Cage Implant System to Evaluate Mechanisms of *in vivo* PLGA Microsphere Drug Release for IVIVC Model Development

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Background

- *In vitro-in vivo* correlations (IVIVCs) serve to accurately predict the *in vivo* performance of a drug product based on data gathered through *in vitro* drug release tests.
- IVIVCs are used extensively during development of conventional, immediate release dosage forms during development and to support bio-waivers.
- The lack of predictive release testing methods creates a significant challenge during development of controlled released drug products.
- **Currently, no formal FDA guideline exists for assessment of injectable controlled release products in terms of dissolution/release and establishing IVIVC models.**

Objectives

- The environment at the site of microparticle administration *in vivo* is complex and its effects on polymer degradation and drug release are poorly understood.
- **The purposes of this study were to:**
  1. **Study mechanisms of drug release as a function of *in vitro* release conditions.**
  2. **Develop a cage system to allow retrieval of PLGA microparticles following *in vivo* administration in order to study mechanisms of drug release *in vivo* for future IVIVC development.**
- Drug release from PLGA microspheres is typically governed by drug diffusion and polymer erosion. By studying these phenomenon concurrently with the dynamic *in vivo* environment, we can determine which individual mechanisms contribute to drug release from PLGA microspheres *in vivo*, and the time scales over which they are relevant.
- **The ultimate goal of this project is to design mechanism-based *in vitro* release conditions for PLGA microparticles to result in rational IVIVCs for these formulations.**

Methods

Microsphere Preparation:

- A model steroid, triamcinolone acetonide (Tr-A), was encapsulated in two PLGA 50/50 microsphere formulations (63-90 µm) using solid-in-oil-in-water double emulsion solvent evaporation:
  - **Tr-A_1:** free acid terminated (MW = 18 KDa); 5.4 ± 0.2 w/w % Tr-A
  - **Tr-A_2:** ester end capped (MW = 54 KDa); 5.2 ± 0.1 w/w % Tr-A
- Scanning electron microscopy (SEM) was used to observe size and morphology of prepared microspheres.
- Loading was determined by dissolving 10 mg microspheres in 2 mL acetonitrile. Total Tr-A content was measured by ultra-performance liquid chromatography (UPLC) using UV detection at 254nm.

Cage Construction and Implantation

- Stainless steel wire mesh (37µm openings) and silicone rubber were used to construct a small cage (Fig. 3) for microsphere restraint in the subcutaneous space. Microspheres were loaded into cage by injection through the silicone rubber.
- Cages were autoclaved for sterilization prior to surgical implantation in subcutaneous pockets formed on the backs of healthy, male Sprague-Dawley rats (see Figure 3).
- Cages were retrieved at selected time points and microspheres were collected for future analyses.

In vivo Drug Release:

- Microspheres retrieved following cage implantation were rinsed, freeze dried, and dissolved in acetonitrile. Tr-A content remaining in the microspheres was determined by UPLC.

In vitro Drug Release:

- 5 mg microspheres (Tr-A_1 and Tr-A_2) were suspended in buffer under mild agitation at 37°C. Multiple release media were used to determine effect of release conditions on drug release (PBST pH 7.4, PBST pH 6.5, PBS + 1.0% triethyl citrate, 10mM HEPES buffer pH 7.4).
- Media was completely removed and replaced at designated time points and analyzed for Tr-A content by UPLC.

PLGA Molecular Weight Determination:

- Microspheres were incubated in release media as described above. Samples were removed weekly, freeze-dried, then dissolved in tetrahydrofuran and analyzed by gel permeation chromatography (GPC).

Particle Morphology During Release--Laser Scanning Confocal Microscopy (LSCM):

- During Tr-A release *in vitro* and *in vivo*, small aliquots of Tr-A_1 and Tr-A_2 microspheres were separated and incubated in a solution of the fluorescent probe bodipy for LSCM.

BODIPY Diffusion in Degrading Tr-A_2 Microspheres[1]:

- Using LSCM images obtained above, normalized pixel intensity was plotted against radial position in degrading microspheres (ImageJ, National Institute of Health)
- Data was fit to the solution of Fick's second law of diffusion (DataFit, Oakdale Engineering):

$$\frac{C}{C_0} = \frac{1}{r/a}\sum_{n=0}^{\infty}\left(erfc\frac{(2n+1)-r/a}{2\sqrt{Dt/a^2}} - \mathrm{erfc}\frac{(2n+1)+r/a}{2\sqrt{Dt/a^2}}\right)$$

Results

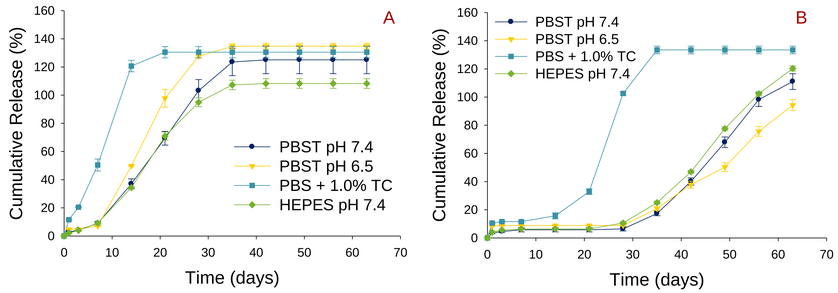

Figure 1. Tr-A release from Tr-A_1 (A) and Tr-A_2 (B) microspheres *in vitro* in a variety of release media. Data represent mean ± SE, n=3.

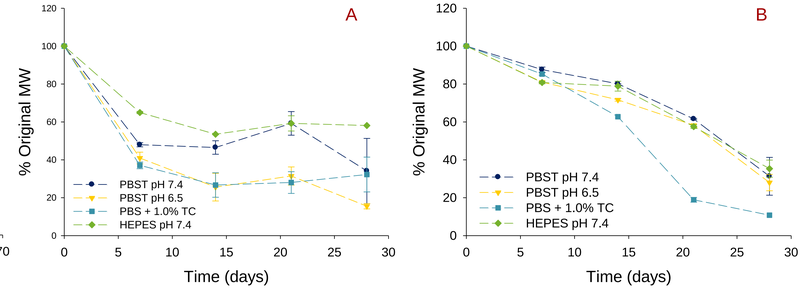

Figure 2. PLGA degradation kinetics in Tr-A_1 (A) and Tr-A_2 (B) microspheres in vitro in a variety of release media. Data represent mean ± SE, n=2.

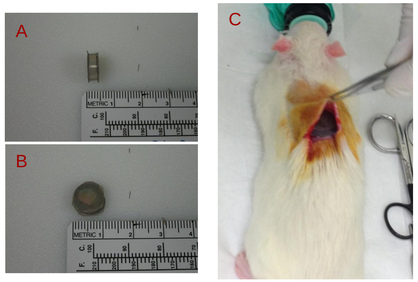

Figure 3. Cages constructed from silicone and stainless steel wire mesh (A, B) and subsequent implantation into Sprague Dawley rats. Subcutaneous pocket is created using surgical instruments (C) and the incision is closed using veterinary adhesive for minimal irritation.

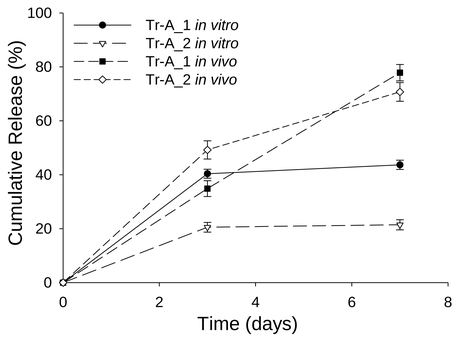

Figure 4. Tr-A release from particles restrained in cages over one week *in vitro* and *in vivo*. *In vitro* release was measured in PBST pH 7.4. Data represent mean ± SE, n=3.

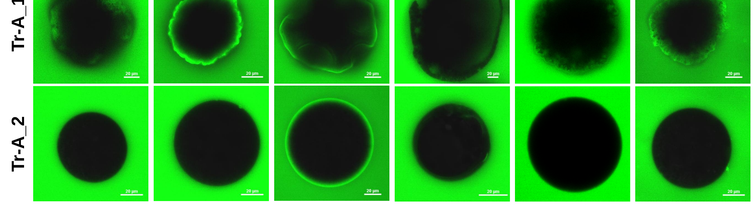

Figure 5. Representative LSCM images of Tr-A_1 and Tr-A_2 following 3 days incubation in various release media and following implantation *in vivo*.

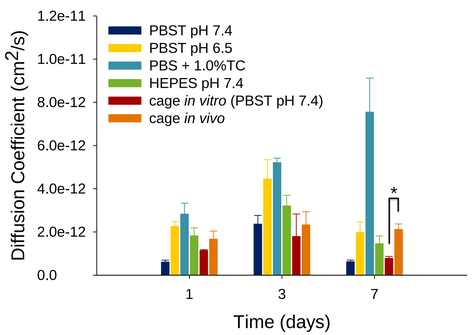

Figure 6. Diffusion coefficients calculated for the molecular probe bodipy following Tr-A_2 incubation in various release media and *in vivo* over one week. Data represent mean ± SE, n=3. *p<0.05.

Discussion & Conclusions

- Tr-A release, PLGA MW, microsphere morphology, and bodipy diffusion in Tr-A_1 and Tr-A_2 microspheres is release media dependent.
- Over one week, Tr-A release was faster *in vivo* than *in vitro* from both formulations investigated.
- We investigated one of the primary mechanisms responsible for drug release from PLGA microspheres, drug diffusion, *in vivo.*
- Following one week release, bodipy diffusion was significantly ($p<0.05$) higher *in vivo* than in particles suspended in PBST pH 7.4 and in particles restrained in cages *in vitro*.
  - This fluorescent probe can be used as a marker of solid state diffusion of triamcinolone acetonide, as it partitions into the polymer phase during incubation[1].
- **Increased diffusion coefficient of bodipy may offer a possible explanation for faster Tr-A release related to increased steroid diffusion in PLGA.**
- **Further evaluation of the mechanisms responsible for increased drug diffusion and other possible causes of increased drug release will suggest ways we can modify *in vitro* release conditions to be more predictive and develop IVIVCs.**

References & Acknowledgements

[1] Kang, J. and S. P. Schwendeman (2003). "Determination of Diffusion Coefficient of a Small Hydrophobic Probe in Poly(lactide-co-glycolide) Microparticles by Laser Scanning Confocal Microscopy." Macromolecules **36**(4): 1324-1330.

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