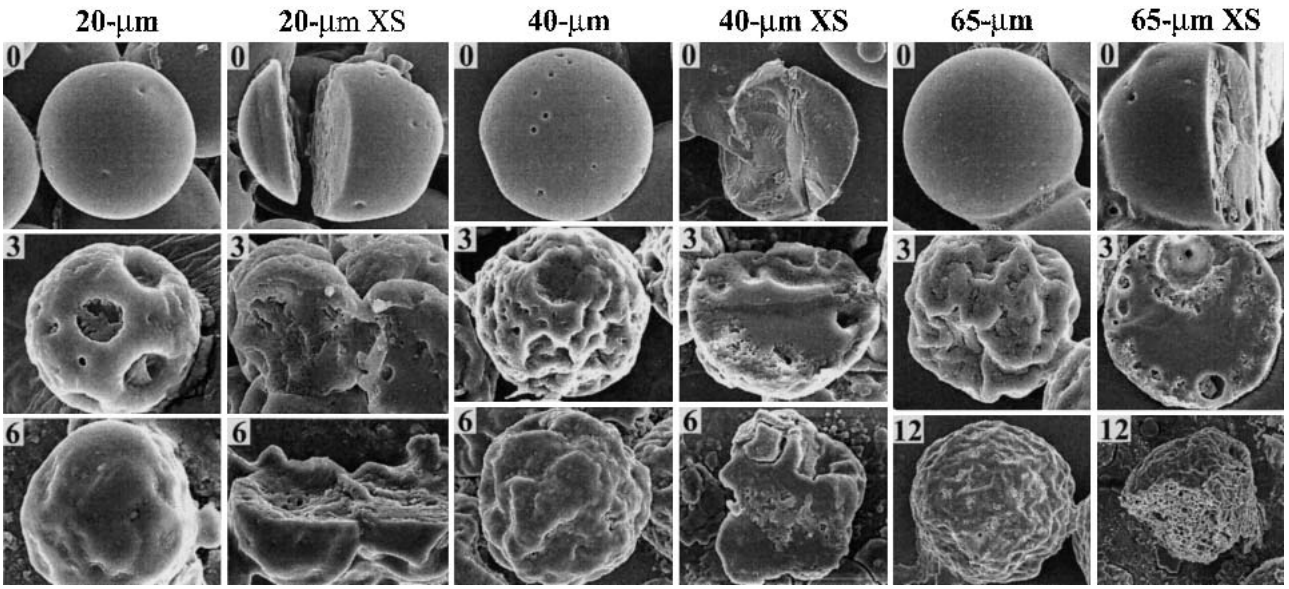

Fig. 5. Scanning electron micrographs of whole and cross-sectioned (XS) rhodamine-loaded microspheres during *in vitro* degradation. Numbers in the insets indicate the time point, in number of days, at which the samples were imaged. Microsphere size is indicated at the top of each column, and the magnification is the same for all images of the same microsphere size.

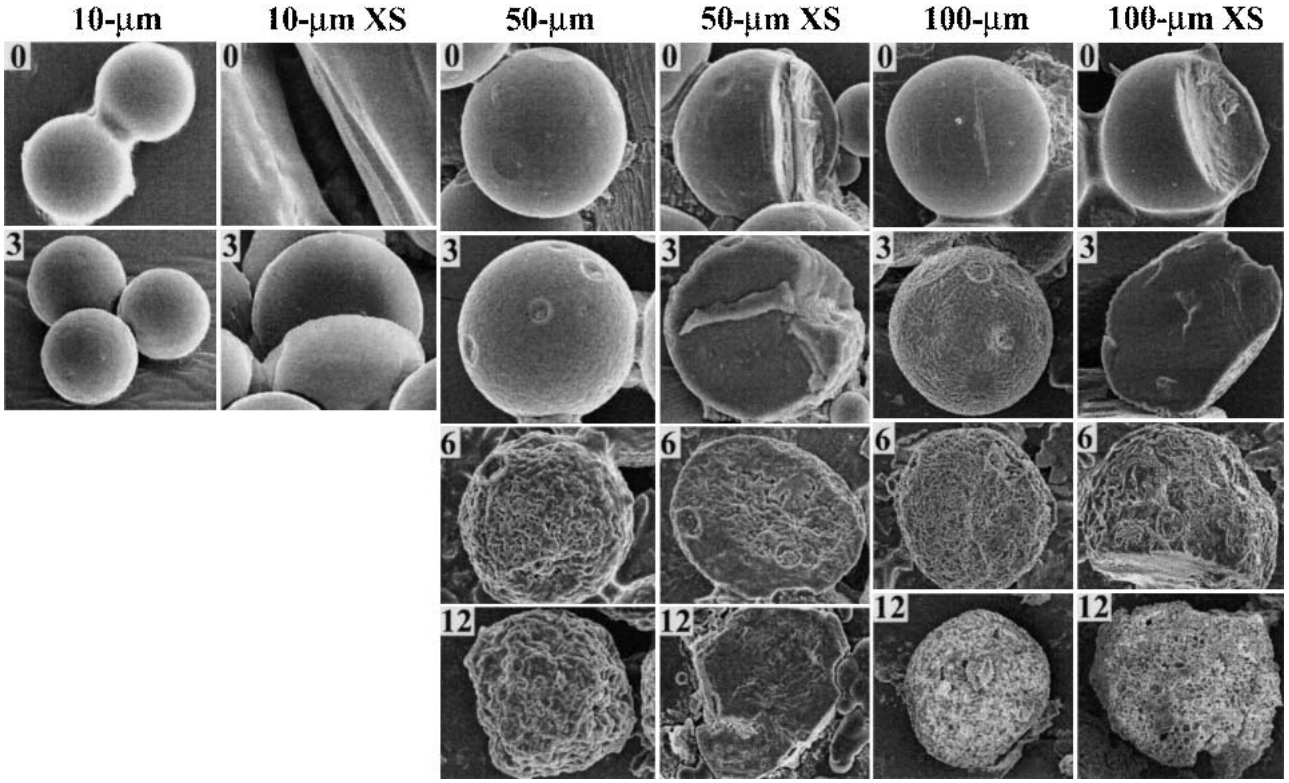

Fig. 6. Scanning electron micrographs of whole and cross-sectioned (XS) piroxicam-loaded microspheres during *in vitro* degradation. Numbers in the insets indicate the time point, in number of days, at which the samples were imaged. Microsphere size is indicated at the top of each column, and the magnification is the same for all images of the same microsphere size.

Following this logic, methods that decrease polymer phase-inversion time or reduce the mobility of drug in the nascent polymer solution should increase drug loading while providing a more uniform drug distribution.

To summarize the results thus far, microsphere size affects release both directly, via decreased surface area:volume ratio with increasing size, and indirectly, via increased partitioning of drug to the surface (or core) with increasing size and more rapid polymer degradation in larger microspheres. The effect of surface area:volume ratio on release is to decrease the drug release rates with increasing microsphere size. Superimposed on this is the effect of the drug distribution. Rhodamine partitions toward the surface. The decreased diffusion distance would be expected to increase rhodamine's release rate in comparison to particles of the same size but with a uniform drug distribution. In this case, the effects of surface area:volume ratio and drug redistribution work against one another, and the overall effect of microsphere size on release rate profiles is muted. The increased degradation rate of polymer in larger microspheres adds slightly to the release rate. Piroxicam, in contrast, partitions toward the core, increasing the diffusion distance as microsphere size increases. Thus, the effects of decreasing surface area:volume ratio is accentuated by drug redistribution. Indeed, drug partitioning, in addition to time-dependent diffusivity (16), may be responsible for the sigmoidal release profile observed for piroxicam release from microspheres >50 μm in diameter.

The SEM investigation of microsphere surface and interior morphology during degradation and release corroborates the explanations offered thus far. Early pore formation (days 3–6) apparent in rhodamine-loaded microspheres provides additional evidence of an increased rate of water penetration as compared to delayed (day 12) formation of smaller pores in piroxicam-loaded microspheres. Also, increased pore formation for larger microspheres suggests an increased polymer degradation and erosion rate, in agreement with the chromatographic analysis of PLG degradation. The notable early formation of large pores in rhodamine-loaded microspheres that subsequently collapse and/or are covered with a dense polymer "skin" (17) may be a result of the high concentration of rhodamine distributed toward the surface of these microspheres. The increased surface concentration of hydrophilic rhodamine may allow rapid water uptake translating into rapid polymer degradation forming a porous structure near the surface. Continued polymer degradation at or near the surface lowers polymer glass transition temperature in that regime, producing a malleable PLG, which collapses to the porous interior or forms a dense skin covering the eroding interior.

CONCLUSIONS

Microsphere size is an effective means of controlling drug release kinetics. Primarily, microsphere size determines the surface area:volume ratio, thereby dictating the amount of surface available for releasing drug via diffusion. However, microsphere size affects drug release by several secondary effects as well. By studying a range of microsphere sizes from ~10 to ~100 μm, we have verified that large microspheres degrade more quickly than small microspheres, probably because of an increased buildup of the acidic byproducts of polymer hydrolysis in large microspheres. SEM showed in-

creased PLG erosion in larger microspheres even when loading drugs that resulted in very different degradation rates such as rhodamine and piroxicam. Finally, microsphere size affected the distribution of drug inside the polymer matrix, with smaller microspheres exhibiting more uniformly distributed drug. The ability to fabricate uniform microspheres provides an improved way to study these effects while investigating ways to optimize microsphere administration and control drug release.

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