A cage implant system for assessing *in vivo* controlled release performance of long-acting release PLGA depots

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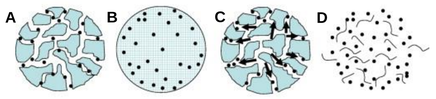
Background

- Drug release from PLGA microspheres *in vivo* is not currently well predicted by standard *in vitro* release tests due to a lack of mechanistic understanding of drug release *in vivo*.
- Controlled release from PLGA microspheres is typically achieved by combinations of[1]:



A: Pore diffusion
B: Diffusion through polymer
C: Swelling / osmotic pumping
D: Hydrolysis → erosion

- A variety of factors may alter the release mechanism, including:

in vitro:	*in vivo:*
Buffering system and capacity	Enzymes and other large molecules
Ionic strength / osmotic pressure	Lipids
pH	Inflammatory response
Volume and flow	Unknown small molecules (e.g. lipids)

- Mechanistic analysis of *in vivo* performance of controlled release poly(lactic-co-glycolic) (PLGA) microspheres has been limited owing to the difficulty of recovering intact microspheres after parenteral administration.
- **The purposes of this study were to:**
  1. **Develop a cage system to allow retrieval of PLGA microparticles following *in vivo* administration**
  2. **Use the cage model to determine mechanisms of release from PLGA microparticles *in vivo***

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) from 1000 mg/mL polymer in CH_2Cl_2
  - **Tr-A_2:** ester end capped (MW = 54 KDa) from 400 mg/mL polymer in CH_2Cl_2
- 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[2]

- Stainless steel wire mesh (37µm openings) and silicone rubber were used to construct a small cage (Fig. 2) 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 2).
- 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 PBST pH 7.4 under mild agitation at 37°C.
- Media was completely removed and replaced at designated time points and analyzed for Tr-A content by UPLC.

Mass Loss and Water Uptake:

- During release *in vitro* and *in vivo*, mass loss and water content of microspheres was determined gravimetrically.

PLGA Molecular Weight Determination:

- Microspheres were incubated in release media or administered via cage model as described above. Samples were rinsed, 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 Microspheres[3]:

- 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

MICROSPHERE FORMULATION

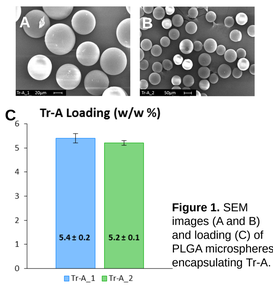


Figure 1. SEM images (A and B) and loading (C) of PLGA microspheres encapsulating Tr-A.

CAGE MODEL DEVELOPMENT

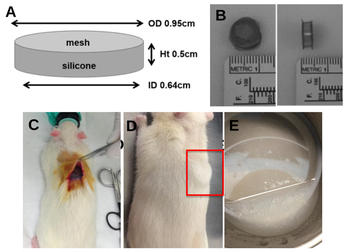


Figure 2. Cage design (A) and product (B). Implantation into subcutaneous space (C and D) and microspheres retrieved from cage and rinsed to remove fluids and tissue (E).

CAGE MODEL VALIDATION *IN VITRO* AND *IN VIVO*

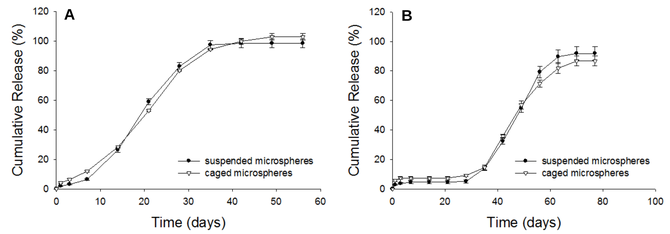


Figure 3. *in vitro* release of Tr-A_1 (A) and Tr-A_2 (B) microspheres either suspended or restrained in cage model in PBST pH 7.4.

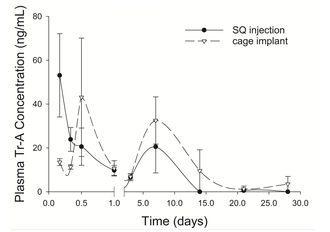
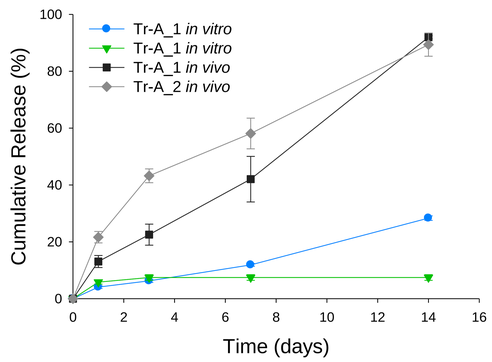


Figure 4. Pharmacokinetics of Tr-A following Tr-A_2 microsphere administration by SQ injection or cage implantation.

IN VITRO VS. *IN VIVO* RELEASE



EROSION, HYDROLYSIS, WATER UPTAKE, DIFFUSION

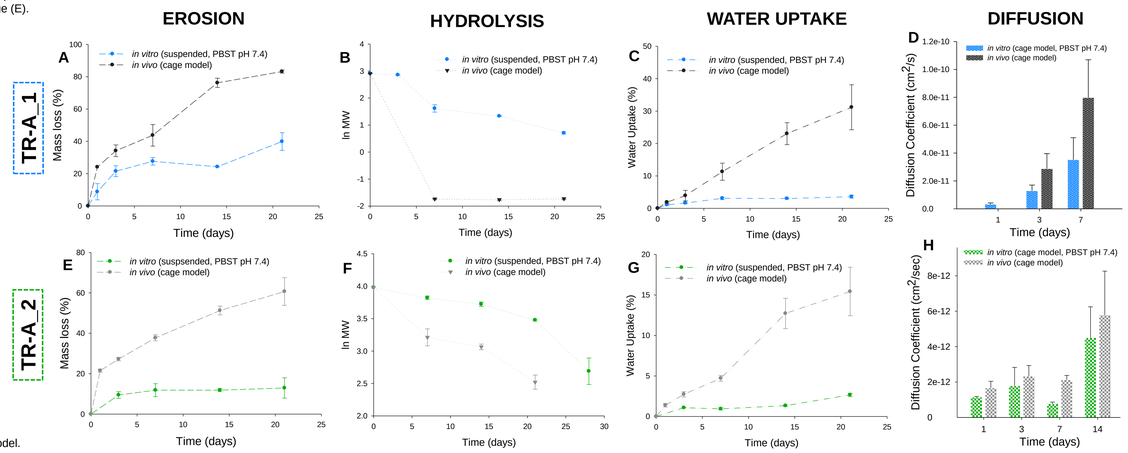


Figure 6. *in vitro* and *in vivo* kinetics of release mechanisms (erosion, hydrolysis, water uptake, solid state diffusion) in Tr-A_1 (A-D) and Tr-A_2 (E-H) microspheres.

Table 1. t_{50s} of release and mass loss *in vitro* and *in vivo*.

	Tr-A_1		*Tr-A_2*	
	in vitro	***in vivo***	***in vitro***	***in vivo***
t_{50} release	19.3 ± 0.5	7.7 ± 0.7	46.9 ± 0.6	6.8 ± 4.4
t_{50} mass loss	24.0 ± 3.9	10.9 ± 1.2	58.1 ± 7.4	14.8 ± 2.1

MORPHOLOGY

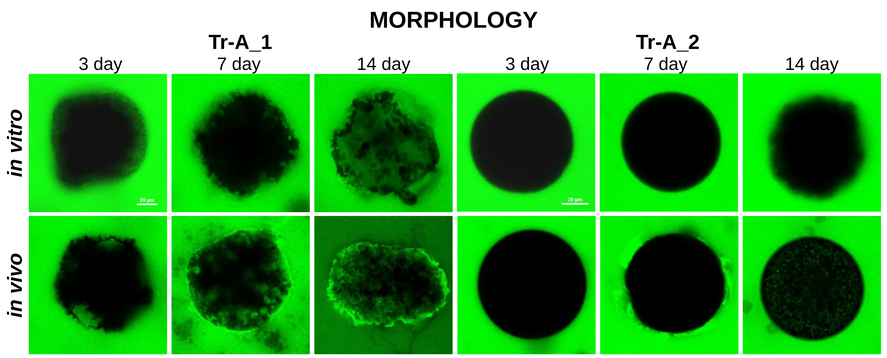


Figure 5. Confocal micrographs of Tr-A_1 and Tr-A_2 microspheres at various times during *in vitro* or *in vivo* release. Particles were incubated in bodipy solution for 3 hours.

Discussion & Conclusions

- Cage implants can be used to uncover valuable mechanistic data concerning *in vivo* release from PLGA microspheres by exposing the polymer to biological fluids and allowing recovery of microspheres after administration.
- Initial data suggest that PLGA release kinetics in the cage is predictive of SQ *in vivo* release after the initial burst.
- Release of steroids from PLGA is generally faster *in vivo* than *in vitro.*
- Some causes of more rapid *in vivo* release:
  - Increased water uptake
  - Increased polymer degradation and erosion kinetics
  - Potential for osmotic pressure-mediated pore diffusion
- This approach may be useful to develop mechanistic *in vitro - in vivo* correlations.

References and Acknowledgements

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