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

Amy C. Doty[a,e], Yan Wang[b], Stephanie Choi[b], Wen Qu[b], Robert Lionberger[b], Meihua R. Feng[a], Anna Schwendeman[d,e], Steven P. Schwendeman[a,c,e]

[a]Department of Pharmaceutical Sciences, University of Michigan, USA; [b]Office of Generic Drugs, U.S. Food and Drug Administration; [c]Department of Biomedical Engineering, University of Michigan, USA; [d]Department of Medicinal Chemistry, University of Michigan, USA; [e]The Biointerfaces Institute, University of Michigan, USA

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 dissolution/release and establishing IVIVC models for PLGA controlled release microparticles.**

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 purpose of this study was to 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 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 50/50s using solid-in-oil-in-water double emulsion solvent evaporation:
  - **Tr-A_1:** free acid terminated (i.v.=0.34 dL/g)
  - **Tr-A_2:** ester end capped (i.v.=0.61 dL/g)
- Scanning electron microscopy (SEM) was used to observe size and morphology of prepared microspheres.

Cage Construction and Implantation

- Stainless steel wire mesh (37µm openings) and silicone rubber were used to construct a small cage (see Figure 1) for microsphere restraint in the subcutaneous space. Tr-A_1 and Tr-A_2 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 1).
- Cages were retrieved at selected time points and microspheres were collected for future analyses.

In vitro Drug Release:

- 5mg microspheres (Tr-A_1 and Tr-A_2) were suspended in phosphate buffered saline + 0.02% Tween 80 (PBST, pH 7.4) under mild agitation at 37°c.
- Release media was completely removed and replaced at designated time points and analyzed for Tr-A content by ultra-performance liquid chromatography (UPLC) using UV detection at 245nm.

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. Multiple release media were used to determine effect of release conditions on particle degradation and drug diffusion (PBST pH 7.4, PBST pH 6.5, PBS + 1.0% triethyl citrate, 10mM HEPES buffer at pH 7.4).

BODIPY Diffusion in Degrading Tr-A_2 Microspheres:

- 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

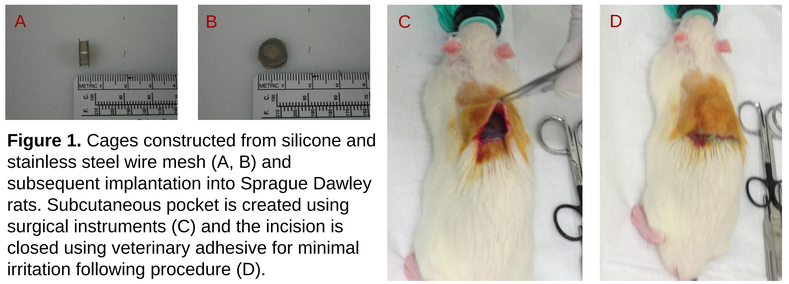


Figure 1. Cages constructed from silicone and stainless steel wire mesh (A, B) and subsequent implantation into Sprague Dawley rats. Subcutaneous pocket is created using surgical instruments (C) and the incision is closed using veterinary adhesive for minimal irritation following procedure (D).

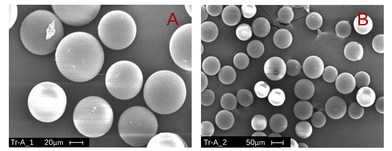
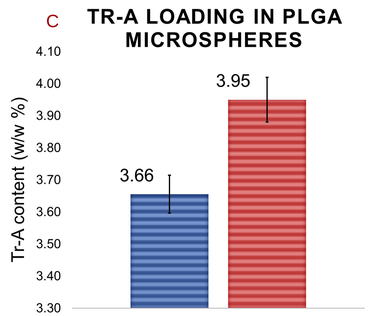


Figure 2. Characterization of Tr-A microspheres prepared by s/o/w double emulsion solvent evaporation. SEM images revealing spherical, non-porous surfaces (A & B).Tr-A loading (w/w %) in microspheres (C). Tr-A was encapsulated in Tr-A_1 and Tr-A_2 with efficiencies of 73 ± 1% and 79 ± 1%, respectively.



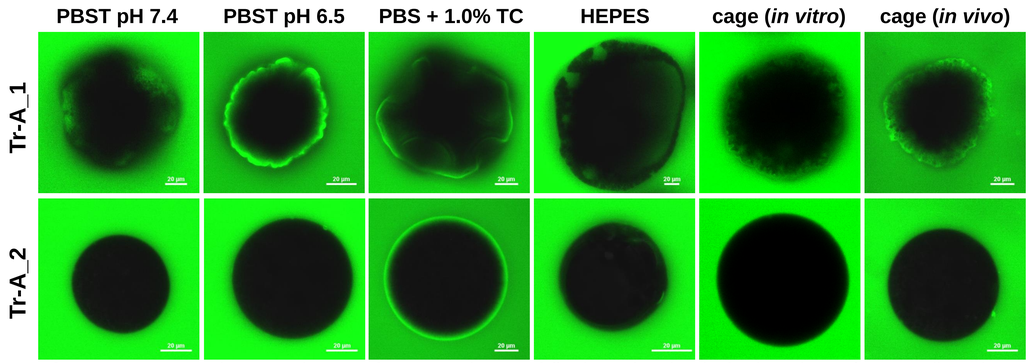


Figure 3. Representative LSCM images of Tr-A_1 and Tr-A_2 following 3 days incubation in various release media and following implantation *in vivo*.

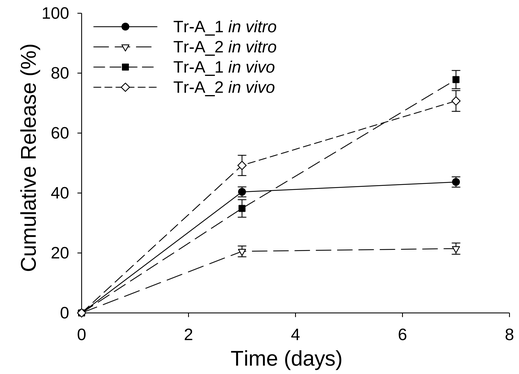


Figure 4. Tr-A release from particles restrained in cages over one week *in vitro* and *in vivo*. *In vitro* release was measured in PBST pH 7.4.

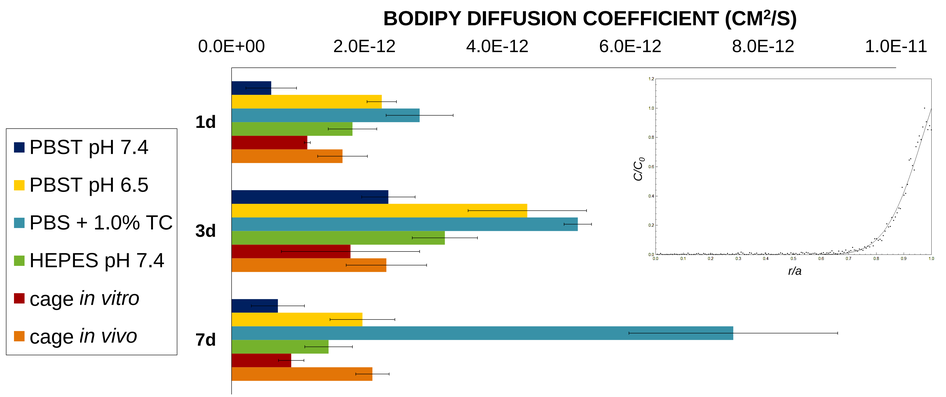


Figure 5. Diffusion coefficients calculated for the molecular probe bodipy following Tr-A_2 incubation in various release media and *in vivo* over one week. Inset: Representative model plot of normalized intensity-position pairs fit to the solution of Fick's Second Law of Diffusion (R^2=*0.984*). Data represent mean ± SE, n=3.

Discussion & Conclusions

- Over one week, Tr-A release was faster *in vivo* than *in vitro*.
- We investigated one of the primary mechanisms responsible for drug release from PLGA microspheres, drug diffusion, *in vivo* and as a function of *in vitro* release conditions.
- Microsphere morphology and bodipy diffusion in degrading microspheres is release-media dependent.
- Following one week release, bodipy diffusion was 3.0x higher *in vivo* than in particles suspended n PBST pH 7.4 *in vitro*, and 2.4x higher than in particles restrained in cages suspended in PBST pH 7.4.
- **Increased diffusion coefficient of bodipy may offer a possible explanation for faster Tr-A release related to increase 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.**

This work is supported by U.S. Food and Drug Administration RFA-FD-13-030