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Mechanisms of *in vivo* release of triamcinolone acetonide from PLGA microspheres

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ABSTRACT

Little is known about the underlying effects controlling *in vitro-in vivo* correlations (IVIVCs) for biodegradable controlled release microspheres. Most reports of IVIVCs that exist are empirical in nature, typically based on a mathematical relationship between *in vitro* and *in vivo* drug release, with the latter often estimated by deconvolution of pharmacokinetic data. In order to improve the ability of *in vitro* release tests to predict microsphere behavior *in vivo* and develop more meaningful IVIVCs, the *in vivo* release mechanisms need to be characterized. Here, two poly(lactic-*co*-glycolic acid) (PLGA) microsphere formulations encapsulating the model steroid triamcinolone acetonide (Tr-A) were implanted subcutaneously in rats by using a validated cage model, allowing for free fluid and cellular exchange and microsphere retrieval during release. Release kinetics, as well as mechanistic indicators of release such as hydrolysis and mass loss, was measured by direct analysis of the recovered microspheres. Release of Tr-A from both formulations was greatly accelerated *in vivo* compared to *in vitro* using agitated phosphate buffered saline + 0.02% Tween 80 pH 7.4, including rate of PLGA hydrolysis, mass loss and water uptake. Both microsphere formulations exhibited erosion-controlled release *in vitro*, indicated by similar polymer mass loss kinetics, but only one of the formulations (low molecular weight, free acid terminated) exhibited the same mechanism *in vivo*. The *in vivo* release of Tr-A from microspheres made of a higher molecular weight, ester end-capped PLGA displayed an osmotically induced/pore diffusion mechanism based on confocal micrographs of percolating pores in the polymer, not previously observed *in vitro*. This research indicates the need to fully understand the *in vivo* environment and how it causes drug release from biodegradable microspheres. This understanding can then be applied to develop *in vitro* release tests which better mimic this environment and cause drug release by the relevant mechanistic processes, ultimately leading to the development of mechanism based IVIVCs.

1. Introduction

Although biodegradable polymers such as poly(lactic-*co*-glycolic acid) (PLGA) are widely used in the literature to achieve controlled drug release, and are even used in over a dozen FDA-approved controlled release (CR) drug products, very few direct measurements of the behavior of these formulations *in vivo* have been reported [1–4]. Typical analysis of these products includes extensive characterization and *in vitro* release, followed by *in vivo* pharmacokinetic and efficacy studies. Surprisingly, *in vivo* analysis of the polymer during drug release is rarely reported and instead, only plasma drug concentrations and therapeutic endpoint markers are discussed [5–14]. This lack of *in vivo* characterization represents a significant issue especially since the *in vitro* tests used to estimate kinetics of *in vivo* release from CR formulations rarely result in accurate predictions. Thus, to date, no FDA guideline exists for establishing *in vitro-in vivo* correlation (IVIVC)

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models to predict *in vivo* performance of CR PLGA formulations, and in particular, the popular microsphere dosage form [15,16].

Release from PLGA microspheres can be controlled by at least three major mechanisms, or combinations thereof: 1) diffusion through the polymer matrix, 2) water-mediated transport processes, and 3) polymer hydrolysis and erosion. While these processes are well studied and fairly well understood in the context of *in vitro* systems, it is unknown whether the same mechanisms are responsible for release *in vivo*. Given the reported discrepancies between *in vitro* and *in vivo* release data, it is likely the *in vivo* environment changes the rates of the mechanistic processes, resulting in differences in release rates. It has been demonstrated that *in vitro* release conditions can affect rates and primary release mechanisms of PLGA microspheres [17]. Thus, a mechanistic understanding of drug release *in vivo* could potentially lead to the development of *in vitro* tests which would more accurately predict *in vivo* performance.

Factors present in the subcutaneous administration environment that are not accurately represented by current *in vitro* release environments have been discussed by some authors, but little work to date has been done in attempt to validate their hypotheses regarding how these factors influence drug release [1,3,18–23]. Biological factors that may influence the way drugs are released from PLGA matrices include the inflammatory response and the presence of enzymes, lipids, organic amines, and other endogenous compounds present in the administration environment [3,18,24,25]. Physical-chemical factors that may alter mechanisms of release from PLGA microspheres *in vivo* as compared to *in vitro* include pH and buffering systems (*e.g.* bicarbonate buffer present *in vivo vs.* common phosphate buffers used *in vitro*), fluid volume, and convection [18,22,23,25,26]. It is clear that the body's reaction to the administration of PLGA microparticles is a complex process made up of a number of factors, which could potentially affect drug release in a variety of ways. What is important to understand is not just how these factors may change drug release rates, but the underlying causes for these changes. That is, what mechanisms of release (*i.e.* water uptake, hydrolysis, erosion, diffusion) are affected by the *in vivo* environment and what the resulting contributions are to drug release rate.

One of the major reasons the *in vivo* release mechanisms of PLGA microspheres are not well understood is due to the difficulty of retrieving the particles following administration. Incubated microspheres *in vivo* no doubt represent the physical evidence to implicate varying factors controlling the kinetics and type of release mechanism. Thus, we recently developed a cage implant used to restrain PLGA microspheres during *in vivo* release. The cage system was validated both by the similar *in vitro* release and *in vivo* pharmacokinetics in the presence or absence of the cage. Using this system, microspheres can be readily retrieved and analyzed at any point during the window of release before significant inflammation results from the presence of the cage, and such inflammation is strongly inhibited when slow release of a corticosteroid is present [27].

The purpose of this paper was to examine the mechanisms of *in vivo* release from PLGA microspheres encapsulating the model drug triamcinolone acetonide (Tr-A). These microspheres have been extensively characterized *in vitro* [17] and herein the same microspheres were implanted in cage implants subcutaneously, recovered, and then subjected to the same mechanistic analyses during *in vivo* release. From this direct and comparative analysis we sought to understand the underlying physical chemical processes and how they influence the rate-controlling release mechanisms *in vivo* and how these differ relative to those observed previously under standard *in vitro* release conditions. Ultimately such analysis may lead to an understanding of the sources of *in vitro* and *in vivo* differences and a more rational development of IVIVCs.

2. Materials

Triamcinolone acetonide (Tr-A), PLGA RESOMER® 502H (i.v. = 0.19 dL/g, free acid terminated), and carboxymethyl cellulose (CMC) were purchased from Sigma-Aldrich. Poly vinyl alcohol (PVA, 88% hydrolyzed, MW ~ 25,000) was purchased from Polysciences, Inc. (Warrington, PA). PLGA (i.v. = 0.61 dL/g, ester terminated) was purchased from Lactel. BODIPY® FL (4,4-Difluoro-5,7-Dimethyl-4-Bora-3a,4a-Diaza-s-Indacene-3-Propionic Acid) was purchased from Life Technologies. Stainless steel wire cloth (type 316, 400 mesh; 38 μm openings) was purchased from Grainger Industrial Supply (Lake Forest, IL). Silicone tubing (1.59 cm inner diameter × 1.27 cm outer diameter) was purchased from Cole Parmer (Vernon Hills, IL). Liquid silicone elastomer was purchased from NuSil Technology (Carpinteria, CA). 7–9 week old male Sprague-Dawley (SD) rats were purchased from Charles River Laboratories (Wilmington, MA). All solvents used were HPLC grade and were purchased from Fisher Scientific and unless otherwise noted, all other chemicals were purchased from Sigma-Aldrich.

3. Methods

3.1. Microsphere preparation

PLGA microspheres encapsulating Tr-A were prepared by solid-in-oil-in-water (s/o/w) double-emulsion solvent evaporation, as previously described [17]. First, Tr-A powder was cryo-milled to obtain drug particle sizes < 10 μm. Two different PLGAs were used to encapsulate Tr-A. The first formulation (Tr-A_1) was prepared with a low molecular weight, free acid terminated PLGA 502H (18 kDa); Tr-A_2 was prepared from a moderate molecular weight, ester terminated (e.t.) PLGA (54 kDa). Microspheres were screened between 63 and 90 μm, lyophilized, and stored at − 20 °C before use.

3.2. Cage construction and microsphere loading

Cages made from silicone tubing, surgical grade stainless steel wire mesh, and silicone elastomer were constructed as previously described [27]. To load microspheres into the cages prior to implantation, the microspheres were suspended in a sterile injection medium (0.9% saline + 3.0% CMC) then injected through the silicone tubing into the cage using a 20 g needle. Cages were then flushed and filled with sterile saline until the time of implantation (< 1 h) with little escape of fluid before implantation.

3.3. Surgical procedures

The treatment of experimental animals was in accordance with the terms of the University Committee on Use and Care of Animals (University of Michigan UCUCA). As previously described [27], male SD rats were housed in cages and given free access to food and water, and were allowed 1–2 weeks to acclimate prior to study initiation. Rats were anesthetized with 2–4% isoflurane gas administered by a vaporizer (Midmark, Orchard Park, NY) before surgical preparation including shaving and sterilizing the surgical area using repeated swabs of alcohol and betadine solutions. An incision approximately 2 cm in length was made across the back of each rat and a pocket was formed in the subcutaneous space using surgical scissors. The cage was placed into this pocket, then the incision was closed using ETHILON® nylon sutures. Animals were allowed to recover from anesthesia on a heated water pad and then returned to their cages where they were monitored until suture removal 7 days after surgery. At each time point, animals were euthanized by CO_2 overdose prior to surgical cage retrieval during necropsy.

3.4. Assessment of drug release in vitro from cage implant

In vitro drug release and mechanistic analyses of Tr-A_1 and Tr-A_2 microspheres was carried out in PBS (137 mM NaCl, 3 mM KCl, 7.74 mM Na_2HPO_4, 2.26 mM NaH_2PO_4) + 0.02% Tween 80 + 0.05% NaN_3 (PBST pH 7.4). Microspheres (~50 mg) were loaded in a cage implant as described above and the cage was then submerged in 30 mL of media and shaken mildly at 37 °C for the duration of the experiment. At each time point (1, 3, 7 days and weekly thereafter), the cage was transferred into fresh media and drug content in the media was measured by UPLC, as described below.

3.5. Microsphere retrieval and measurement of drug content

Following cage removal from the subcutaneous space, the cage was opened using fine scissors and the microspheres were collected onto a 20-μm sieve. The particles were washed thoroughly on the sieve with ddH_2O to remove cellular debris and exudate. The particles were then collected and dried to constant weight under vacuum. A small aliquot of dried microparticles (3–5 mg) was then dissolved in 20 mL acetonitrile to determine the remaining Tr-A content, measured by UPLC.

3.6. Release and mechanistic analyses in vivo

Mechanistic analyses were performed on the particles retrieved by the cage using the methods previously described in detail for *in vitro* experiments [17]. Water uptake and mass loss were determined using the wet weight of microspheres following explantation/washing and the final weight measured after drying under vacuum [28]. Molecular weight (hydrolysis) of the PLGA was measured by gel permeation chromatography (GPC) using polystyrene standards (Mw 820–450,000 Da). Finally, the explanted microspheres were incubated in BODIPY FL dye and imaged with using Laser Scanning Confocal Microscopy (LCSM). In addition to providing insight as to the morphology of the microspheres while implanted subcutaneously, these images allow for an estimation of effective diffusion in the polymer phase by fitting the solution of Fick's second law of diffusion to normalized dye intensity (I/I_0) – position (r/a) pairs [28,29]. All of these methods have been described in detail previously.

3.7. Tr-A quantification by UPLC

Tr-A content in microspheres or release media was quantified using UPLC (Acquity UPLC, Waters, USA). The mobile phase was composed of 40:60 v/v (acetonitrile:ddH2O) or 70:30 v/v (methanol:water) and the flow rate was set to 0.5 mL/min. Samples and standards prepared in either acetonitrile or PBST were injected onto a C18 (Aqcuity BEH C18, 1.7 μm, 2.1 × 100 mm) column maintained at 30 °C. Tr-A was detected at 254 nm.

3.8. Statistical and regression analysis

Statistical analyses and regressions were performed using Prism (Graphpad, San Diego, CA). Rate constants, t_{50} values, and diffusion coefficients *in vivo* were compared to *in vitro* results (PBST pH 7.4) using unpaired student t-tests to determine two-tailed *P*-values. The level of significance was established at the 95% confidence interval ($\alpha < 0.05$).

4. Results and discussion

Tr-A release from both PLGA microsphere formulations was much faster *in vivo* than *in vitro* (Fig. 1). *In vitro*, Tr-A_1 exhibited mostly continuous release lasting approximately 35 days [17]. Release *in vivo* was also continuous, but release was 99.1 ± 0.4% complete after just 21 days. Release was similarly fast from Tr-A_2 *in vivo*, with 95 ± 3% drug released in 21 days relative to only 7.4 ± 1.0% release at the same time point in PBST pH 7.4. The lag phase observed in this formulation *in vitro* was absent *in vivo*, resulting in continuous, near linear release until completion. It is also interesting to note that although there were distinct differences in the release rate and profile between the two formulations *in vitro*, *in vivo* release was very similar from the low molecular weight, acid-terminated PLGA and the moderate molecular weight, ester end-capped PLGA. Given the significant differences observed between *in vitro* and *in vivo* release kinetics for both of these microsphere formulations, it is important to understand what mechanisms of release are responsible for the accelerated release and how these may be different from the operative mechanisms *in vitro*.

Mechanistic analyses were performed on microspheres during *in vivo* release and compared to the *control* data generated and previously reported with these microsphere formulations during *in vitro* release testing in PBST [17]. All *in vitro* data (release and mechanistic) has been reproduced here for this important direct and easy *in vitro-in vivo* comparison. First, the degradation kinetics were determined by measuring the molecular weight at each time point by GPC. Hydrolysis was significantly faster *in vivo* than *in vitro* (Fig. 2), as seen by the initial first order degradation rate constants listed in Table 1.

Total erosion of microspheres was also accelerated *in vivo* (Fig. 3). Erosion was found the be the major mechanism of release from microspheres *in vitro*, as release and mass loss occurred generally on the same time scale. For example, if other mechanisms such as diffusion and water-mediated effects were minimal and not rate-controlling, one would expect for a homogenously distributed drug in a polymer matrix that as polymer loses mass, drug would in proportion to its loading also be released at roughly the same rate. Therefore, for *ideal* mass loss (or erosion)-controlled release, one expects fractional mass loss and drug release to coincide.

The erosion and release comparison was analyzed quantitatively by performing nonlinear regression analysis on release and mass loss to determine t_{50} values, or the time taken to reach 50% release ($t_{50,release}$) and 50% mass loss ($t_{50,erosion}$). If these two processes occurred generally on the same time scale ($t_{50,release} / t_{50,erosion} \approx 1$), then erosion was implicated as the dominant mechanism of release. Table 2 shows the estimated t_{50} values as well as the ratios of these values both in PBST pH 7.4 and *in vivo*. In the case of Tr-A_1, both the $t_{50,release}$ and the $t_{50,erosion}$ were both slightly lower *in vivo* than *in vitro*, but the ratio of the two did not change (0.77 *vs.* 0.71). This slightly low, but similar, ratio suggests that although release was accelerated *in vivo*, erosion was still dominant mechanism of release and this process is also accelerated following SC administration. It should be noted that during the process of collecting microspheres from the cage implant, there is a limited potential for loss of microspheres or related polymer debris that would be smaller than the 20-μm sieve used for collection. Previously we have shown that release from cages *in vitro* (exchanged with fresh media at each time point) is identical to that observed for release kinetic evaluation in centrifuge tubes (particles spun down) without cages and that cage-measured release *in vivo* occurs over the same time frame as the exposure of drug plasma levels [27]. These data provide good confidence that the initially sieved 63–90-μm microspheres remain larger than the cage opening during most of their release life times, and if some small portion of the microspheres break down and could possibly escape the cage or sieve, that this small fraction if present is not a strong concern. Thus the interpretation of these *in vivo* mass loss data is reliable. This conclusion drawn from the mass loss data is also supported by the degradation kinetics, as the rate of hydrolysis *in vivo* was nearly double that *in vitro* for Tr-A_1. The rapid production of shorter, lower molecular PLGA chains leads to faster transport of these oligomers out of the microsphere, causing overall erosion of the polymer matrix.

Tr-A release from Tr-A_2 microspheres was similarly greatly accelerated *in vivo* ($t_{50,release} \approx 7$ d *vs.* 47 d), as was erosion ($t_{50,erosion} \approx 15$ d *vs.* 46 d). In this formulation, however, the $t_{50,release}/t_{50,erosion}$ was much

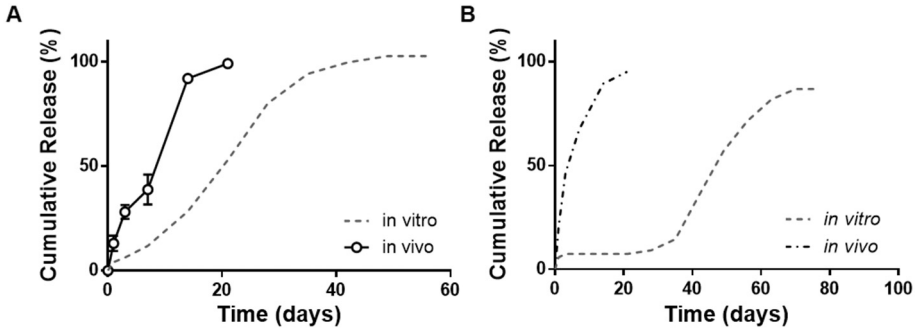

Fig. 1. Contrasting *in vitro* and *in vivo* release from Tr-A_1 (A) and Tr-A_2 (B) microspheres. *In vivo* release was measured using a SC cage model. Data represent mean ± SEM, $n = 3$–4. *In vitro* data from [17] and *in vivo* release from Tr_2 is reproduced from [27].

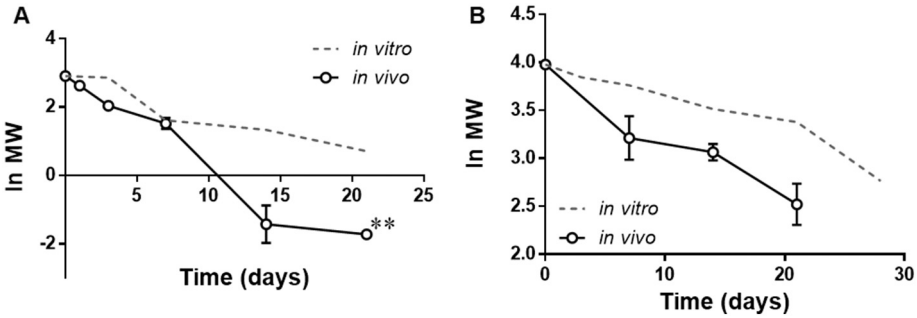

Fig. 2. *In vitro* and *in vivo* PLGA hydrolysis kinetics in Tr-A_1 (A) and Tr-A_2 (B) microspheres. Data represent mean ± SEM, $n = 3$–5. *In vitro* data from [17]. **indicates the measured MW value falls below the polystyrene standard curve range.

Table 1
Initial first order rate constants (day^{-1}) of PLGA hydrolysis in Tr-A_1 and Tr-A_2 microspheres as determined by linear regression analysis of data shown in Fig. 2. Values were taken from regression over the first 14 days. *In vitro* data from [17].

	In vitro	*In vivo*
Tr-A_1	0.125 ± 0.018	0.301 ± 0.022[†]
Tr-A_2	0.034 ± 0.001	0.065 ± 0.010[*]

[†] $p < 0.0001$.
[*] $p < 0.05$.

Table 2
Characteristic times (in days) of release and erosion from Tr-A_1 and Tr-A_2 microspheres. Values represent mean ± SEM, $n = 3$.The t_{50} ratios were calculated from nonlinear regression of release and erosion data. *In vitro* data from [17].

	Tr-A_1		*Tr-A_2*	
	In vitro	*In vivo*	*In vitro*	*In vivo*
$t_{50,release}$	19.0 ± 0.4	7.9 ± 0.8	46.8 ± 0.6	6.8 ± 4.4
$t_{50,erosion}$	25 ± 8	11 ± 1	46 ± 3	15 ± 2
$t_{50,release} / t_{50,erosion}$	0.77	0.72	1.01	0.46

reduced *in vivo* relative to that observed *in vitro* (1.01 *vs.* 0.46), as release occurred on a faster time scale than erosion *in vivo*. This disparity in the ratios suggested that some other mechanism was contributing to release *in vivo* more prominently than *in vitro*.

Further investigation into the mechanisms causing accelerated Tr-A release *in vivo* included measurement of water content in the microspheres over the time course of release. As has been well described by previous authors, water uptake into microspheres can have multiple effects on degradation and drug release, both direct and indirect. In addition to indirectly causing polymer erosion by initiating polymer chain hydrolysis and dissolving small monomers/oligomers, water uptake of polymer matrices can directly trigger drug release by multiple processes. First, polymer swelling can create new percolating polymer pore networks for rapid drug diffusion through pores or through the polymer pathways made less tortuous by rapid swelling. Secondly, osmotic pressure can cause osmotic pumping and convective drug transport through the aqueous pore network which has formed [30–32].

Very soon after release began, water content was dramatically increased at each time point *in vivo* relative to that observed during release *in vitro* (Fig. 4). Increased water uptake into the microspheres if also in the polymer phase could have also increased PLGA hydrolysis

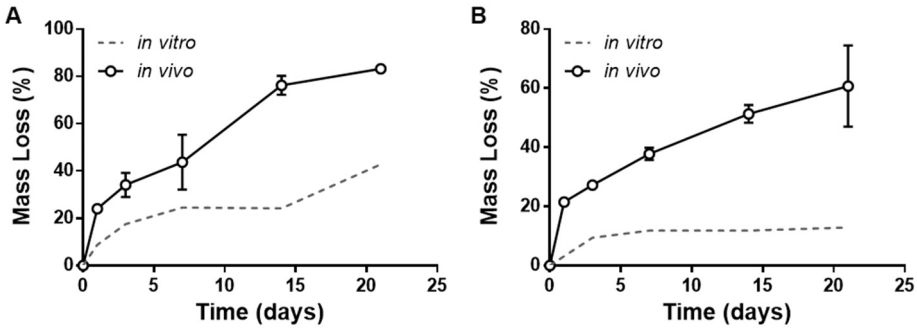

Fig. 3. *In vitro* and *in vivo* mass loss of Tr-A_1 (A) and Tr-A_2 (B) microspheres. Data represent mean ± SEM, $n = 3$–5. *In vitro* data plotted from [17].

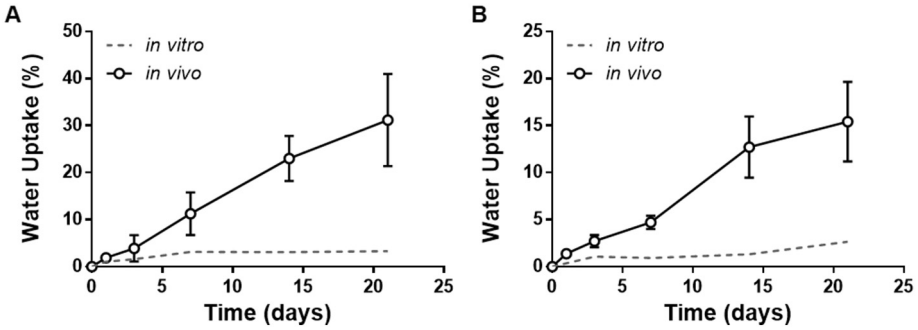

Fig. 4. *In vitro* and *in vivo* water uptake in Tr-A_1 (A) and Tr-A_2 (B) microspheres. Data represent mean ± SEM, n = 3–5. *In vitro* data plotted from [17].

and subsequent erosion, consistent with the hydrolysis kinetics and mass loss data described above.

Indeed, the substantial water uptake in both Tr-A_1 and Tr-A_2 *in vivo* is surprising and was more pronounced in the more hydrophilic acid-capped and lower MW PLGA formulation. The origin of this behavior, which has been also documented in leuprolide/PLGA microspheres (data not shown) will be the subject of future studies. In addition, the extent to which water uptake represents "pore water" and "polymer phase" water would appear to be a significant question for future study, as the former would be expected to contribute to increased osmotic-mediated release and the latter increased polymer hydrolysis.

Finally, for an even more complete understanding of how the *in vivo* environment affects the Tr-A/PLGA microspheres, the morphology of the polymer matrices was imaged by LCSM during various stages of drug release after brief exposure of the microspheres to bodipy FL (Fig. 5). This fluorescent dye is capable of diffusing through both the polymer phase and aqueous pores, although typically *in vitro* solid state diffusion is limiting [29]. Overall, no major changes in morphology were observed in Tr-A_1 microspheres over 2 weeks. By contrast After one week, microspheres recovered from SC space showed some evidence of a percolating pore network near the surface more advanced than the pore network developed *in vitro* over the same time, which may partially account for the $t_{50,release} / t_{50,erosion} < 1$. While the hydrolysis and resulting erosion of Tr-A_1 microspheres does occur faster *in vivo* then *in vitro*, at similar stages of erosion the overall changes in visible microsphere morphology over time appeared similar *in vitro* and *in vivo*.

By contrast, one major observation was seen in the Tr-A_2 microspheres implanted subcutaneously for 14 days (Fig. 5B). The water-soluble and PLGA-soluble bodipy FL dye had entered pores throughout the polymer but without entering significantly into the polymer phase. Hence, these microspheres appear to have developed an internal pore network not evident in microspheres incubated in *in vitro* release media. This pore network strongly implicates higher water penetration in the polymer matrix and that Tr-A may also be released from this formulation *in vivo* by osmotically induced aqueous pore diffusion and/or

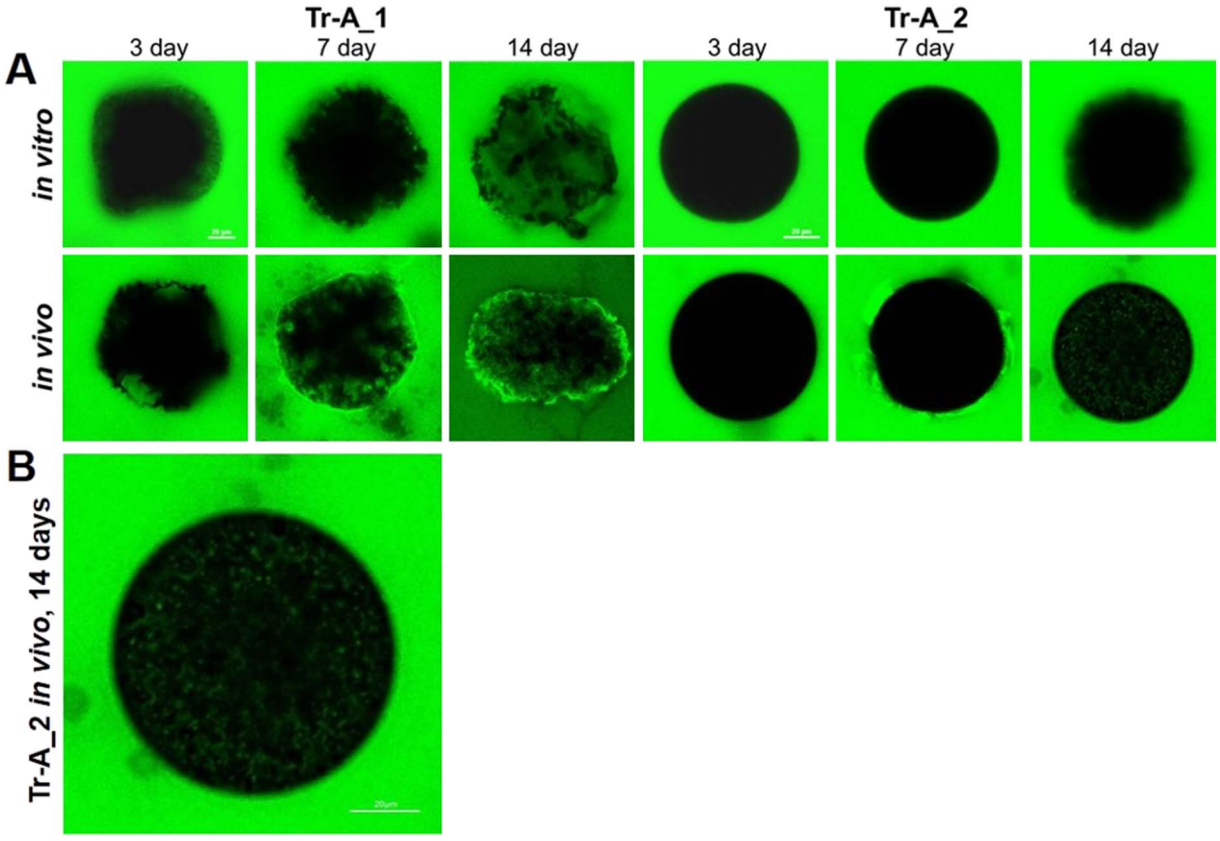

Fig. 5. Representative confocal images of Tr-A_1 and Tr-A_2 microspheres during *in vitro* and *in vivo* release (A). Enlarged image of Tr-A_2 microspheres recovered from cages after 14 days *in vivo* (B). Images were taken following incubation in aqueous solution of BODIPY FL for 10 min or 3 h.

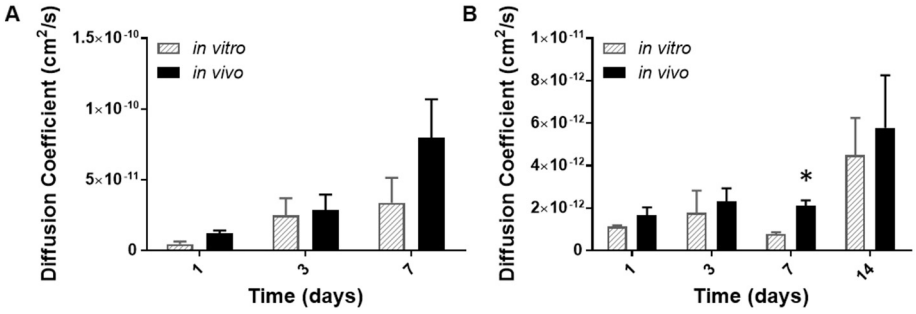

Fig. 6. BODIPY effective diffusion coefficients in degrading Tr-A_1 (A) and Tr-A_2 (B) microspheres *in vitro* and *in vivo*. Data represent mean ± SEM, $n = 6$. *$p < 0.05$. *In vitro* data taken from [17].

convection. This hypothesis is supported by the release and erosion kinetics, as $t_{50,release} \ll t_{50,erosion}$, and reasonably explains the second mechanism causing rapid drug release *in vivo*. The accelerated release kinetics from Tr-A_2 *in vivo* are evident well before these morphology changes can be seen using the confocal imaging technique, indicating that the acceleration at early time points is likely due to a combination of the increased hydrolysis, mass loss, and water uptake. The much higher water uptake observed in Tr-A_2 microspheres at early and later time points *in vivo* also supports a water-mediated mechanism causing accelerated release as compared to what was observed *in vitro*.

The effective diffusion of bodipy FL was measured at various times over one week in Tr-A_1 microspheres and over two weeks in Tr-A_2 microspheres by analyzing the confocal images, as previously described (Fig. 6). In Tr-A_1, diffusion was slightly increased *in vivo* at 7 days but the difference was not significant. A significant increase ($p < 0.05$) was observed at 7 days in Tr-A_2 microspheres but not at any other time points. The similarity of the effective diffusion coefficient values, unlike release kinetics, strongly supports very little difference in the polymer phase resistance to diffusion of Tr-A and a lack of significant contributions of diffusion alone to the release mechanism *in vitro* and *in vivo*.

5. Conclusions

In accordance with other literature reports on steroid release from PLGA, Tr-A microspheres exhbit accelerated release from PLGA microspheres *in vivo* as compared to results from standard *in vitro* release tests. Using a novel cage implant system for microsphere retrieval, we are able to assess the major rate-controlling mechanisms of release *in vivo* and compare these results directly with data from previously performed analyses of microspheres during *in vitro* release. Rate of PLGA hydrolysis, mass loss and water uptake all increased *in vivo* compared to in PBST pH 7.4, with the large increase in water uptake *in vivo* particularly interesting and unexpected. Both microsphere formulations studied here exhibit primarily erosion-controlled release *in vitro*, but only Tr-A_1 exhibits the same mechanism *in vivo*. The release of Tr-A_2 *in vivo* displays primarily an osmotically induced/pore diffusion mechanism as indicated by bodipy uptake into percolating pores of the polymer, increased water uptake, and polymer mass loss kinetics that was much slower than release.

This new analytical approach demonstrates the need to investigate release media incorporating biorelevant components in order to create an environment capable of causing drug release by *in vivo* operative mechanisms. Future studies will seek to identify which features present in the *in vivo* environment (interstitial fluid components, mixing, biological response) that contribute to the specific mechanistic differences (*e.g.*, increased water uptake) observed here. Ultimately, comparison of these *in vivo* data with data gained from biorelevant *in vitro* tests may lead to the design of mechanism-based predictive *in vitro* release tests for PLGA microspheres.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jconrel.2017.03.031.

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