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A Cage implant system for assessing in vivo performance of long-acting release PLGA depots

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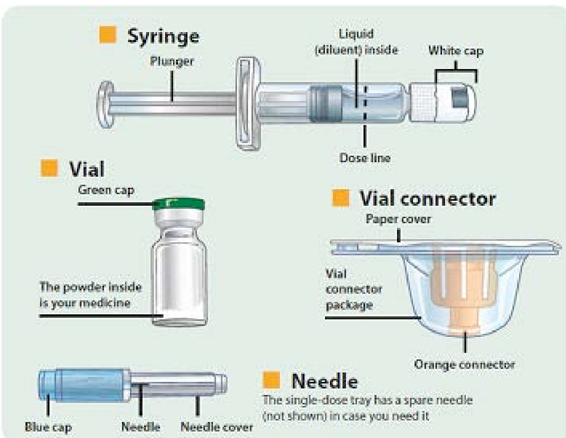
Examples of PLGA microspheres used clinically

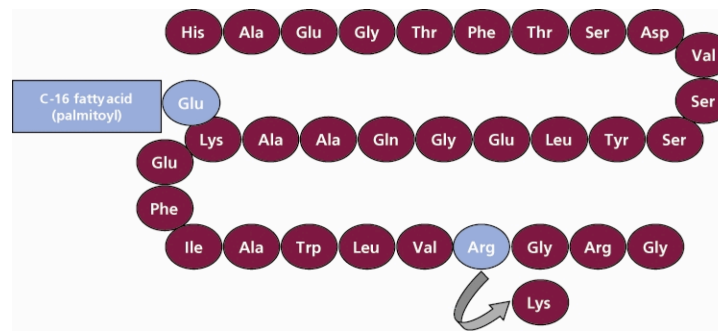
	Name	*Company*	*Disease*
Peptides	Sandostatin© LAR©	Novartis	Acromegaly
	Lupron©	TAP	Prostate and breast cancer
	Decapeptyl© Depot	Ferring	Prostate cancer, endometriosis
	Trelstar©	Pfizer	Prostate cancer
	Pamorelin ©	Ipsen	Prostate cancer
	Somatuline© LA	Ipsen	Acromegaly
	Suprecur MP© (Japan)	Mochida	Endometriosis
Proteins	Nutropin Depot®	Genentech	Pediatric GH deficiency
Small Mol.	Vivitrol©	Cephalon	Alcoholism
	Risperidal© Consta©	Janssen	Schizophrenia
	Arestin©	OraPharma	Peridontal disease
	Parlodel LA ©	Sandoz	Parkinson's, acromegaly

Wischke & Schwendeman, *Int. J. Pharm.*, **364**, 298-327 (2008)

Minimally invasive delivery of large molecules - Battle of GLP-1s



VS.



- ✧ **Bydureon** – Once-weekly injection Exenatide-encapsulated in PLGA microspheres (FDA approved 2012)
- ✧ **Victoza** – Once-daily injection Liraglutide-lipid/AA modification for increasing circulation time

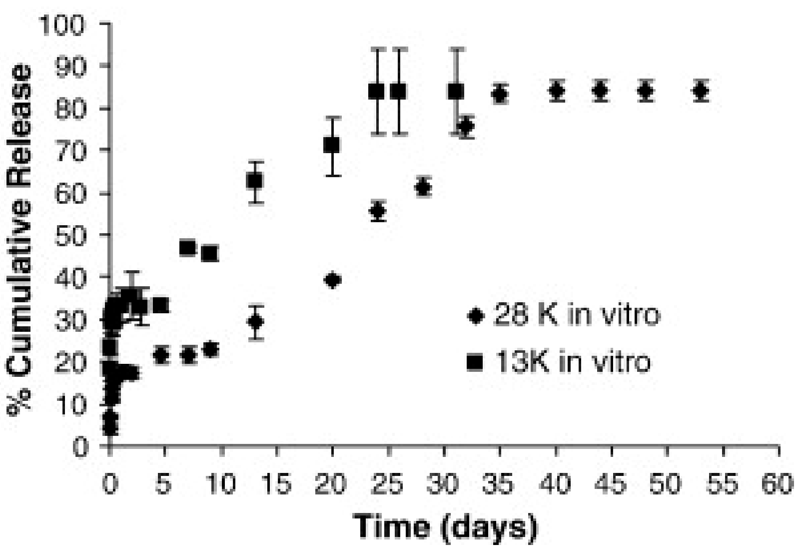
Controlled release *in vivo* often faster than *in vitro*

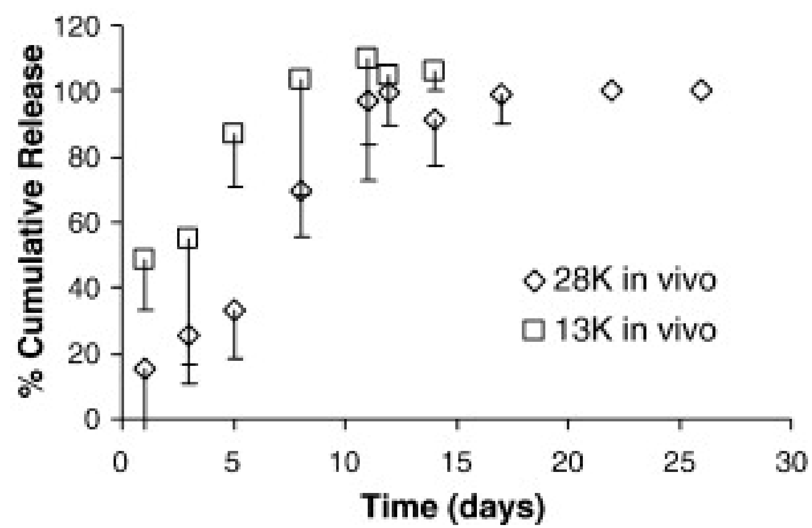
IN VITRO

- Triphasic release
- Initial Burst 20-30%
- Lag phase 5-9 days
- Total release in 30-35 days

IN VIVO

- Biphasic Release
- Initial Burst 15-45%
- No apparent lag phase
- Total Release in 12-16 days





Polymer degradation can also be faster *in vivo*

ELSEVIER

Biomaterials 20 (1999) 1057–1062

Biomaterials

Factors affecting the degradation rate of poly(lactide-co-glycolide) microspheres in vivo and in vitro

M.A. Tracy*, K.L. Ward, L. Firouzabadian, Y. Wang, N. Dong, R. Qian, Y. Zhang

Alkermes Inc., 64 Sidney Street, Cambridge, MA 02139, USA

Received 20 January 1998; accepted 30 December 1998

Table 2
In vivo vs. in vitro degradation results for PLG 50:50 microspheres

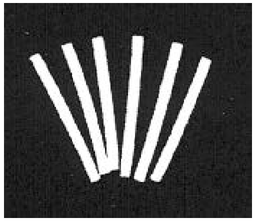
Microsphere polymer type	In vivo rate constant ($\times 10^{-2}$) (1/days)#	In vitro rate constant ($\times 10^{-2}$) (1/days)#	Rate constant ratio in vivo/in vitro	Microsphere duration in vivo (days)	Microsphere duration in vitro (days)
Capped, 9.5 kD	-3.3 ± 0.6	-1.8 ± 0.3	1.8	50–60	⩾60*
Capped, 12.7 kD	-4 ± 1	-2.4 ± 0.7	1.7	42–49	⩾60*
Uncapped, 8 kD	-13 ± 5	-5 ± 1	2.6	14–21	~35
Uncapped, 21 kD	-7.9 ± 0.8	-4.4 ± 0.8	1.8	21–28	>60*

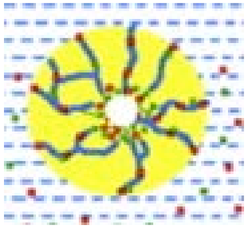
* Last time point taken.
# Errors are 95% confidence limits.

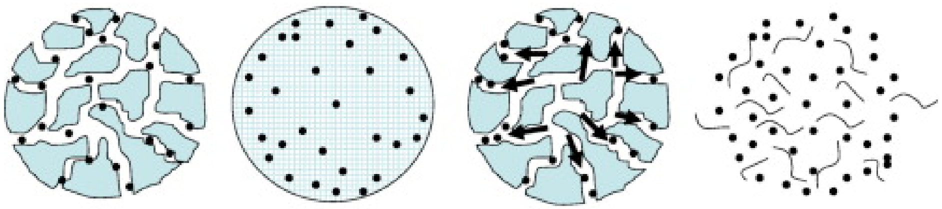
How is slow release commonly achieved from PLGA?

Combination of 3 basic phenomena —

- ✧ Diffusion
- ✧ Osmotic pressure/swelling
- ✧ Bioerosion when polymer chains become small enough to give way to stresses and/or dissolve







(Fredenberg et al., *Int. J. Pharm.*, **415**, 34–52 (2011))

What factors can affect the release mechanism?

- Buffering system and capacity
- Ionic strength/osmotic pressure
- pH
- Volume/flow
- Enzymes
- Lipids
- Inflammatory response
- *Unknown* small molecules present *in vivo*

Long-term objective: Understand mechanistically *in vivo* controlled release from PLGA microspheres and develop mechanism-based IVIVCs

Drug release from PLGA microparticles *in vitro*

Knowledge Gap

How do these differ?
Why do these differ?

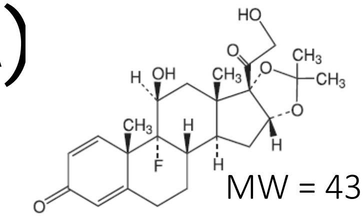
Drug release from PLGA microparticles *in vivo*

Measure relevant time scales—

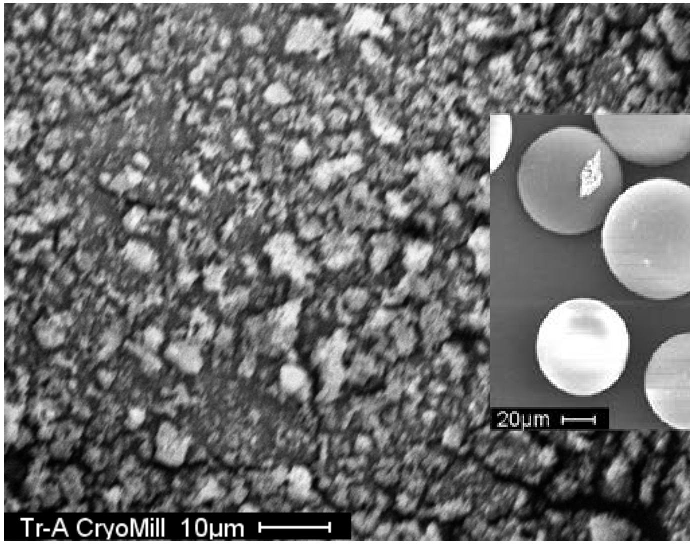
$\tau_{release}$

- $\tau_{erosion}$
- $\tau_{water\ uptake}$
- $\tau_{hydrolysis}$
- $\tau_{diffusion}$

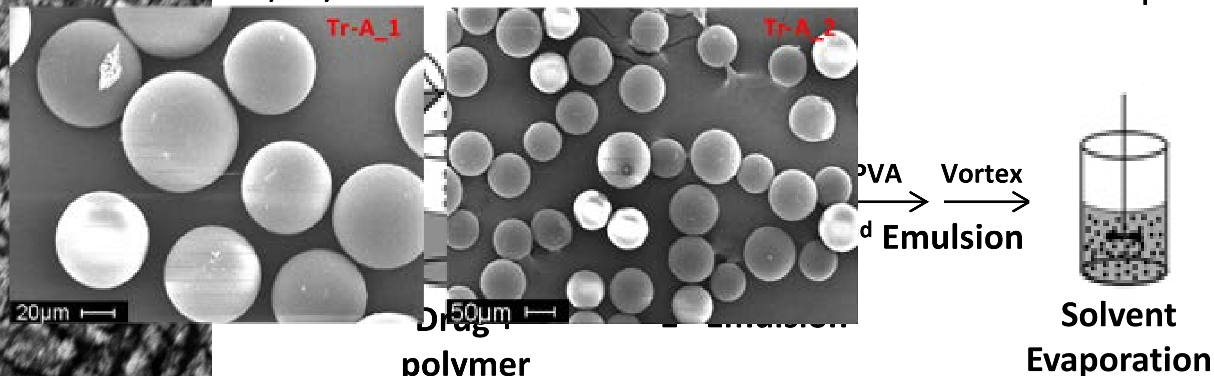
Two Triamcinolone acetonide (Tr-A) PLGA Microsphere Formulations



MW = 435 g/mol



s/o/w double emulsion solvent evaporation



Tr-A_1	Tr-A_2
Low molecular weight (18 kDa) acid terminated PLGA 50/50 (502H) 1000 mg/mL PLGA	Moderate molecular weight (54 kDa) ester end-capped PLGA 50/50 400 mg/mL PLGA

In vitro release methods

Release media

- Phosphate buffered saline + 0.02% Tween 80 (PBST)
  - pH 7.4 (**standard condition**)
  - pH 6.5
- HEPES buffered saline pH 7.4 + 0.02% Tween 80
- PBS + 1.0% triethyl citrate (TC)

Method

- 5mg (approx) incubated in 50mL media
- 37°C, mild agitation
- Particles centrifuged and media completely removed and replaced

Analyses (release and mechanisms of release)

- Release media analyzed for drug content by HPLC — $\tau_{release}$
- Molecular weight determined by GPC — $\tau_{hydrolysis}$
- Mass loss and water uptake determined gravimetrically — $\tau_{mass\ loss}$; $\tau_{water\ uptake}$
- Particles incubated in BODIPY to determine diffusion coefficient — $\tau_{diffusion}$

In vivo release methods

- Measure PK (indirect)
- Recover microspheres after injection

Problem!! – How to recover microspheres intact after administration – we don't see them??

We want to to understand **mechanism** and therefore want to measure—

$\tau_{release}$

$\tau_{hydrolysis}$

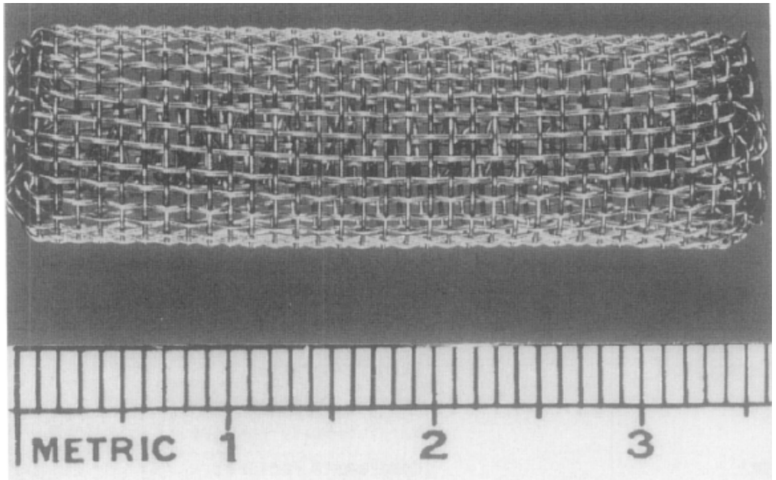
$\tau_{mass\ loss}$; $\tau_{water\ uptake}$

$\tau_{diffusion}$

How can we recover microspheres simply *In vivo*?

The Cage implant—

- Developed by Marchant *et al.* for evaluation of biocompatibility of biomaterials



Cage Model Design

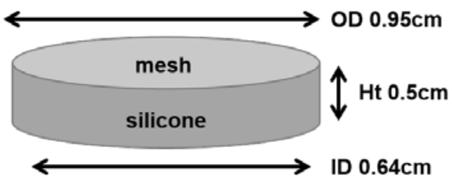
Cages:

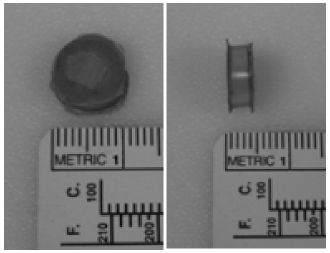
Stainless steel mesh (37µm openings)

Silicone tubing

Silicone elastomer

Vulcanize and by autoclave





Loading of Microspheres into Cages:

1. Microspheres are suspended in an injection medium containing 1% CMC
2. Suspension is injected (via 20g needle) through silicone tubing into cage
3. Loaded cages are suspended in saline solution until implantation

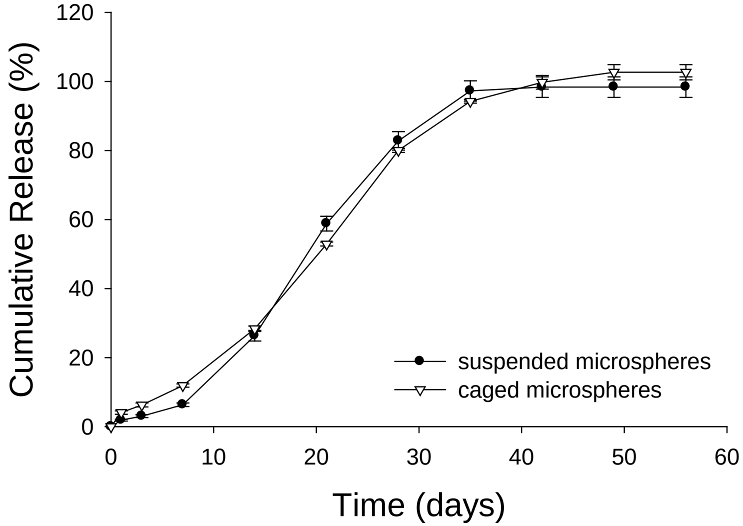
Cage Implantation:

1. Animals are anesthetized and surgical site is sterilized
2. Single incision is made on the flank and a subcutaneous pocket is created
3. Cage is implanted into the pocket
4. Incision is closed using veterinary adhesive

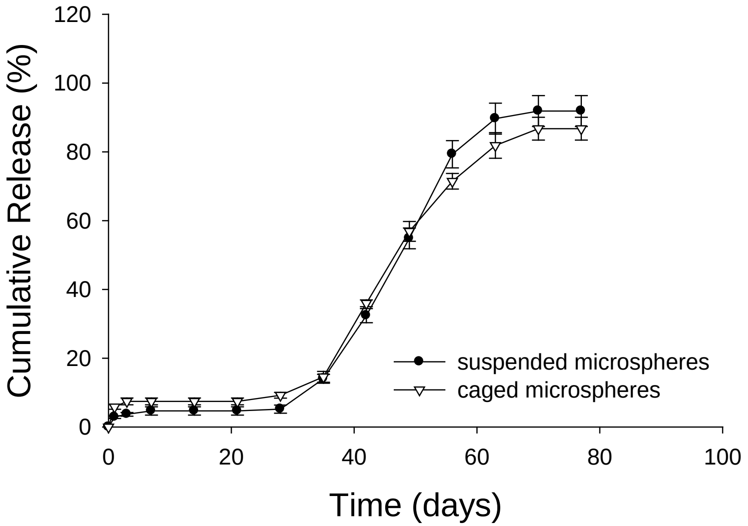
Validation of Cage Model *in vitro*

caged vs. suspended release *in vitro*

Tr-A_1

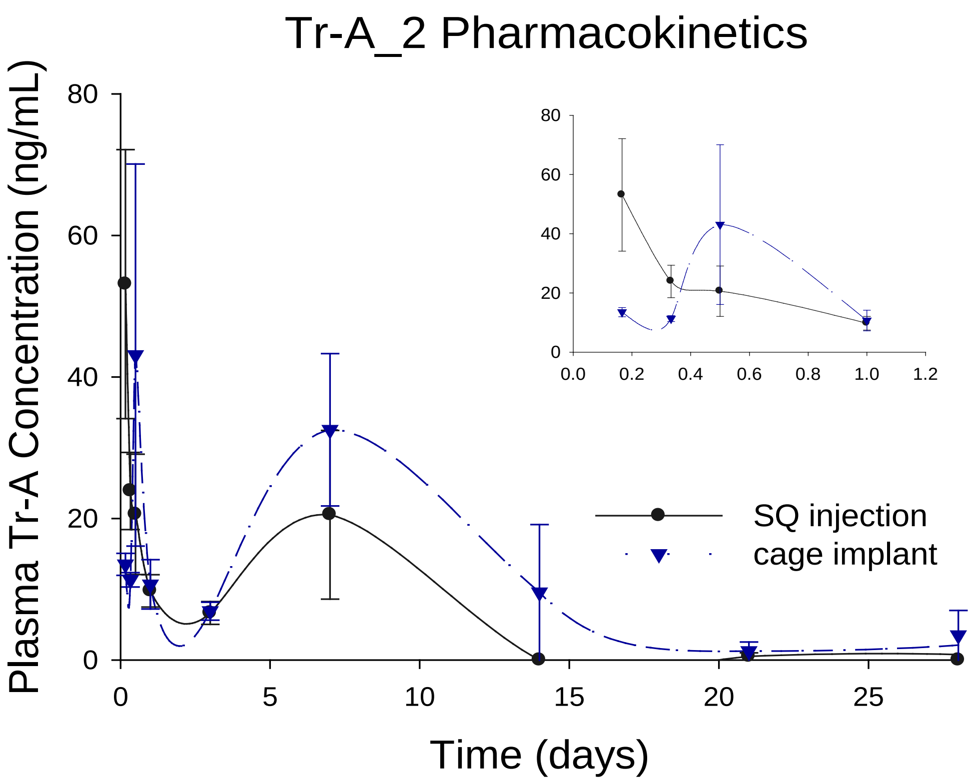


Tr-A_2



In vitro release from cage (suspended in PBST pH 7.4) is similar to release of particles freely suspended in PBST pH 7.4

Validation of Cage Model *in vivo*



- Delayed burst from cage (within first 24 hours)
  - Overall similar kinetics through one month
- Very low drug levels seen after 14 days (suggests release within this time frame)
  - Faster release in vivo as compared to in vitro is observed in cage as well as from freely suspended particles

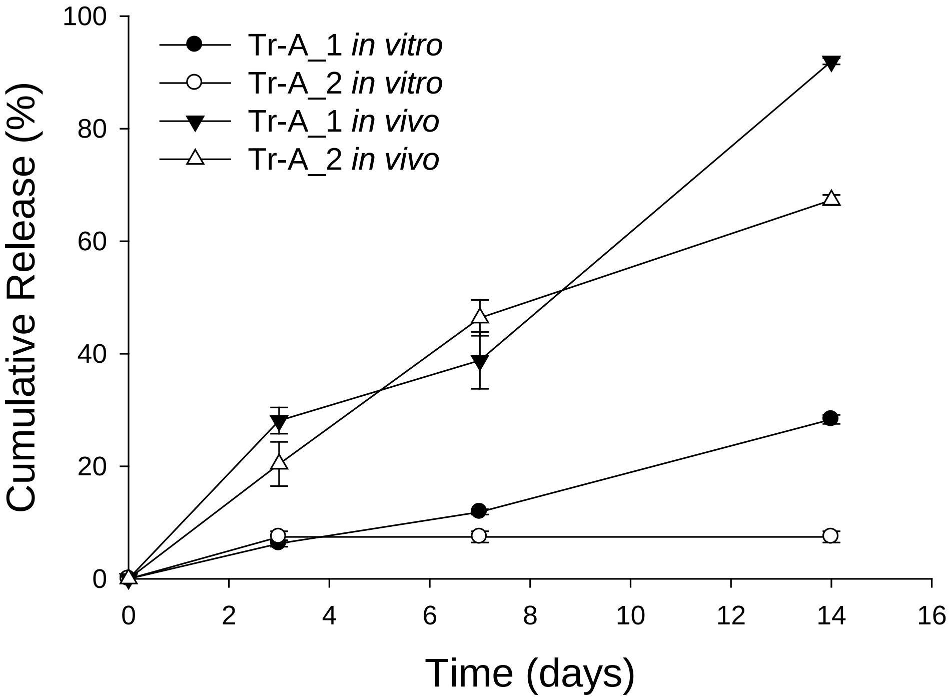
Tr-A release is much faster *in vivo* than *in vitro*

- Release *in vivo* was measured using **cage model**
- Drug release determined by measuring drug remaining in microspheres
- Tr-A_1 release thru 14d:

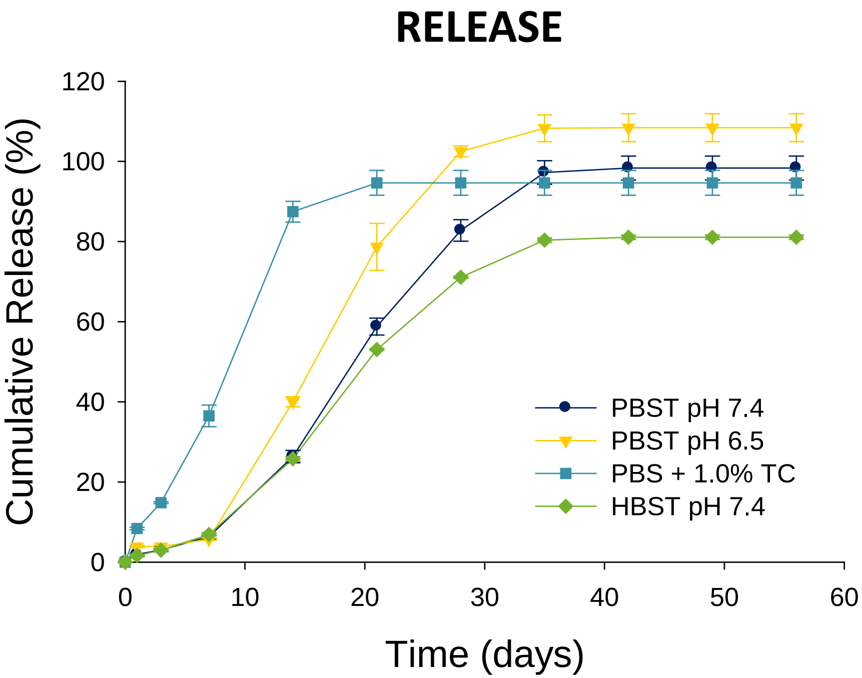
in vitro	28.4 ± 0.8%
in vivo	**91.9 ± 0.8%**

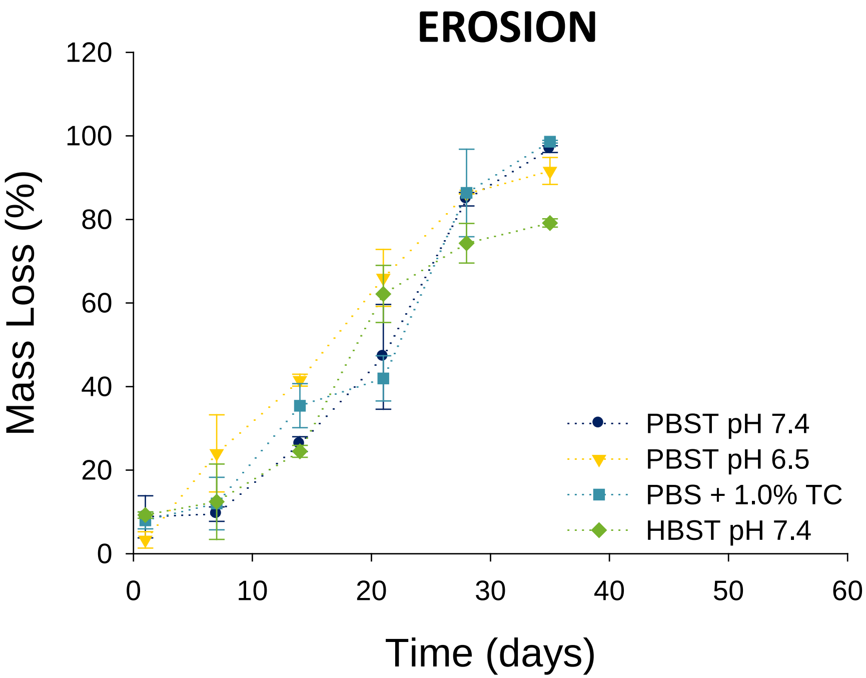
- Tr-A_2 release thru 14d:

in vitro	7.4 ± 1.0%
in vivo	**67.3 ± 1.3%**



Tr-A_1: Reduced pH and plasticizer accelerate release and erosion *in vitro*



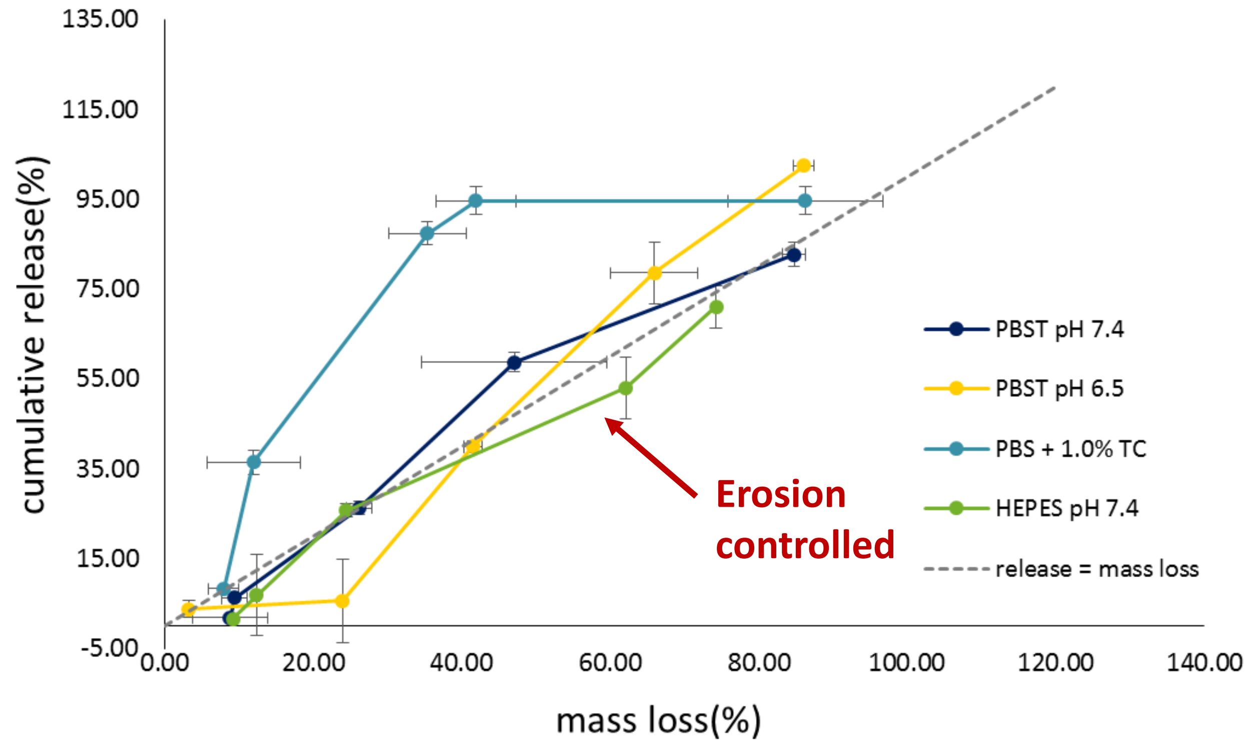


- Two conditions of slightly accelerated Tr-A release:
    1. PBS + 1.0% TC
    2. PBST pH 6.5

TC changes mechanism of *in vitro* release

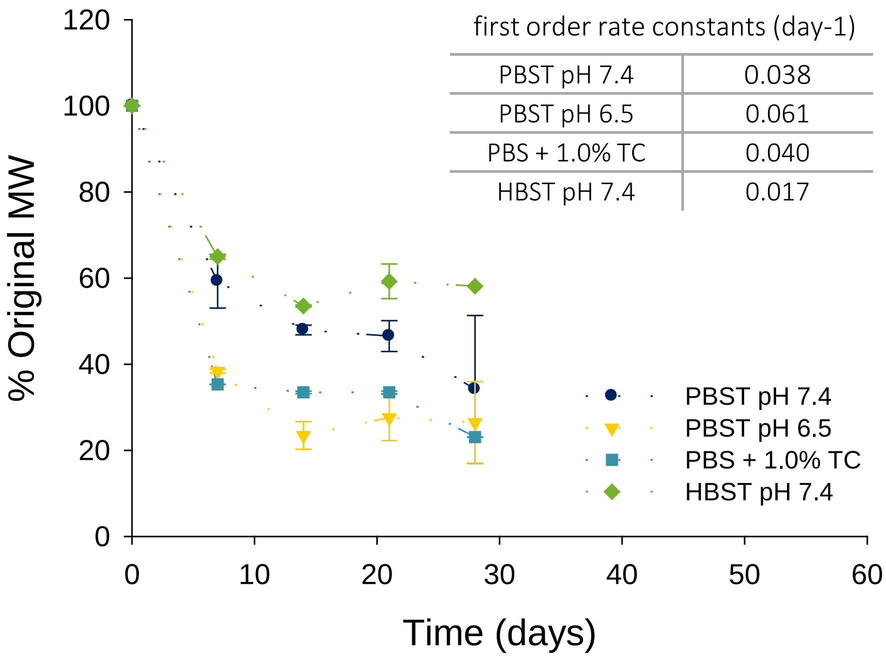
Half times to release and mass loss (days)

	PBST pH 7.4	PBST pH 6.5	PBS + 1.0% TC	HEPES pH 7.4
t_{50} release	19.3 ± 0.5	16.6 ± 0.4	8.5 ± 0.3	18.1 ± 0.2
t_{50} mass loss	24.0 ± 3.9	16.1 ± 1.3	19.3 ± 1.1	17.6 ± 1.1

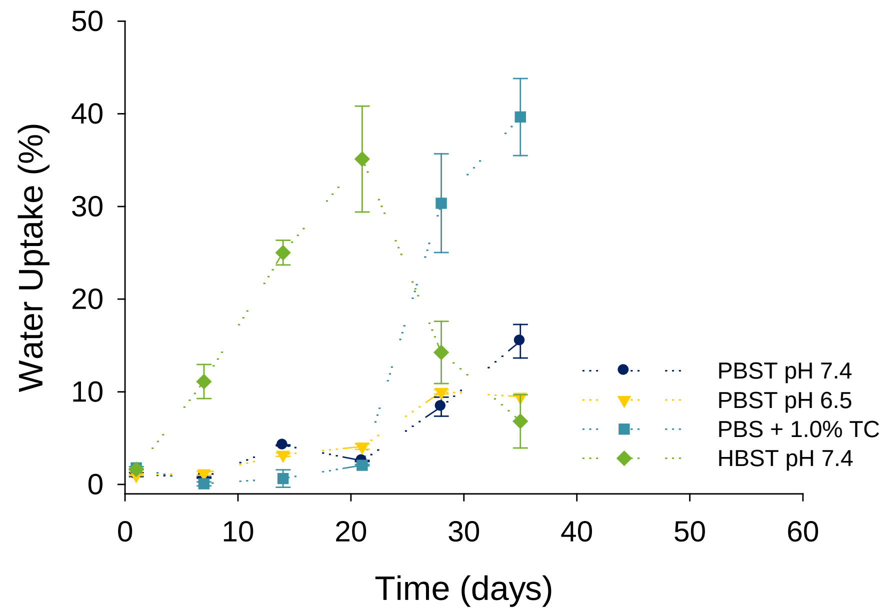


Tr-A_1: *in vitro* kinetics of PLGA MW and water uptake

HYDROLYSIS

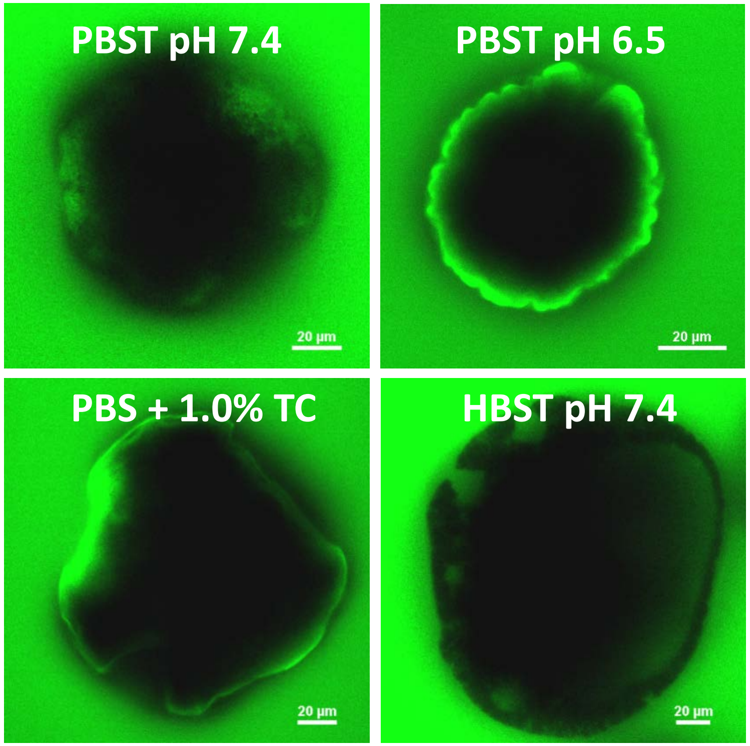


WATER UPTAKE

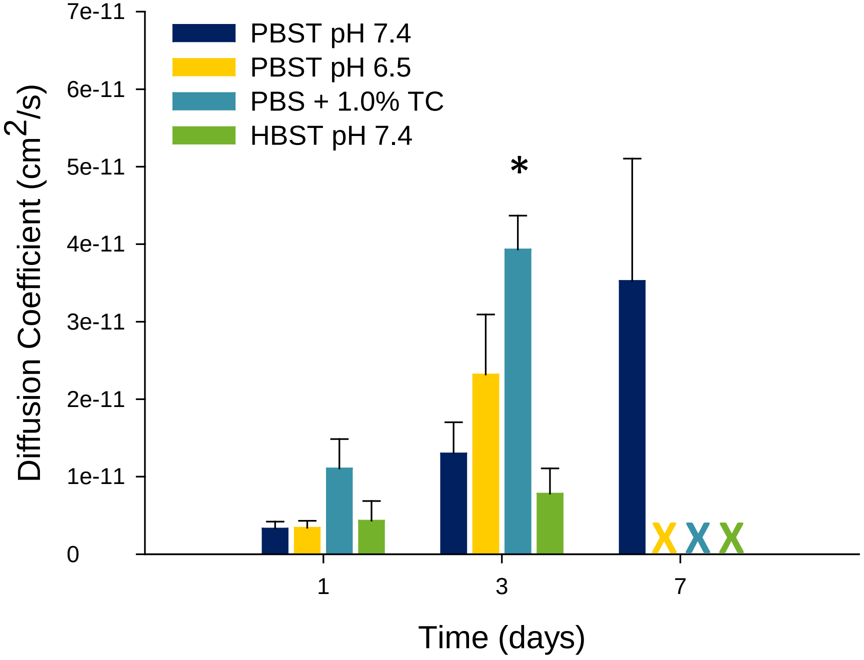


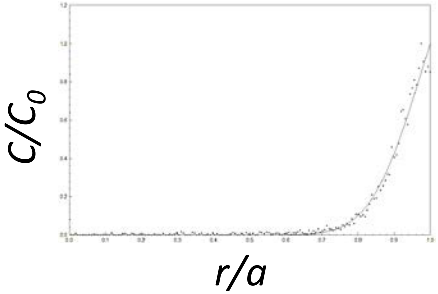
- Slightly accelerated hydrolysis in two conditions:
  1. PBS + 1.0% TC
  2. PBST pH 6.5
- Water uptake kinetics appear not to influence release from Tr-A_1

Tr-A_1: Diffusion of bodipy in degrading microspheres



Representative LCSM images following 3 days incubation

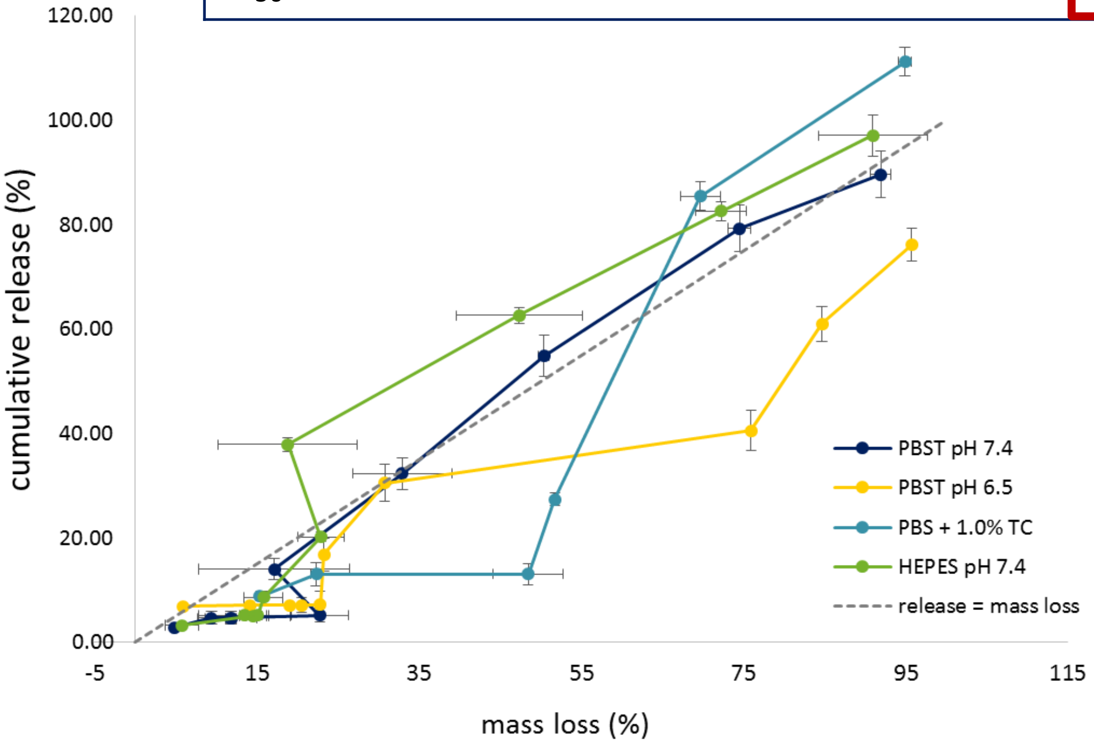




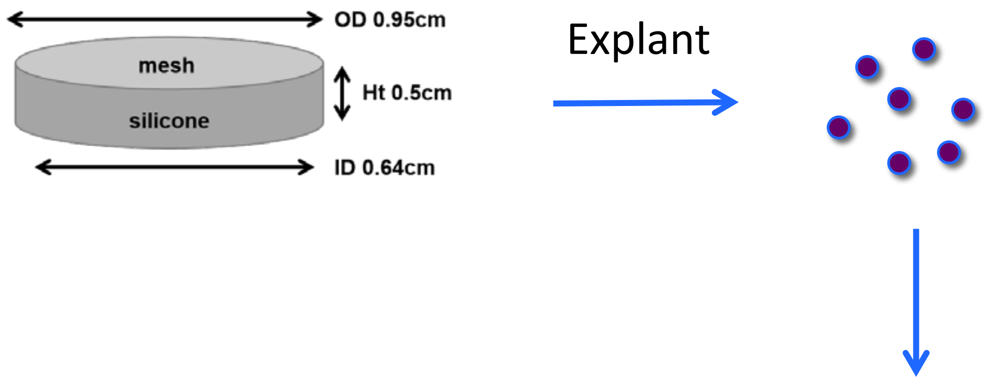
Tr-A_2: Most formulations also erosion-controlled *in vitro*

Half times to release and mass loss (days)

	PBST pH 7.4	PBST pH 6.5	PBS + 1.0% TC	HEPES pH 7.4
t_{50} release	46.9 ± 0.6	52.1 ± 1.1	25.1 ± 0.2	46.5 ± 0.4
t_{50} mass loss	58.1 ± 7.4	46.1 ± 1.2	17.5 ± 1.7	50.7 ± 2.0

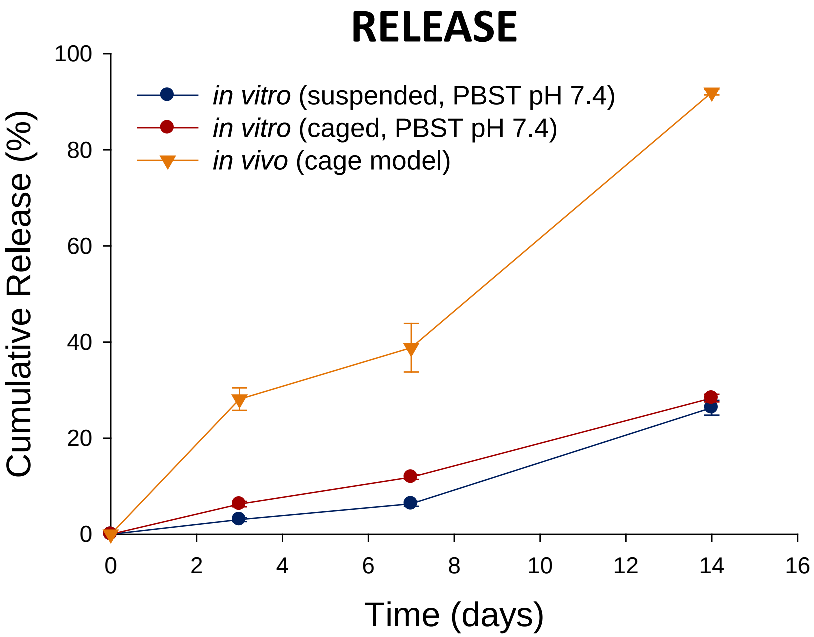


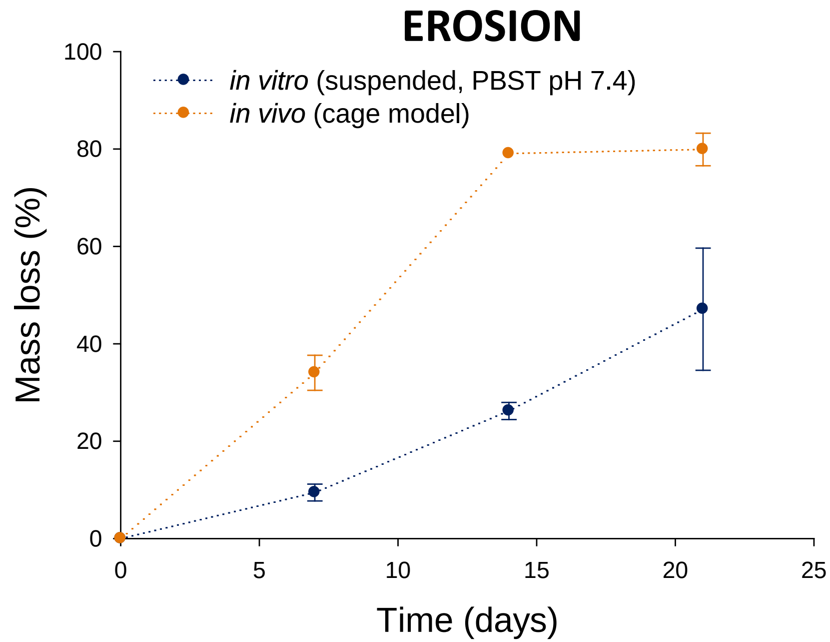
Assessment of release mechanisms from microspheres recovered from in vivo cage implantation



Mechanistic experiments

Tr-A_1: release and mass loss accelerated *in vivo*



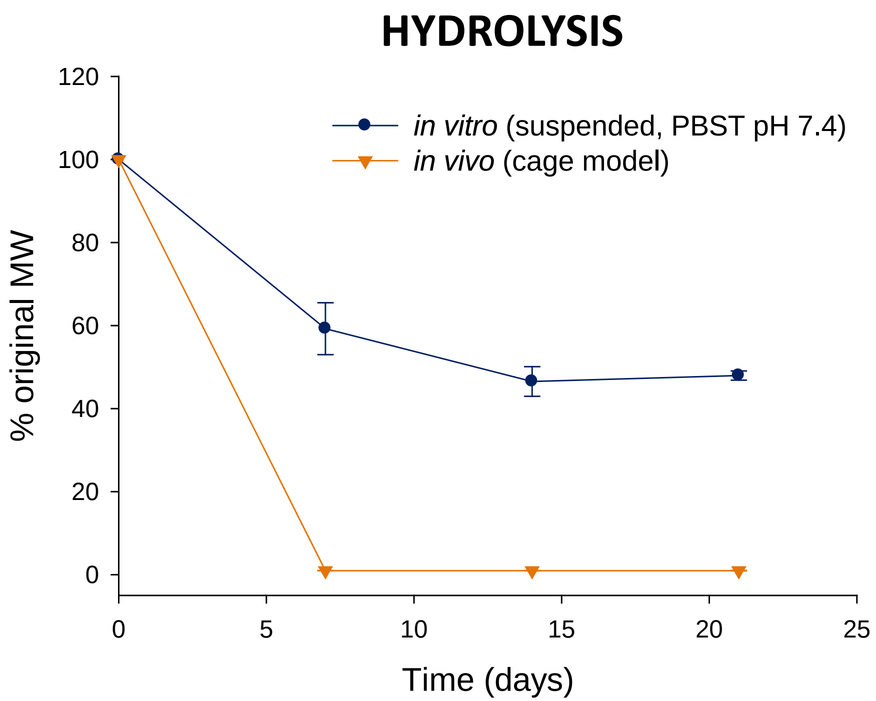


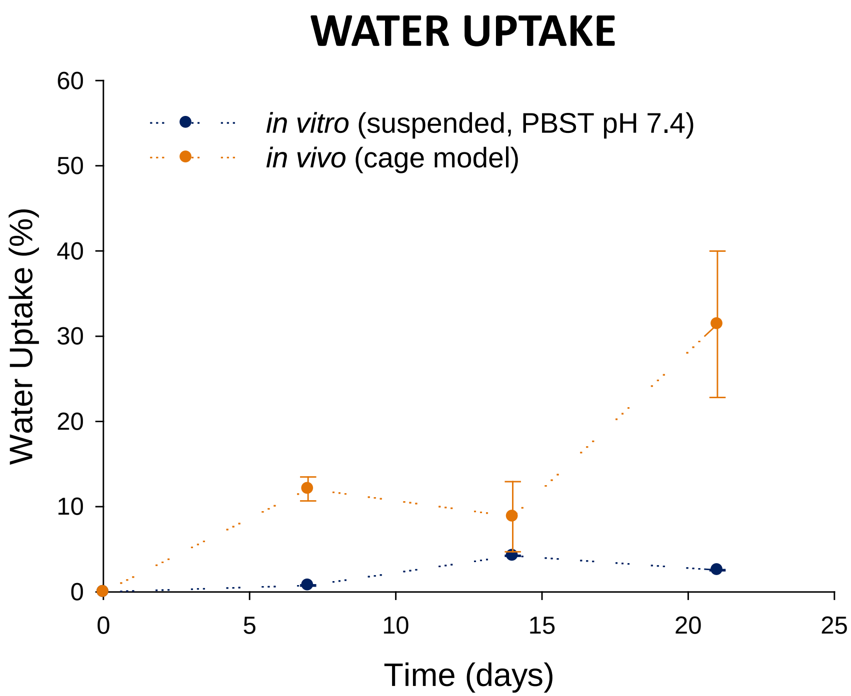
Half times to release and mass loss

	in vitro	in vivo
t_{50} release	19.3 ± 0.5	8 (approx.)
t_{50} mass loss	24.0 ± 3.9	10 (approx.)

- Release and mass loss are faster *in vivo* than *in vitro*
  - Accelerated erosion *in vivo*
- t_{50} release ≈ t_{50} mass loss in both cases : suggests erosion-controlled release

Hydrolysis kinetics and water uptake increased *in vivo*

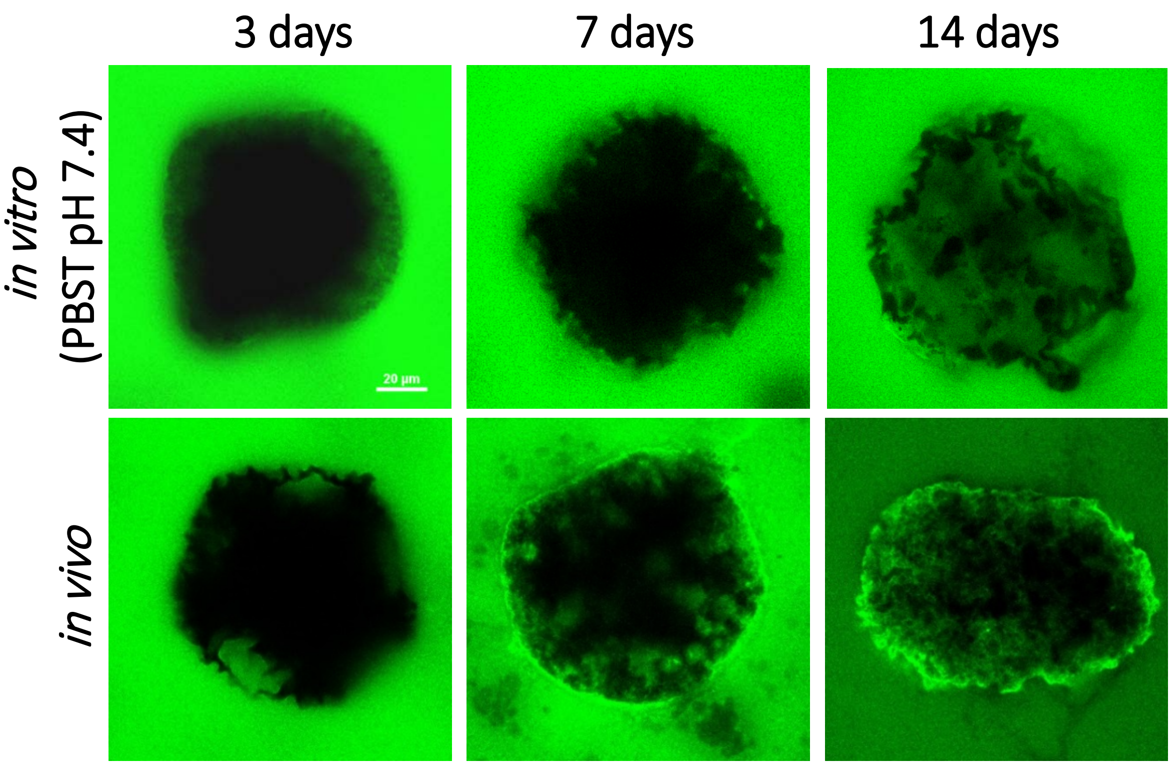


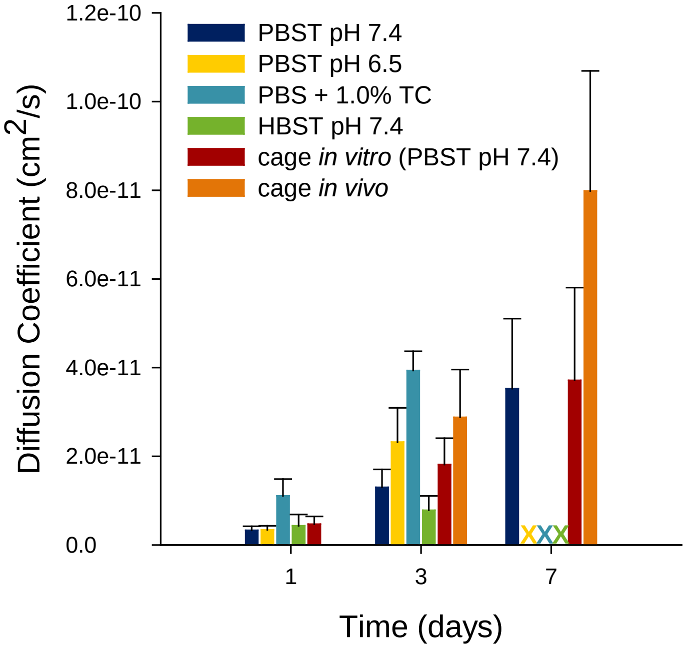


- Water uptake much higher *in vivo* than *in vitro* (PBST pH 7.4)
- Hydrolysis of PLGA faster *in vivo* than *in vitro*
  - Likely contributes to accelerated mass loss and release

	in vitro	in vivo
First order rate constant (k)	0.038 day^{-1}	TBD

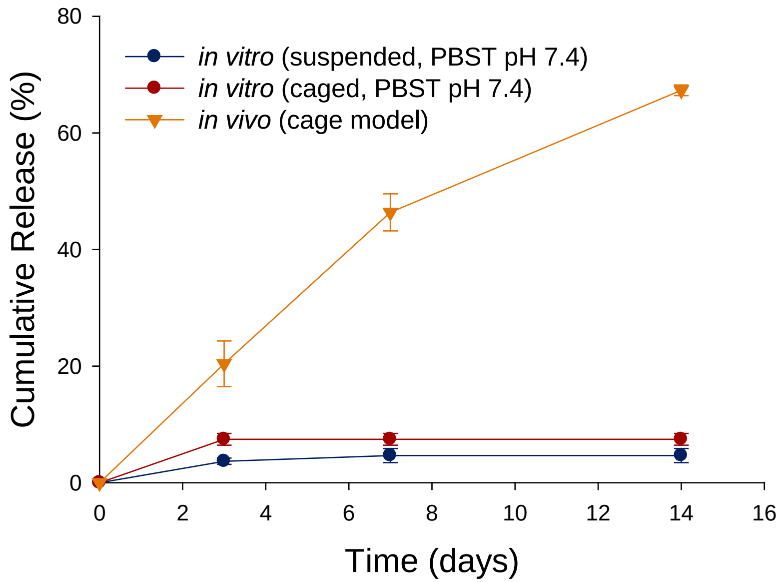
Tr-A_1: Diffusion of bodipy in degrading microspheres not so different



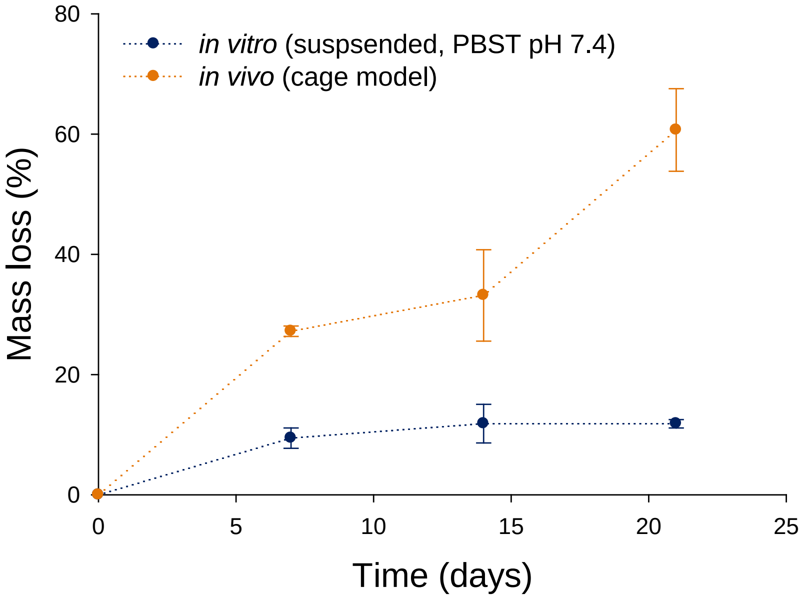


Tr-A_2: release and mass loss accelerated *in vivo*

RELEASE



EROSION

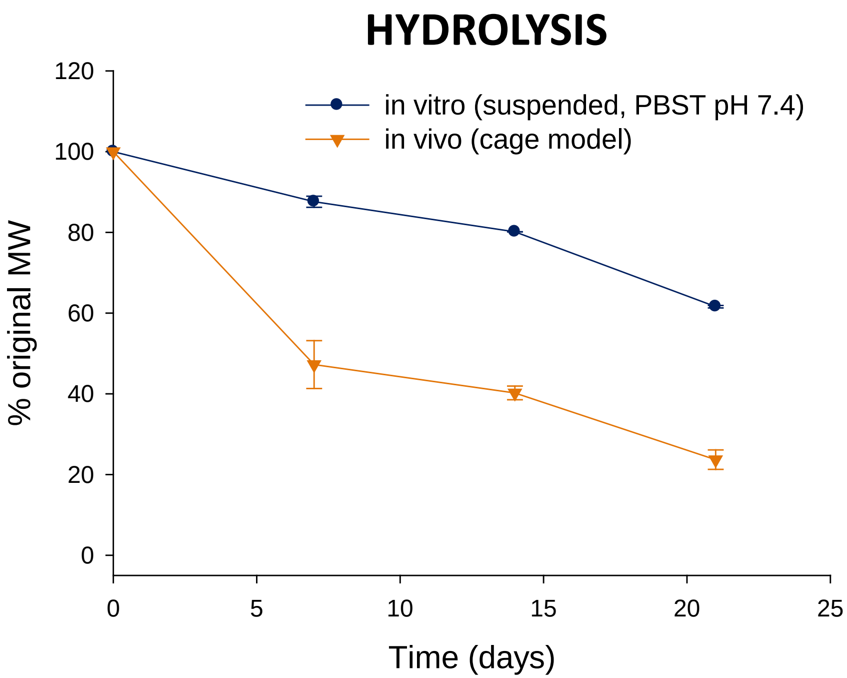


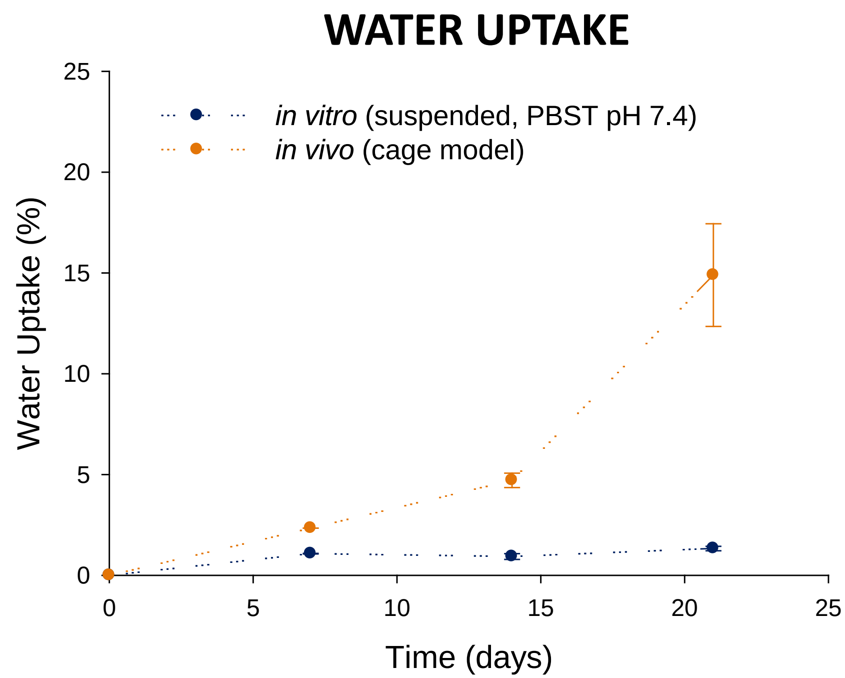
Half times to release and mass loss

	in vitro	in vivo
t_{50} release	46.9 ± 0.6	6.5 ± 2.1
t_{50} mass loss	58.1 ± 7.4	18 (approx.)

- Release and mass loss are faster *in vivo* than *in vitro*
  - Accelerated erosion *in vivo*
- t_{50} release << t_{50} mass loss *in vivo*
  - <u>suggests another mechanism may contribute to accelerated release</u>

Degradation and water uptake increase *in vivo*

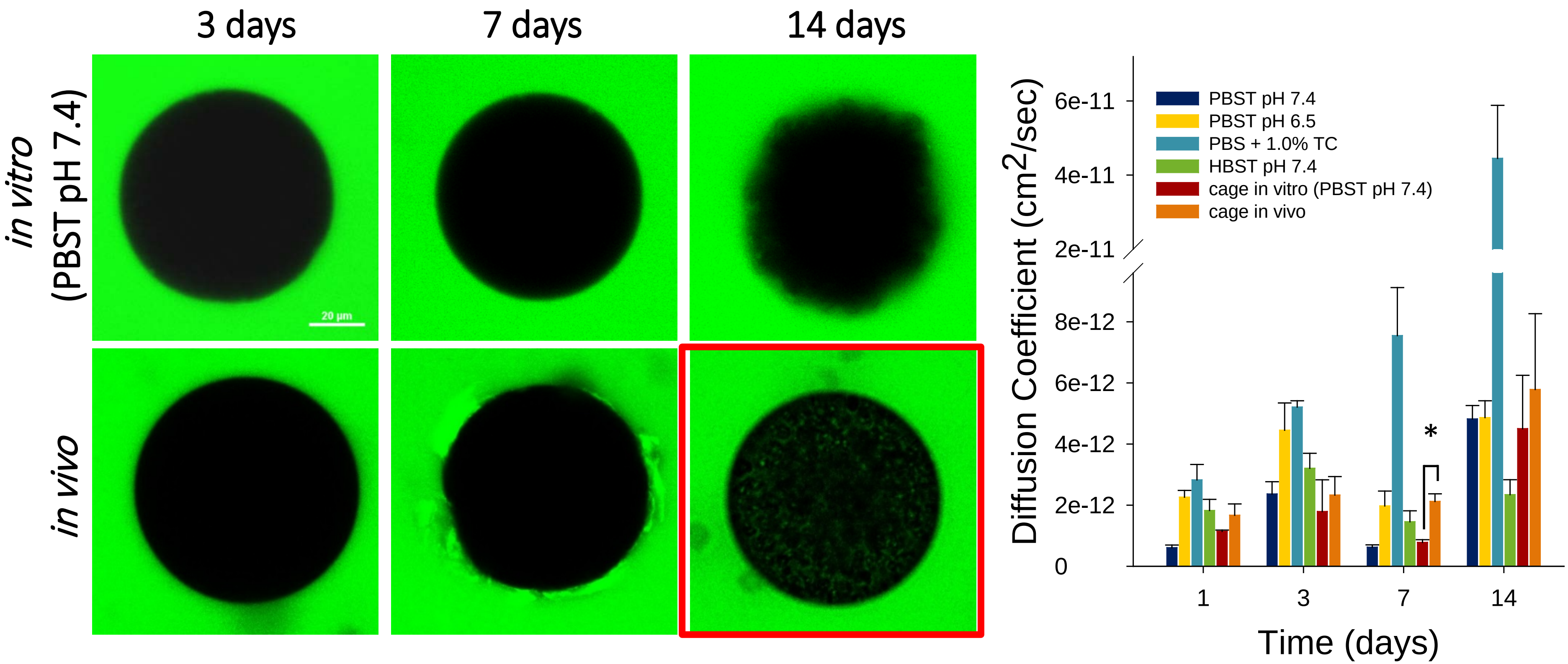




- Water uptake much higher *in vivo* than *in vitro* (PBST pH 7.4)
- Hydrolysis of PLGA faster *in vivo* than *in vitro*
  - Likely contributes to accelerated mass loss and release

	in vitro	in vivo
First order rate constant (k)	0.040 day^{-1}	0.065 day^{-1}

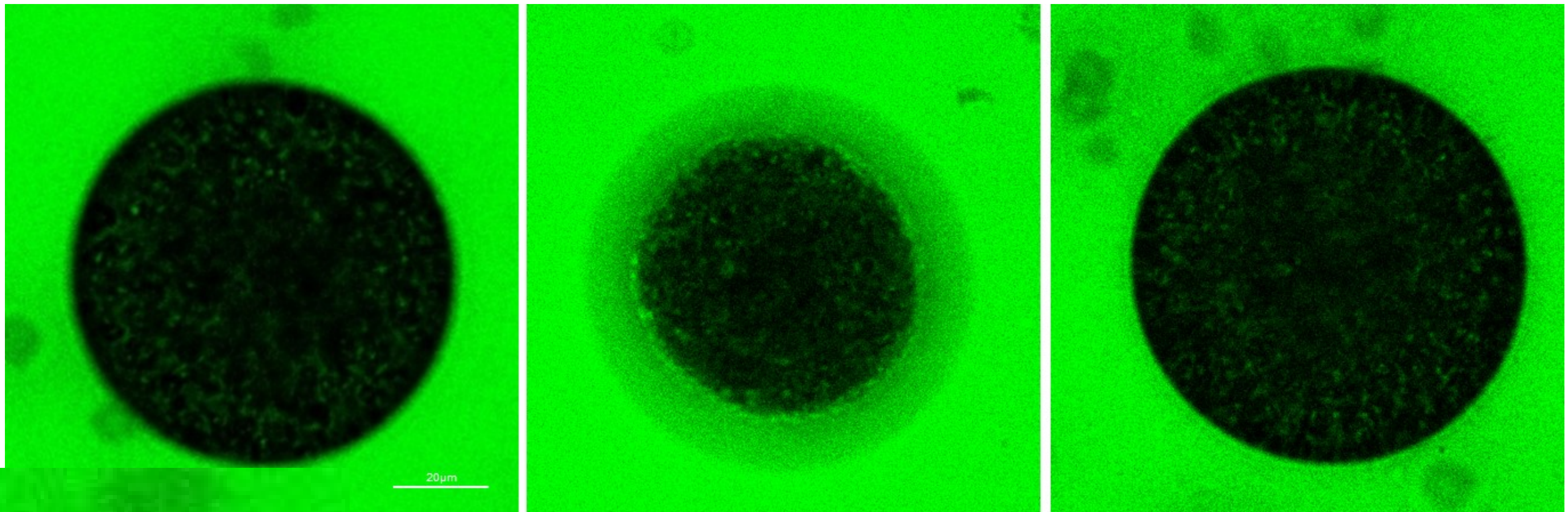
Tr-A_2: Solid state diffusion of bodipy not so different

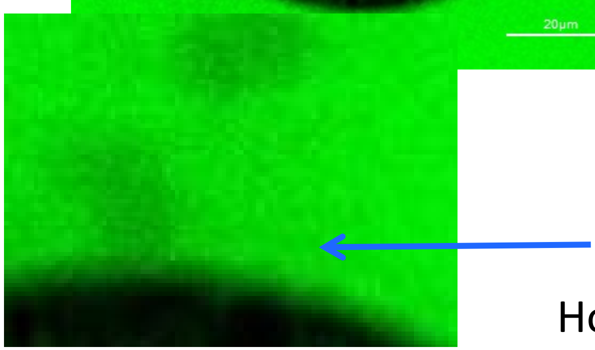


- Internal pore formation visible following 14 days *in vivo*
    - Not evident following 14 day release *in vitro*

Pore localization of Bodipy suggests osmotically induced aqueous pore diffusion as a mechanism of release *in vivo*

Tr-A_2 particles following 2 weeks in subcutaneous cage implant





Holes visible at surface connecting percolating network

Conclusions

- Cage implants can be used to uncover valuable mechanistic data concerning *in vivo* release from PLGA LARs -- by solving the problem of difficult recovery of intact microspheres after administration
- Initial data suggest that PLGA release kinetics in the cage is predictive of *SC in vivo* release after the initial burst (similar PK w/ and w/o cage)
- Release of steroids from PLGA is generally faster *in vivo* than common *in vitro* release conditions
- Some causes of more rapid *in vivo* release:
  - Increased water uptake
  - Increase polymer degradation and erosion kinetics
  - Potential for osmotic pressure-mediated pore diffusion
- This approach may be useful to develop mechanistic IVIVCs

Acknowledgements

Lab members

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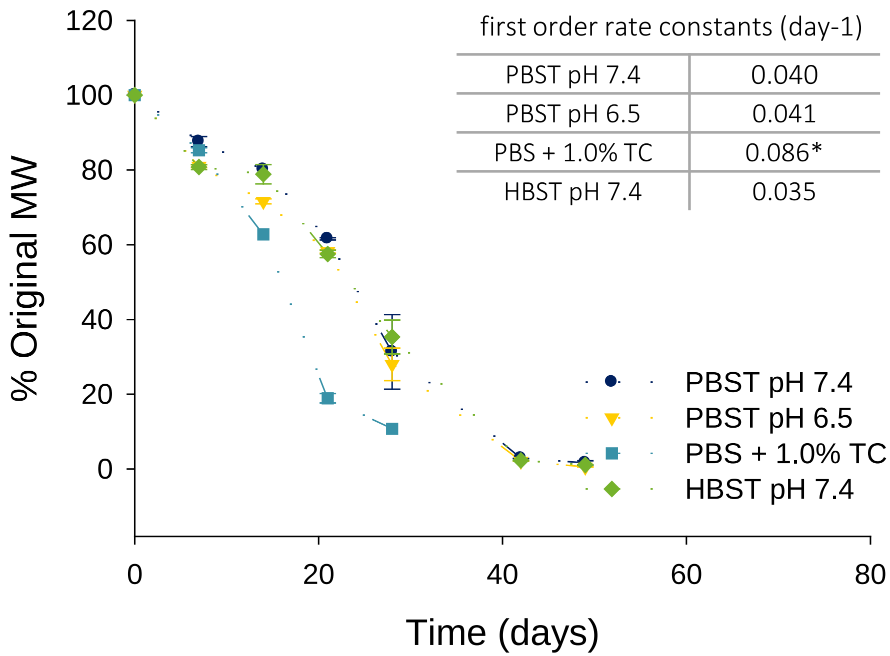
TC accelerates hydrolysis of PLGA in Tr-A_2

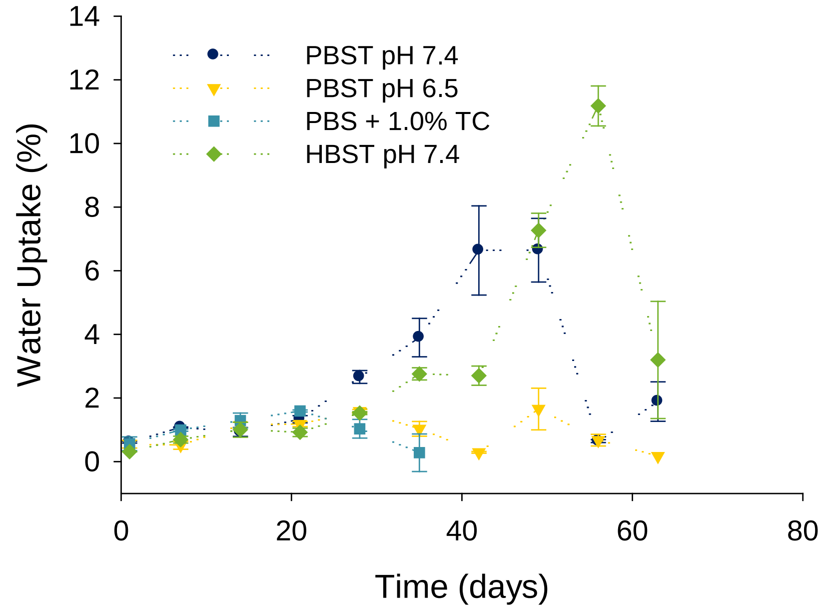
HYDROLYSIS



first order rate constants (day-1)

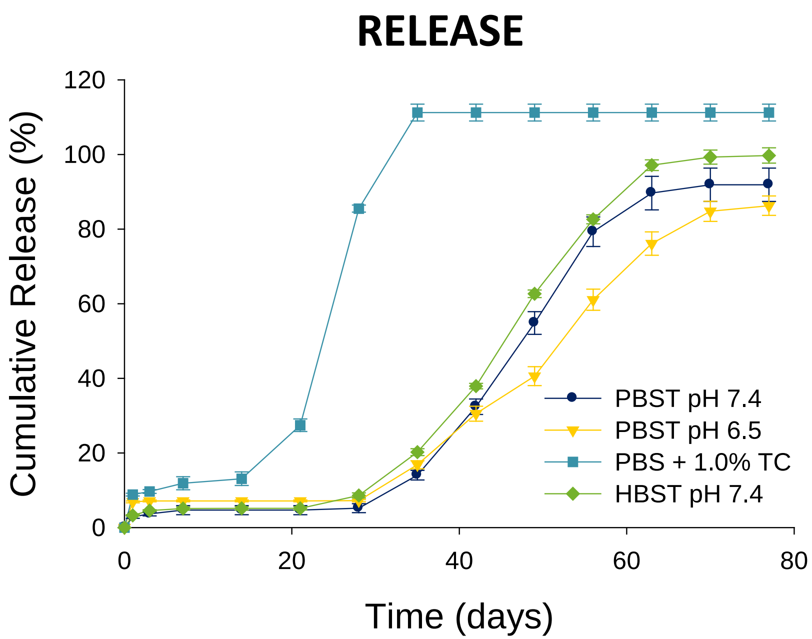
PBST pH 7.4	0.040
PBST pH 6.5	0.041
PBS + 1.0% TC	0.086*
HBST pH 7.4	0.035

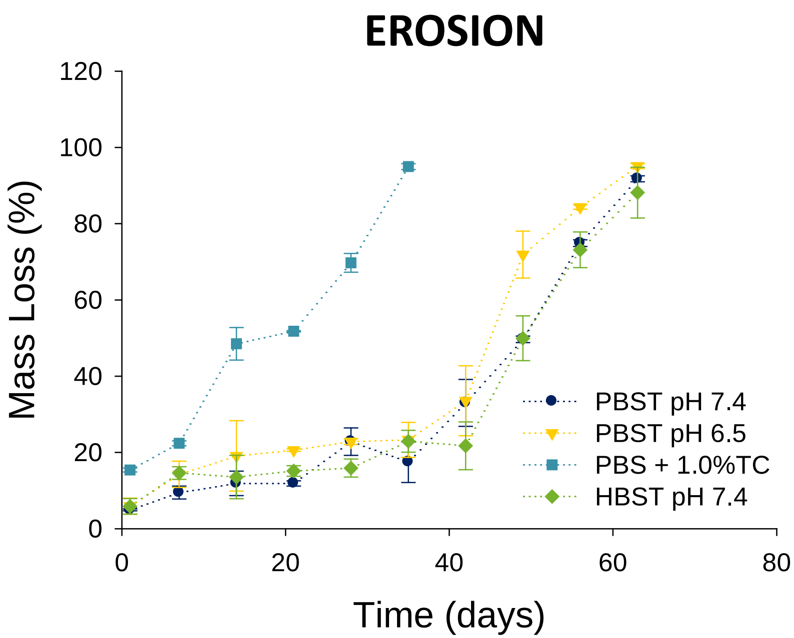
WATER UPTAKE



- Tr-A_2 formulation: PLGA 50:50, ester end capped
  - Original molecular weight (as determined in our lab by GPC) ≈ 54KDa
- Accelerated hydrolysis in one condition:
  1. PBS + 1.0% TC

TC accelerates release and mass loss from Tr-A_2





- Accelerated release in PBS + 1.0% TC
  - Accelerated erosion also evident in this condition