Characterizing Release Mechanisms of Leuprolide-Loaded PLGA Microparticles for IVIVC Development

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Background

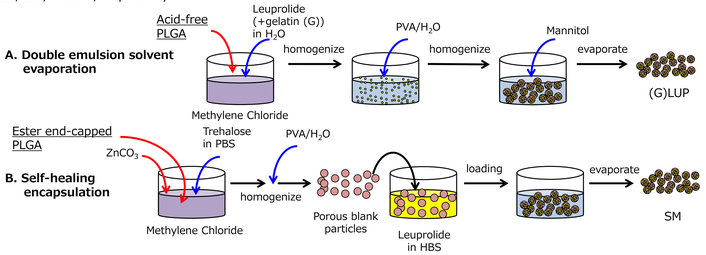
Development of *in vitro-in vivo* correlations (IVIVCs) for long acting release microspheres are always challenging. To date, majority of the literature reports on IVIVCs for microspheres are based on empirical studies[1,2]. This is largely due to the complexity of *in vivo* environment including microsphere-induced foreign body responses and the presence of various endogenous components[3,4]. All these factors may contribute to very different release kinetics *in vivo* relative to that observed *in vitro*, particularly for PLGA microspheres.

Objective

The effect of various incubation media on release kinetics of leuprolide-loaded PLGA microspheres *in vitro* was determined in order to simulate accelerated *in vivo* release rates. Key mechanistic factors governing leuprolide release from PLGA microspheres were also investigated to develop rational mechanism-based IVIVCs.

Methods

1. Preparation of PLGA microspheres loaded with leuprolide
Leuprolide PLGA microspheres were prepared by two methods, double emulsion solvent evaporation method (A) and our newly developed self-healing microencapsulation method (B)[5]. Prepared particles were screened to 63-90 μm and then freeze-dried. Formulations prepared by the double emulsion method with and without gelatin and by self-encapsulation method are referred as GLUP, LUP, and SM, respectively.

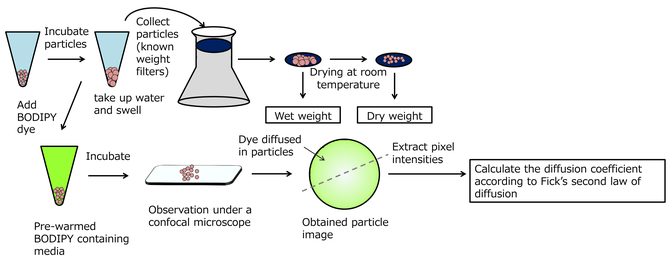


2. Release kinetics of leuprolide form PLGA microspheres
PLGA microspheres were incubated at 37 °C in the following releasing media, all with 0.05% sodium azide; 10 mM phosphate-buffered saline (PBS) with 0.02% Tween 80 (PBST) at pH 7.4 (PBST7.4), 10 mM PBST at pH 6.5 (PBST6.5), 10 mM PBST at pH 5.5 (PBST5.5), 10 mM PBS with 1.0 % (w/w) triethyl citrate (TC) (PBStc) and 10 mM HEPES-buffered saline (HBS) with 0.02% Tween 80 (HBST) at pH 7.4 . Whole media were collected and replaced with 1 mL of the same solutions at days 1, 3, 7 and every week up to week 8. The amount of leuprolide released in the media was determined by UPLC with UV detection at 280 nm.

3. Determination of Mw decline
The microspheres were incubated in the same manner including replacement of media performed in release study. Then, the microspheres were dissolved with tetrahydrofuran and subjected to gel permeation chromatography with refractive index detection.

4. Quantification of water uptake and mass loss
Incubated microspheres were collected by weight-known nylon membrane filters under vacuum and washed salt ingredients off with ddH_2O. Then, the surface water was removed and the wet weight of the microspheres was immediately measured. The samples were then dried at room temperature under vacuum to a constant weight and the dry weight was recorded. A correction to inter-particle water was used[6].

5. Analysis of diffusion coefficient of PLGA microspheres
Incubated microspheres were further incubated in BODIPY FL solution. The probe distribution in PLGA microspheres was imaged by confocal laser scanning microscopy. Consecutive pixel intensities of BODIPY from the edge of the microspheres to the center of the microspheres were extracted using ImageJ software and were fit to the equation of Fick's second law of diffusion using DataFit software to calculate diffusion coefficient[7].



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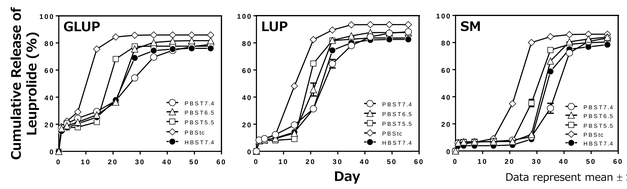
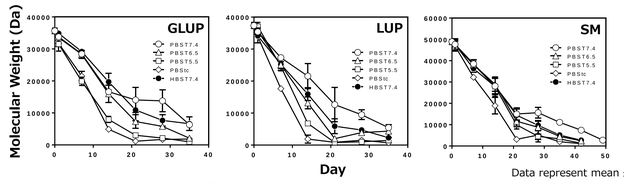
Results and Discussion



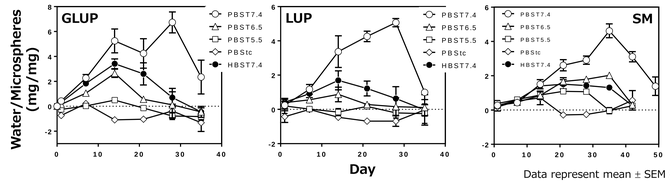
Data represent mean ± SEM (n=3).

Figure 1. Cumulative amount of leuprolide released from PLGA microspheres.
- PBStc accelerated release by shifting the initiation point of the active releasing phase by approx. 14 days earlier than the standard condition buffer, PBST7.4.
- Acidic conditions showed faster release than the neutral PBST and those rates were in between those observed in PBStc and PBST7.4.



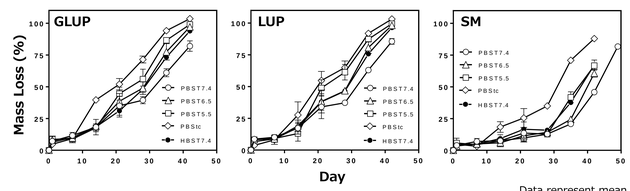
Data represent mean ± SEM (n=3).

Figure 2. Kinetics of Mw decline of PLGA.
- TC accelerated degradation of PLGA regardless of terminal properties of PLGA as compared to PBST7.4.
- Regarding the effect of acidity of the buffer on the degradation, GLUP and LUP were more susceptible than ester end-capped SM.



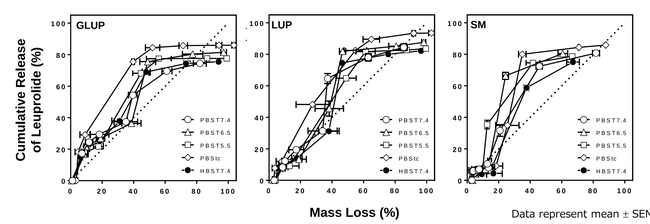
Data represent mean ± SEM (n=3).

Figure 3. Kinetics of water uptake into microspheres.
- TC had little effect on water uptake.
- Acidic buffer condition strongly decreased water uptake.
- Faster degradation resulted in lower water uptake.



Data represent mean ± SEM (n=3).

Figure 4. Kinetics of mass loss.
- From the onset of active releasing phase, mass loss preceded in a proportional manner.
- TC and acidic buffer conditions accelerated mass loss.
- A trend of susceptibility to acid-induced increase in mass loss was similar to that observed in Mw decline study.



Data represent mean ± SEM (n=3).

Figure 5. Visualizing relationship between cumulative release and mass loss.
- The release proceeded in an erosion-dependent manner until the active release phase.
- The release was then accelerated due to formation of pore networks.

Formulation	Item	PBST7.4	PBS6.5	PBS5.5	PBStc	HBST7.4
GLUP	$t_{50,\ release\ (Day)}$	25.5 ± 0.5	23.3 ± 0.3	18.2 ± 0.1	10.5 ± 0.1	22.7 ± 0.3
	$t_{50,\ mass\ loss\ (Day)}$	37.7 ± 9.5	32.2 ± 4.1	26.9 ± 2.1	21.6 ± 1.3	33.2 ± 2.6
	$t_{50,\ release}$ / $t_{50,\ mass\ loss}$	0.68	0.72	0.68	0.49	0.68
LUP	$t_{50,\ release\ (Day)}$	24.5 ± 0.4	21.3 ± 0.2	19.4 ± 0.2	14.3 ± 0.1	23.0 ± 0.2
	$t_{50,\ mass\ loss\ (Day)}$	42.9 ± 12.3	32.5 ± 4.3	24.7 ± 1.7	22.9 ± 1.9	33.0 ± 3.6
	$t_{50,\ release}$ / $t_{50,\ mass\ loss}$	0.57	0.66	0.79	0.62	0.70
SM	$t_{50,\ release\ (Day)}$	37.0 ± 0.2	32.6 ± 0.2	29 ± 0.1	22.3 ± 0.1	33.0 ± 0.1
	$t_{50,\ mass\ loss\ (Day)}$	43.2 ± 0.6	40.3 ± 0.9	38.4 ± 0.9	30.7 ± 2.0	38.5 ± 1.0
	$t_{50,\ release}$ / $t_{50,\ mass\ loss}$	0.86	0.81	0.76	0.73	0.86

Data represent mean ± SEM (n=3).

Table 1. Calculation of $t_{50,\ release}$ and $t_{50,\ mass\ loss}$.
- The presence of TC exhibited lower numbers in $t_{50,\ release}$ / $t_{50,\ mass\ loss}$, while differences in pH and buffer salt had little effect on it.
- Release of leuprolide from PLGA microspheres under different pH conditions proceeded with erosion of PLGA and followed water-mediated release acceleration.

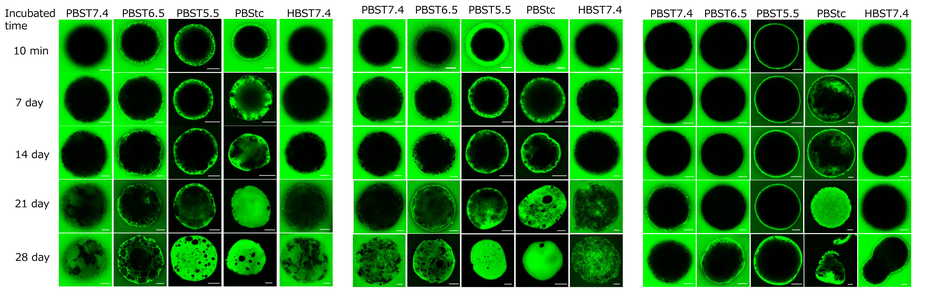
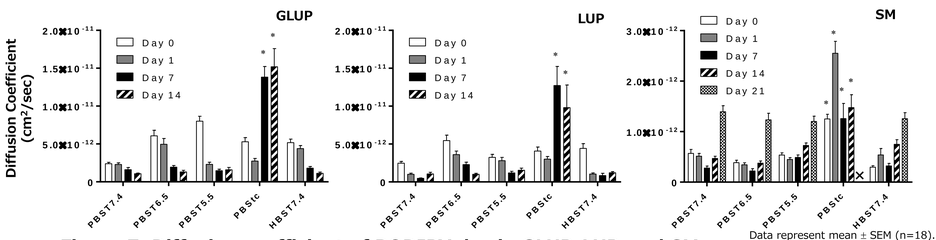


Figure 6. Confocal microscopic images of GLUP, LUP, and SM incubated with BODIPY dye.
- In PBStc and PBST5.5, the microspheres showed significant decrease in porosity and the heavy dye uptake indicated equilibration of the more permeable polymer phase in these cases.
- The dye diffused into the microspheres much faster in the presence of TC.



Data represent mean ± SEM (n=18).

Figure 7. Diffusion coefficient of BODIPY dye in GLUP, LUP, and SM.
- TC significantly increased diffusion coefficient as compared to others after 7 days of incubation for GLUP and LUP and 1 day of incubation for SM.
- Other medium conditions had little effect on diffusion coefficient after 14 days of incubation for all three groups of microspheres.

Conclusions

Lowering pH and addition of a plasticizer in the release media accelerated *in vitro* release of leuprolide from PLGA microspheres. Drug release during days 1 to 14 following the initial burst release was primarily controlled by polymer erosion (i.e., mass loss). From day 14, the drug release was accelerated by a second mechanism as the release rate exceeded mass loss rate. Efforts are ongoing to identify this second mechanism, which may be related to salt formation of leuprolide and the polymer. Mw decline of PLGA proceeded in advance to the erosion. The presence of TC resulted in a large increase in diffusion coefficient of BODIPY, which may accelerate the release of leuprolide from microspheres prepared using PLGA with acid end-cap. These mechanistic studies directly measuring various potential rate-controlling parameters for release can be applied to the microspheres retrieved from *in vivo* studies. Findings between *in vitro* and *in vivo* approached by the mechanistic procedures performed in this study will be promising to establish mechanism-based IVIVCs.