Stabilization of Biotherapeutics in Microparticles Exhibiting Pulsatile Release

Tyler P. Graf, Mei-Li Laracuente, Kadryn Kadasia, Erin Euliano, Sarah Melhorn, Haisong Yang, Brett H. Pogostin, Samantha Brady, Kevin J. McHugh

RICE ENGINEERING
Bioengineering

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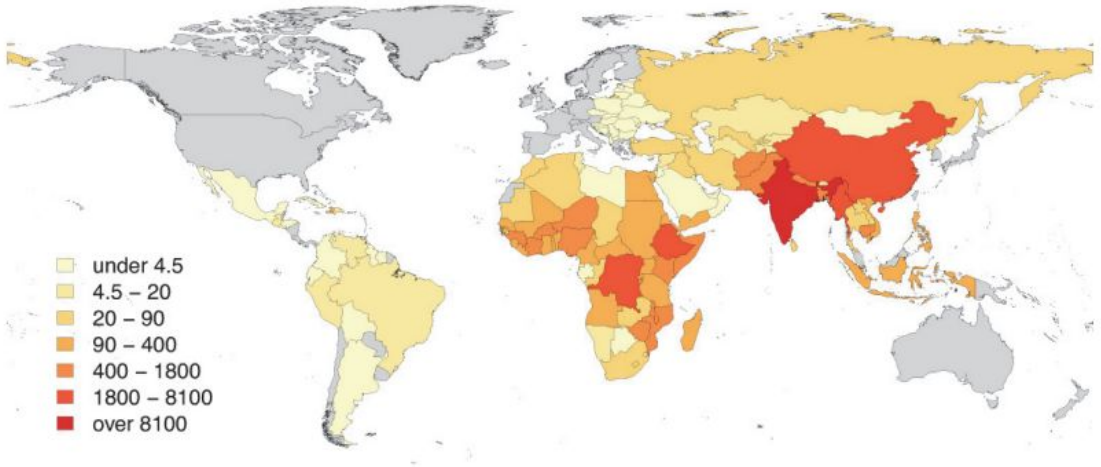
Single Injection Vaccination For Rabies Post Exposure Prophylaxis

- Rabies is 100% treatable with post-exposure administration of the rabies vaccine and rabies IgG
- Yet 59,000 rabies death occur annually, predominantly in low-and-middle income countries
- Deaths occur due to:
  1) Lack of healthcare access, especially in rural communities
  2) Post-exposure prophylaxis requires as many as 5 vaccine doses in a single month

Human Deaths Due to Rabies



Hampson, *PLOS Neglect Trop Dis* (2015)
Radhakrishnan, *Trop Med Infect Dis* (2020)
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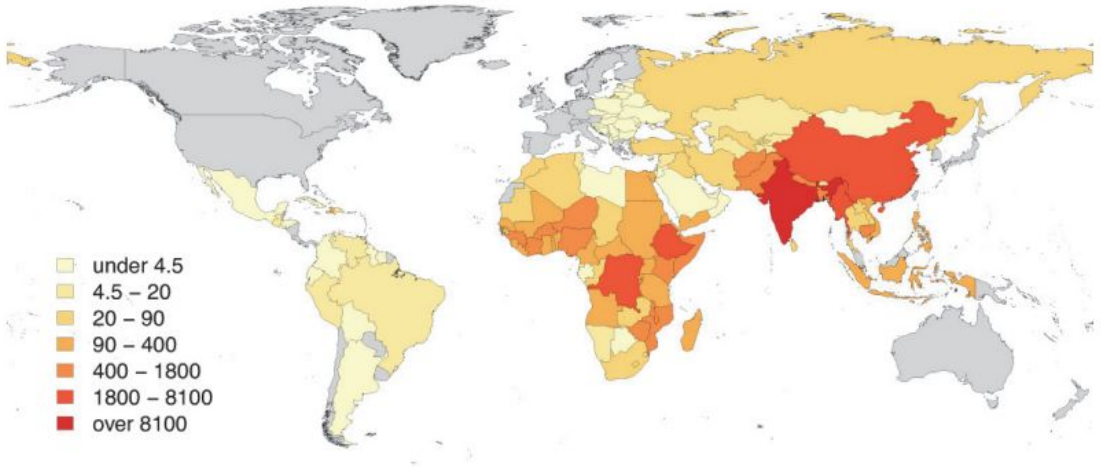
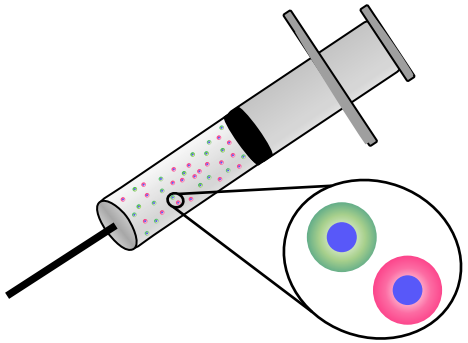
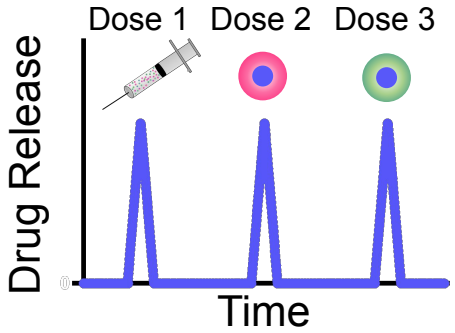


The administration of multiple doses of time-released rabies vaccine in a single injection could reduce barriers to access and compliance issues; however, to work **the rabies vaccine must be stabilized at body temperature prior to release.**





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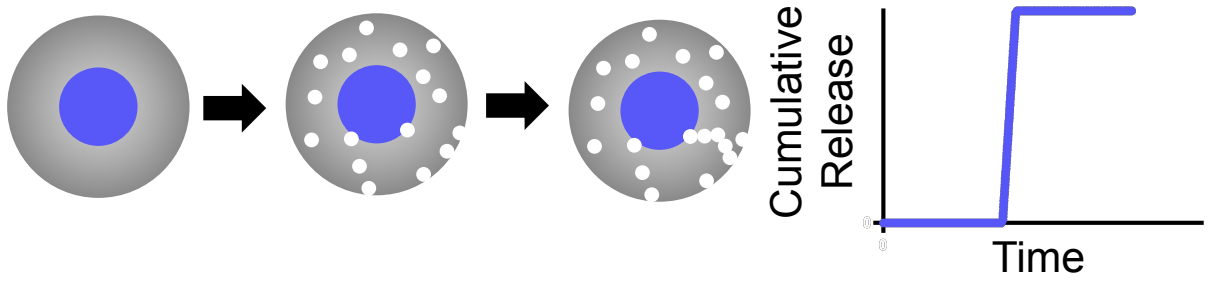
Background: Pulsatile Drug Delivery

Pulsatile Microparticles



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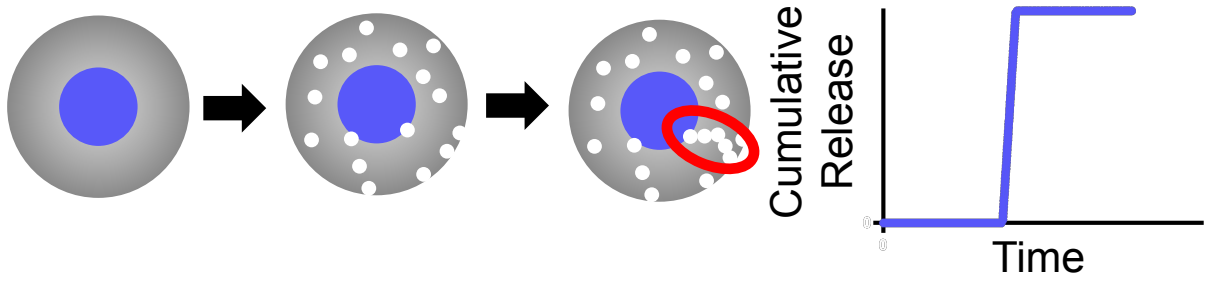

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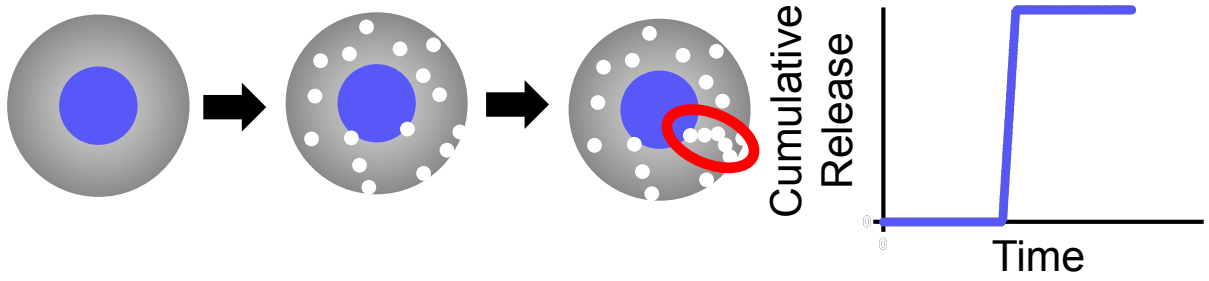
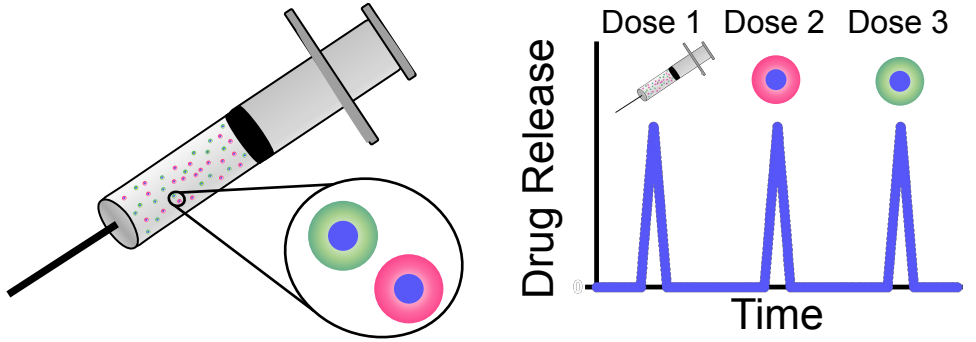


Deliver Multiple Doses in a Single Injection



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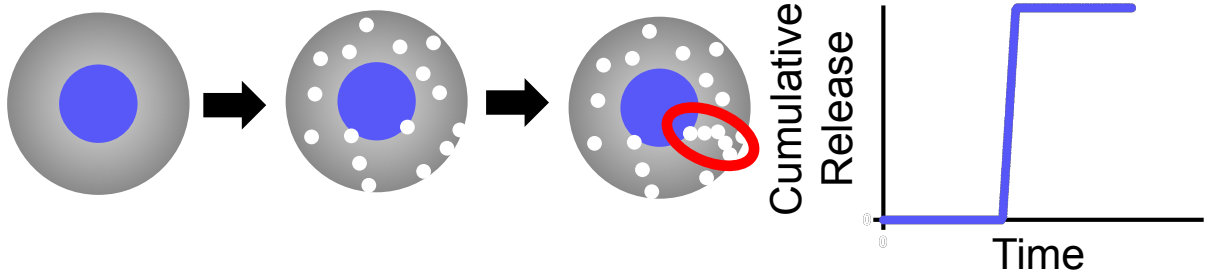

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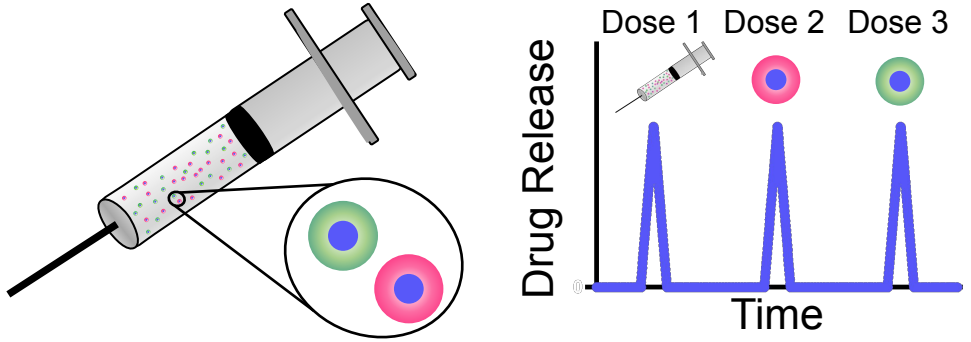
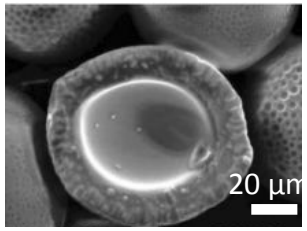
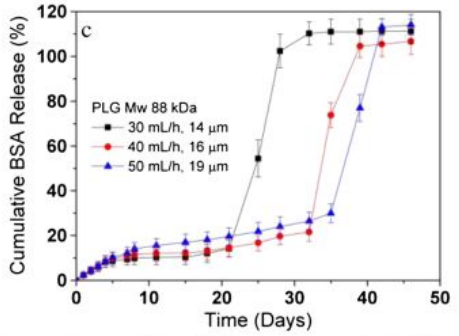
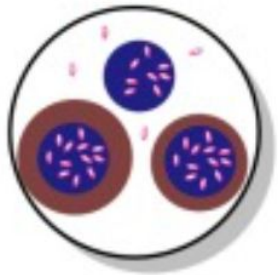


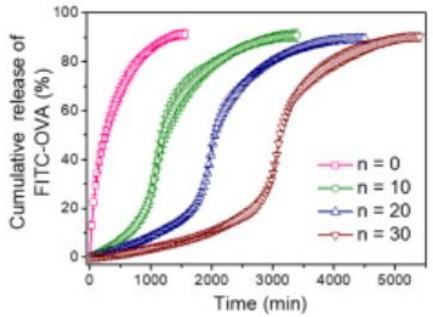
Liquid-Core Microcapsules



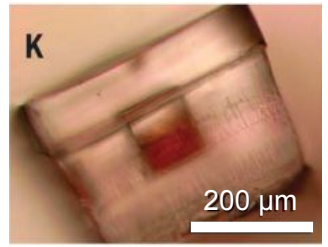


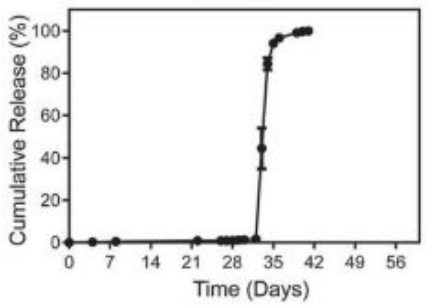
Layer-by-Layer Erodible Coatings





StampEd Assembly of Polymer Layers





Limitations

- **Leakage of cargo prior to release**
- **Stability of biologics prior to release**
- **Scalability poses commercialization challenges**

Xia, *Pharmaceutical Research* (2014)
McHugh, *Science* (2017)
Wang, *Biomaterials Advances* (2022)

Particles Uniformly Liquified and Sealed to Encapsulate Drug (PULSED)

- Poly(lactic-co-glycolic acid) (PLGA) films are compressed into polydimethylsiloxane (PDMS) molds and heated above their glass transition temperature (T_g) to form particles

Particle Molding Single Particle Particle Array

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Particle Filling

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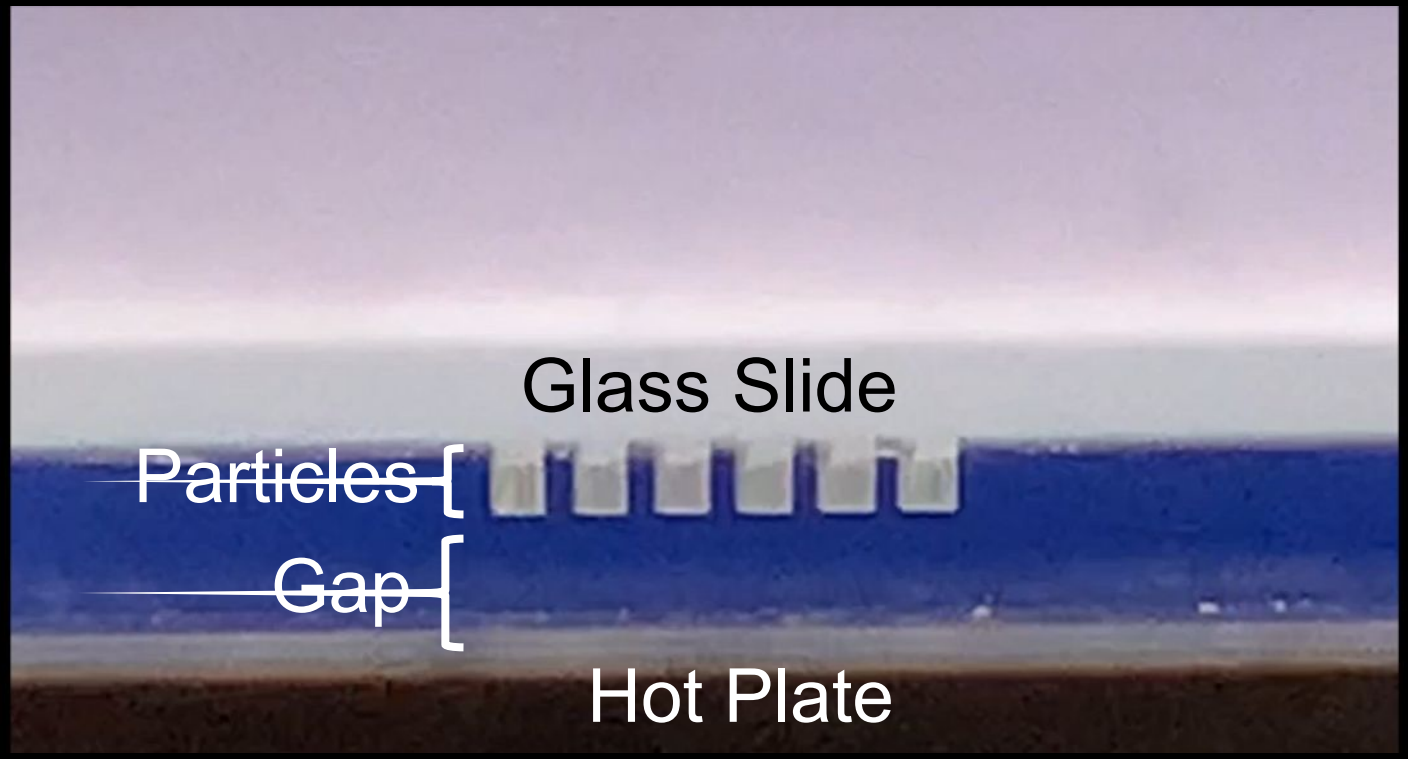
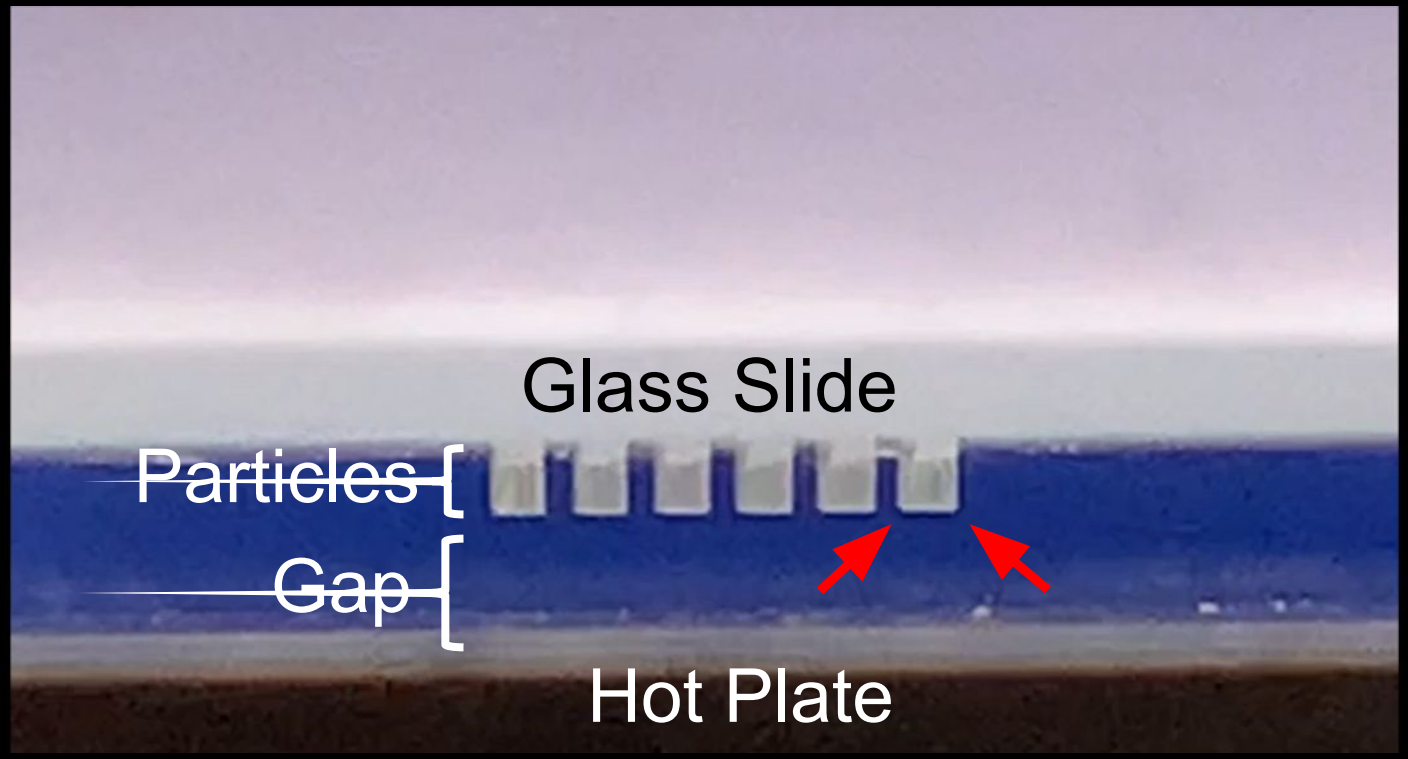
Particle Sealing


Glass Slide
Particles
Gap
Hot Plate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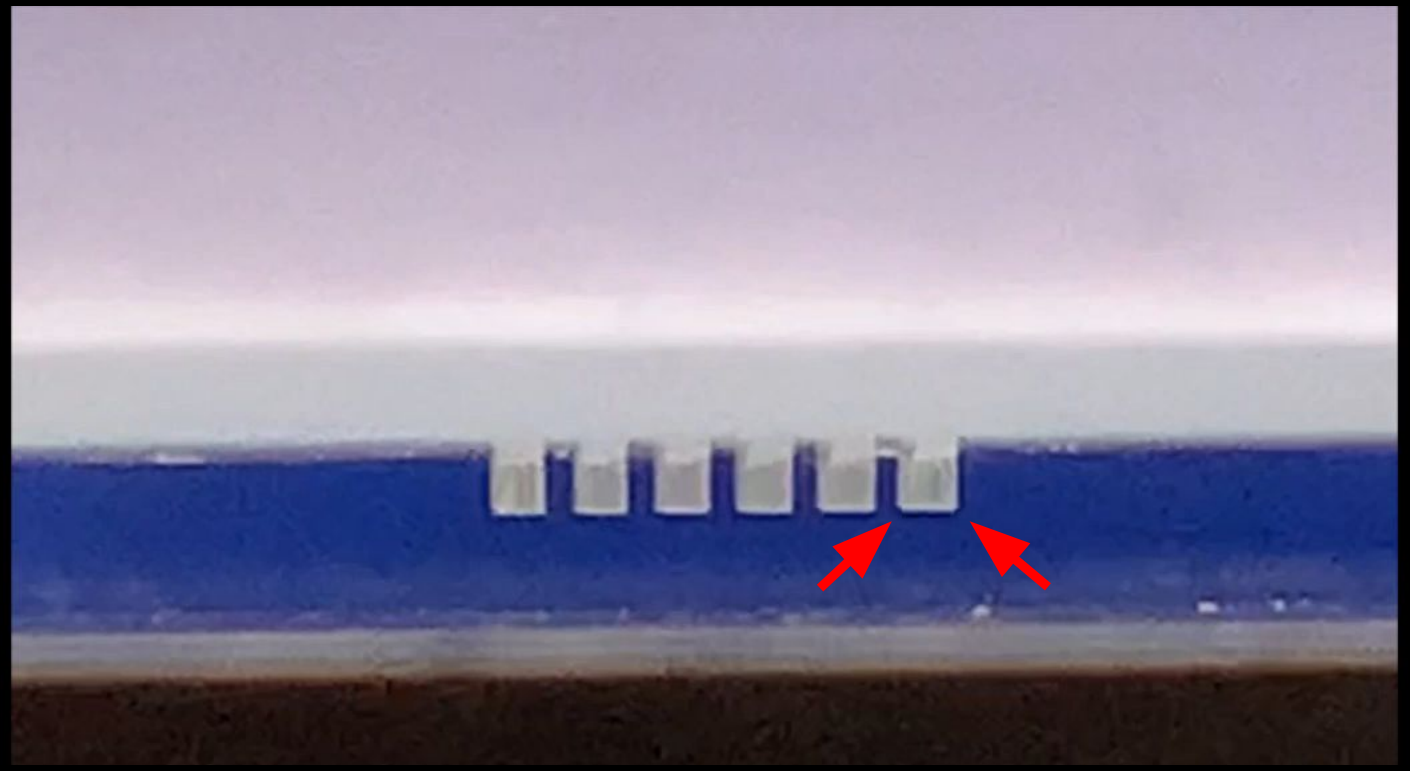
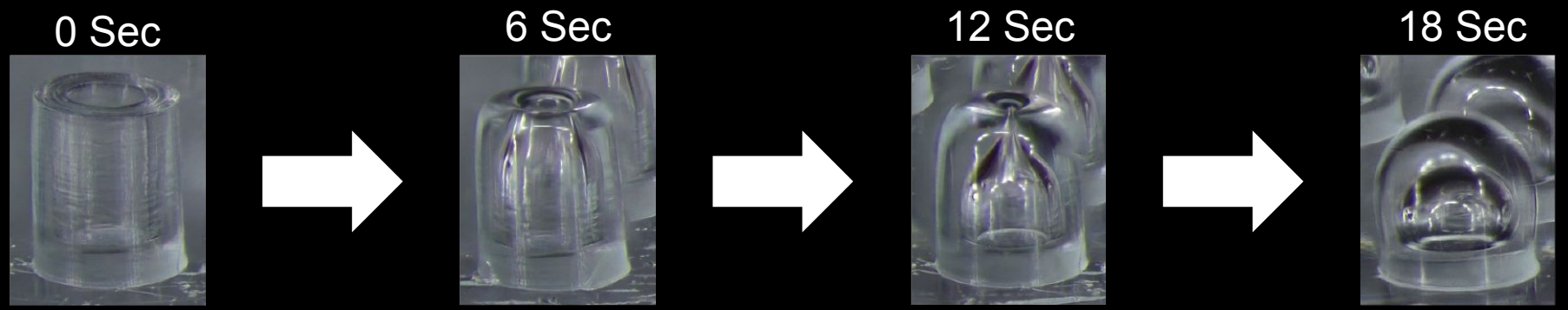



0 Sec
6 Sec
12 Sec
18 Sec

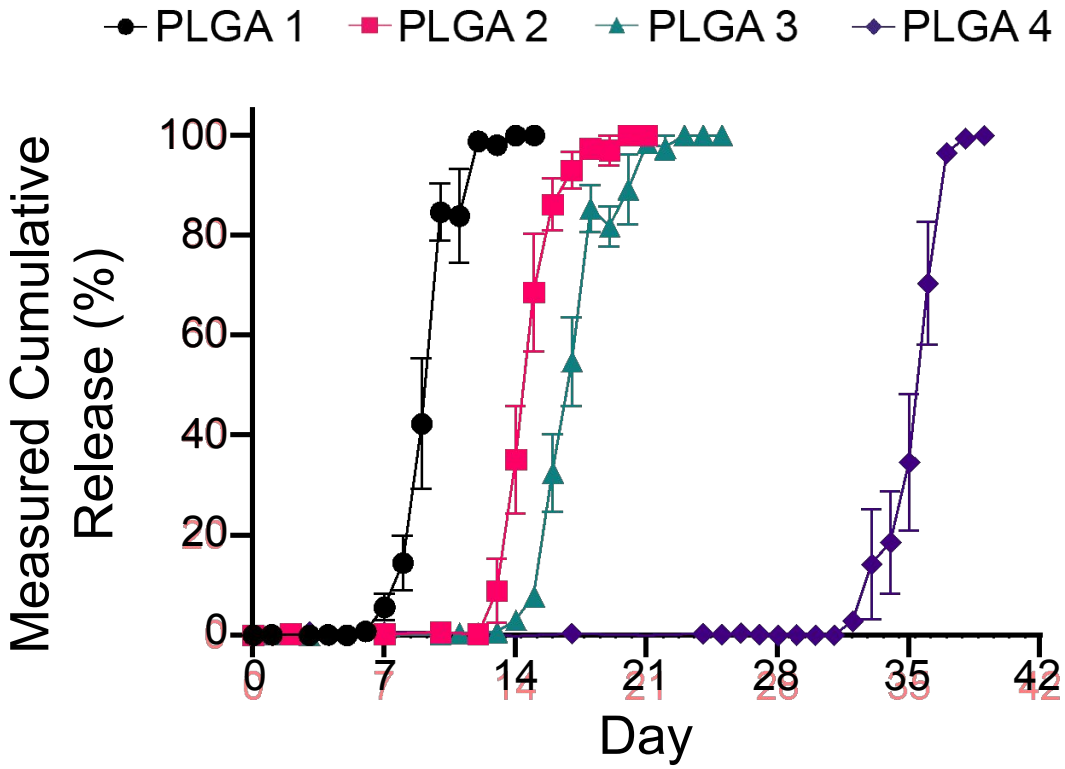
PULSED Microparticles Exhibit Tunable Pulsatile Release *In Vivo*

Method

Different PLGA particles filled with 1 µg of 10 kDa dextran conjugated to Alexa Fluor 647 were injected into the flanks of SKH1-Elite mice (n= 9-10). Release measured using an in vivo imaging system.

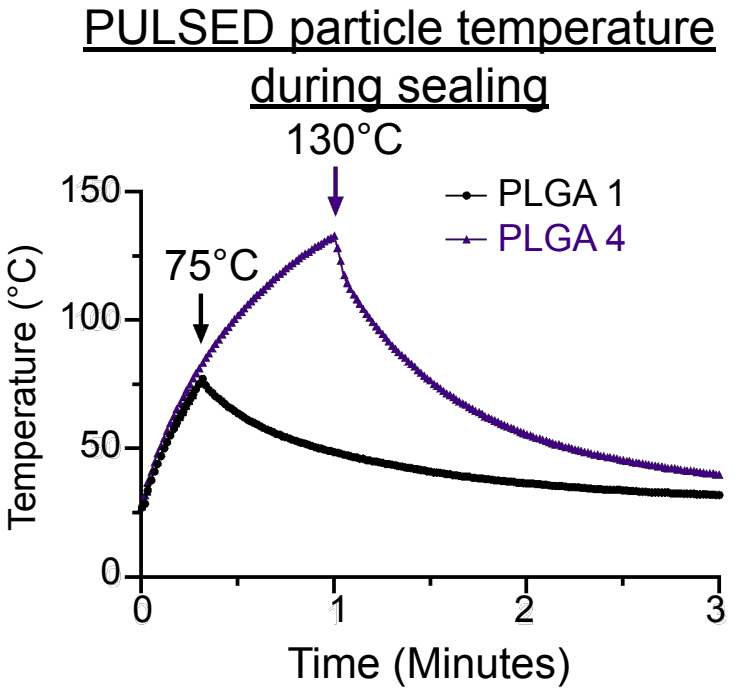
Materials

Type of PLGA	Molecular Weight (kDa)	End Group	Lactic Acid: Glycolic Acid
PLGA 1	13	Carboxylic Acid	50:50
PLGA 2	42	Carboxylic Acid	50:50
PLGA 3	34	Ester	50:50
PLGA 4	87	Ester	50:50



Stabilizing Biologics Through Sealing

- Particles are sealed by suspending the array over a hot plate to heat the PLGA above its T_g allowing the material to flow and form a seal



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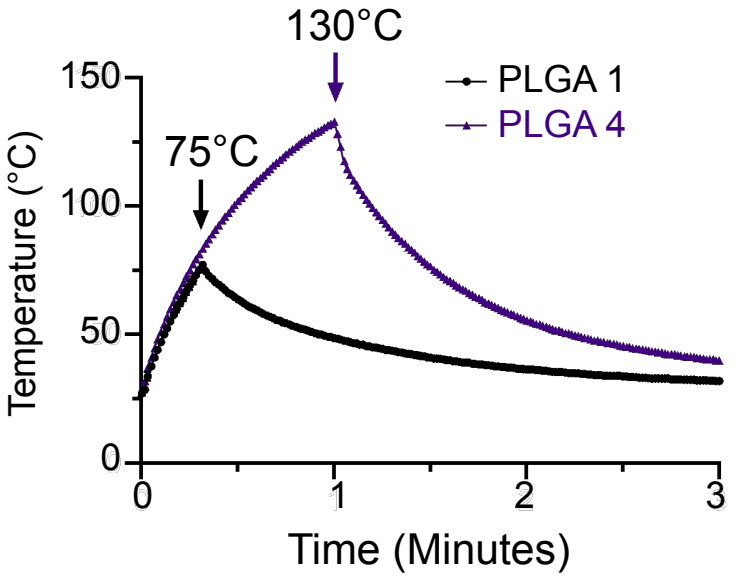
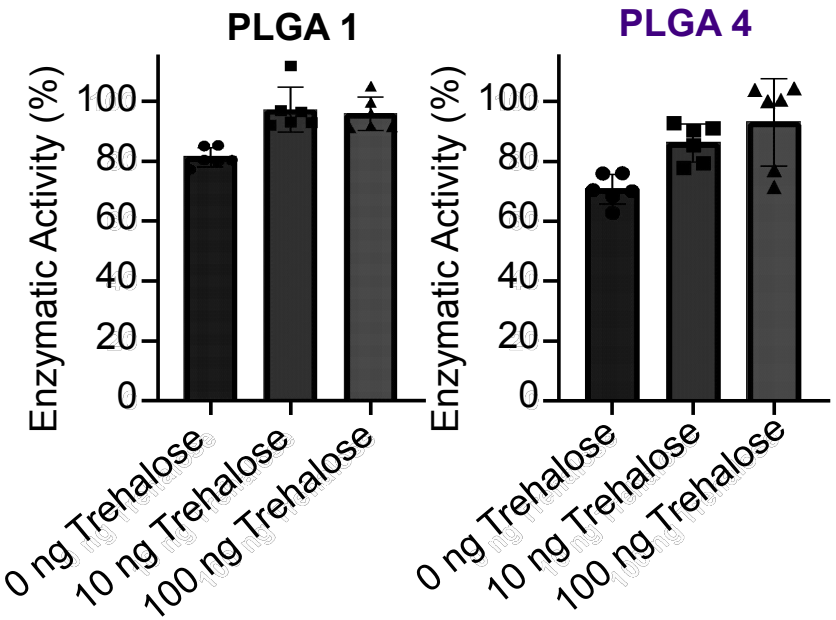
PULSED particle temperature during sealing



Stability of 10 ng of horseradish peroxidase after sealing



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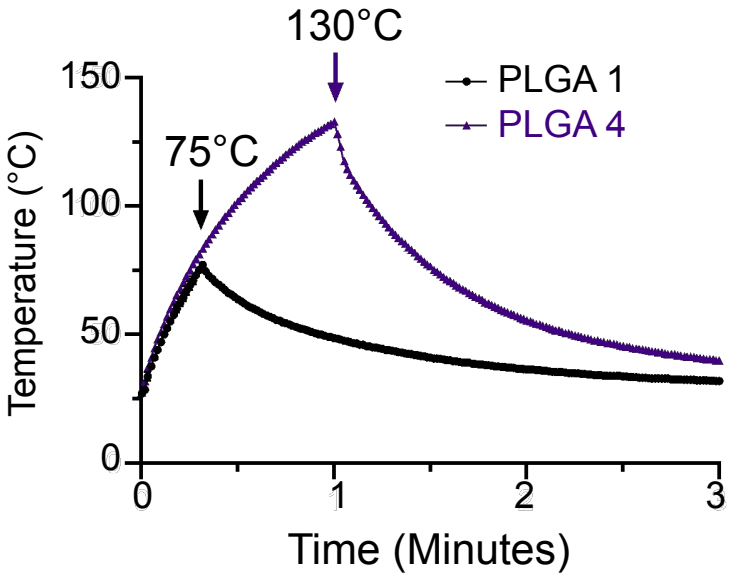

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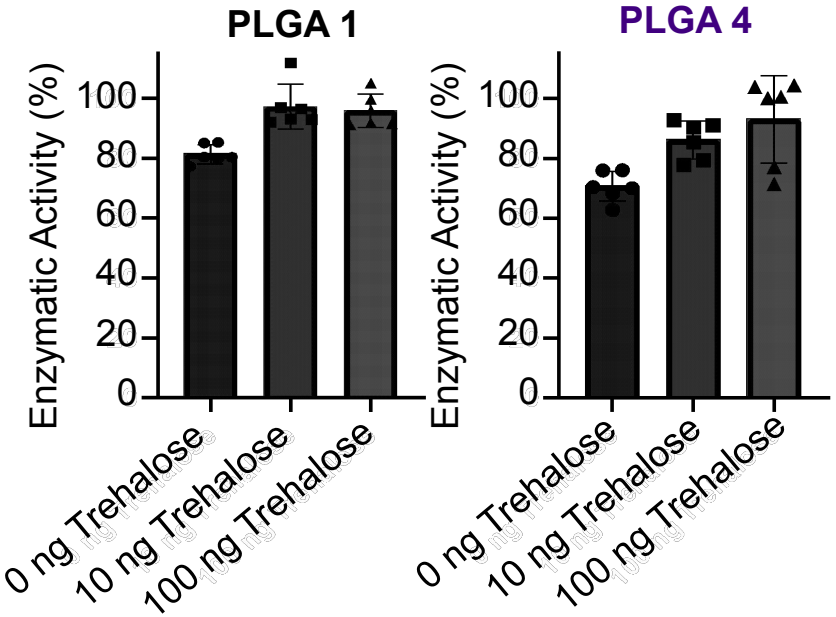
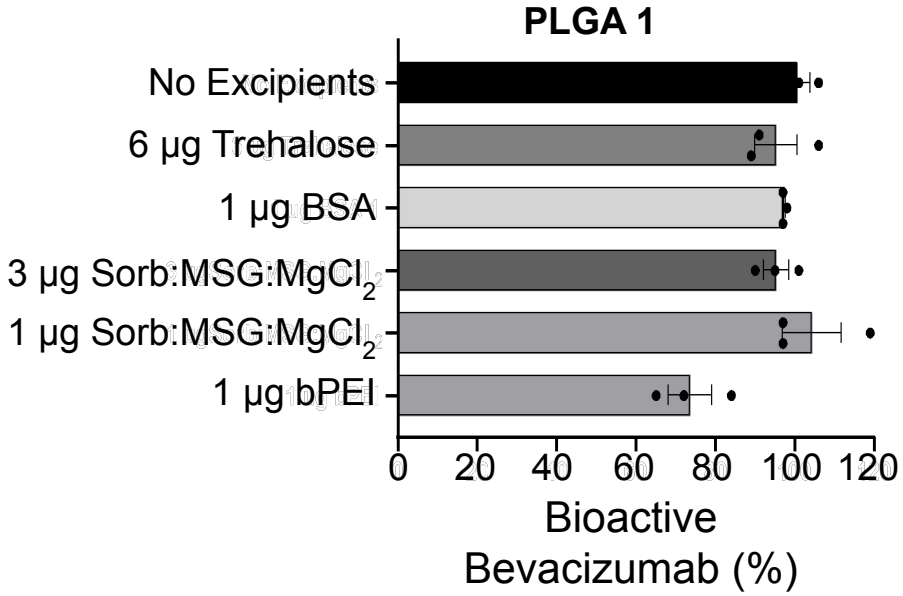


Stability of 10 ng of bevacizumab (FDA-approved anti-VEGF antibody)



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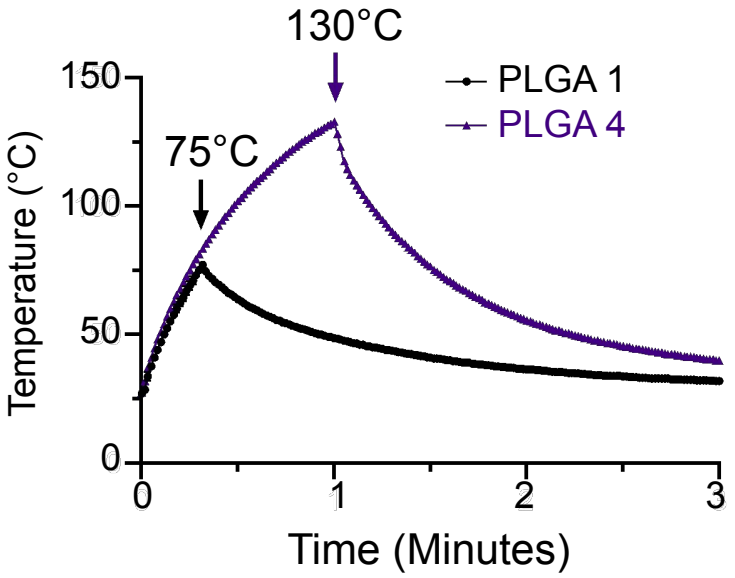

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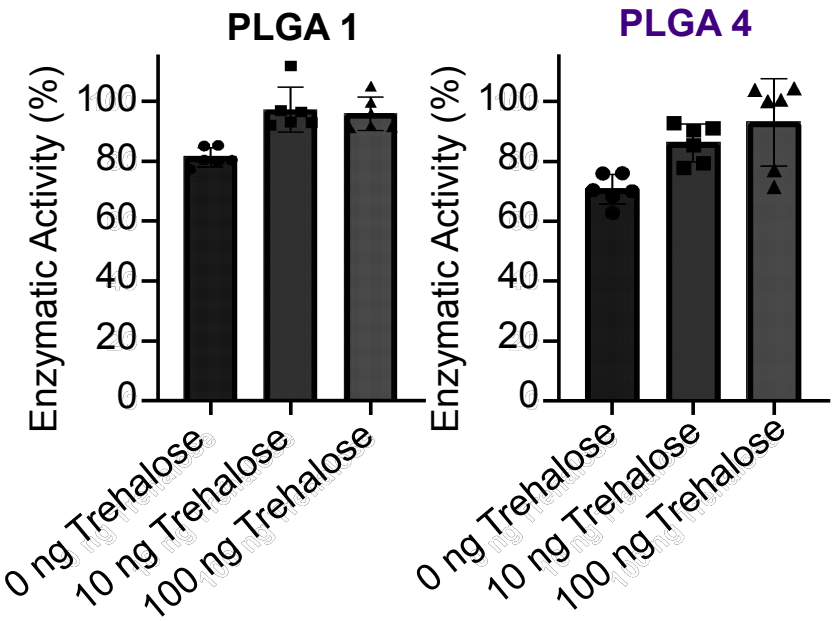

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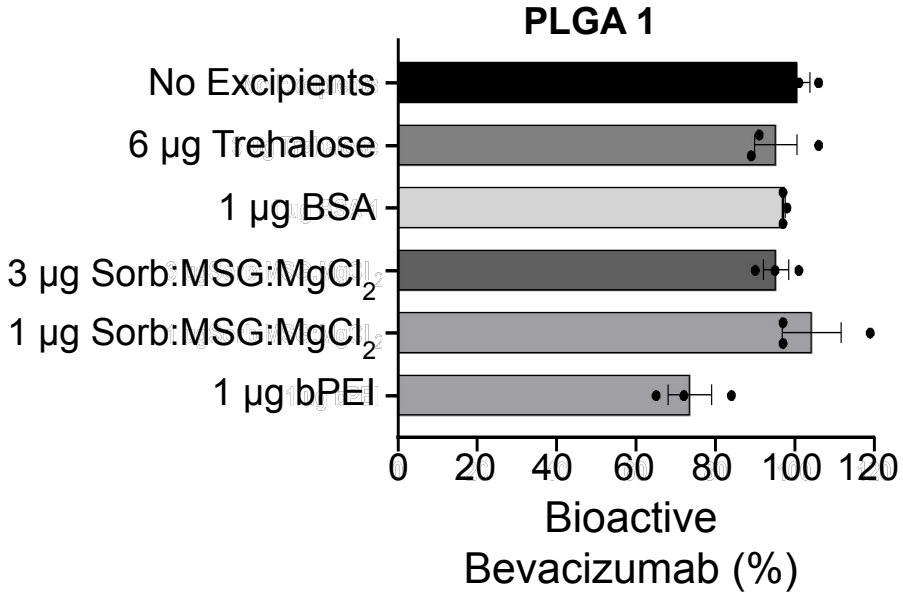
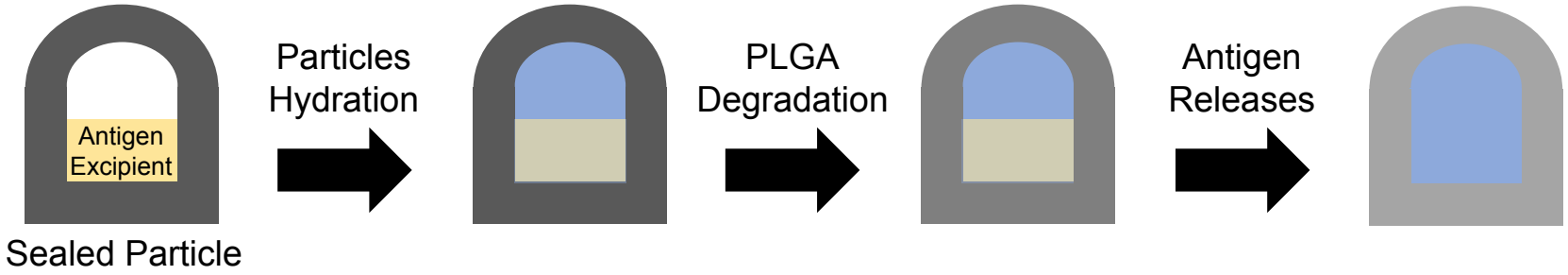


Microparticle sealing causes minimal damage to dried proteins and excipients can be used to prevent or minimize losses in bioactivity

Stabilizing Bevacizumab as a Model Biologic Through Release

Particle filled with antigen and excipient progressing through release



Sealed Particle

Stressors causing biologic degradation

1. Aggregation
2. Aqueous Environment
3. Thermal Stress
4. Low pH

Encapsulated biologics are exposed to multiple stress: (1) aggregation after filling particles, (2) hydration and (3) thermal stress prior to release, as well as (4) acidic microenvironment due to PLGA degradation

Ding, *Pharmaceutical Research* (2014)

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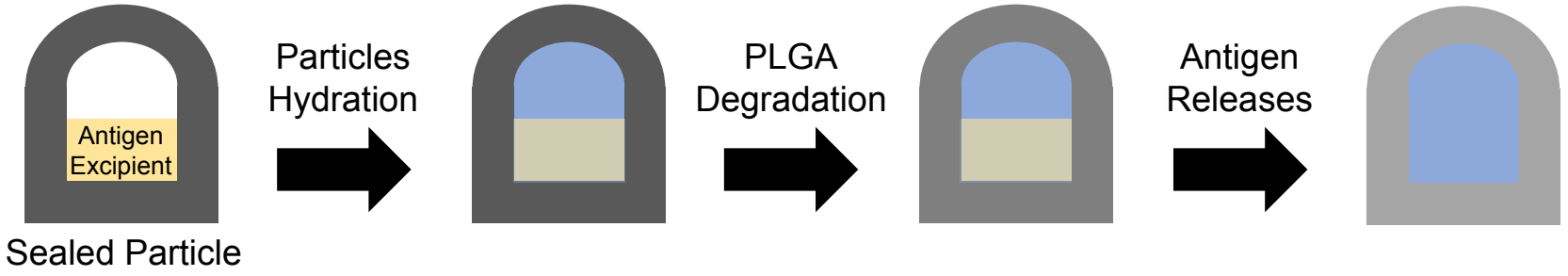

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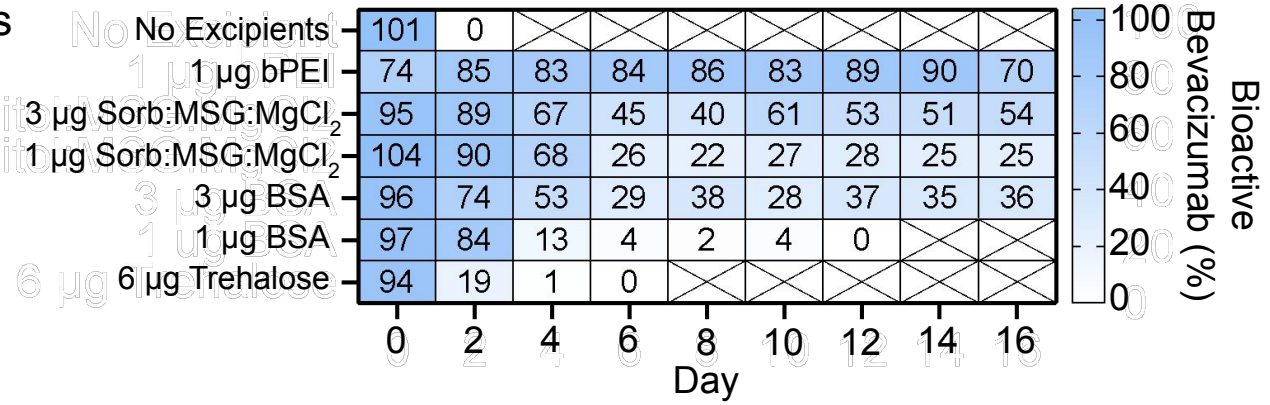
- Particles are broken open to measure a combination of released and encapsulated bevacizumab

Bioactivity of bevacizumab in PLGA 1 (encapsulated and released)



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Tzeng, J. Controlled Release (2016)

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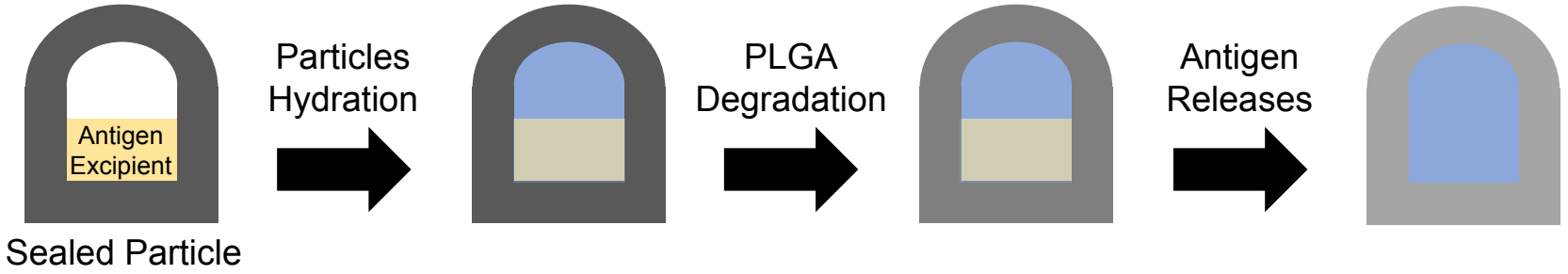

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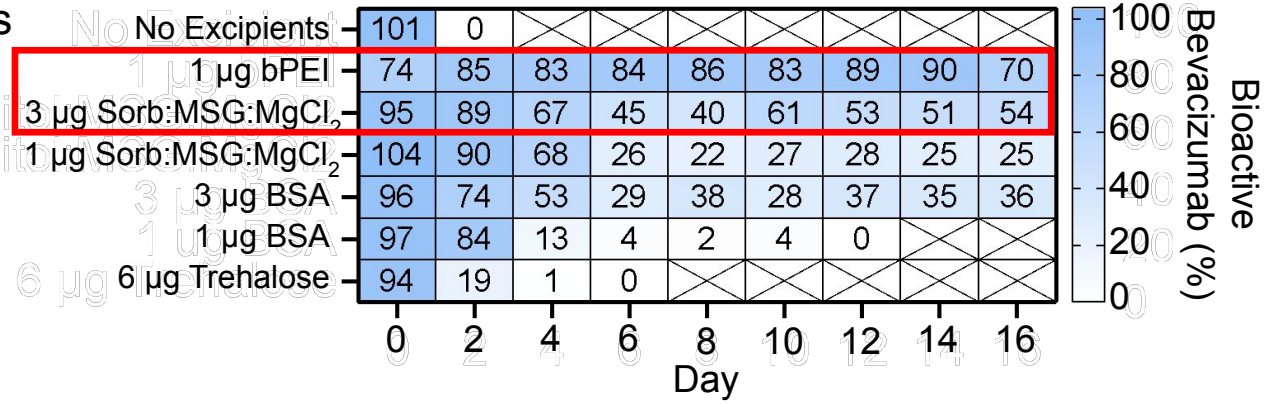

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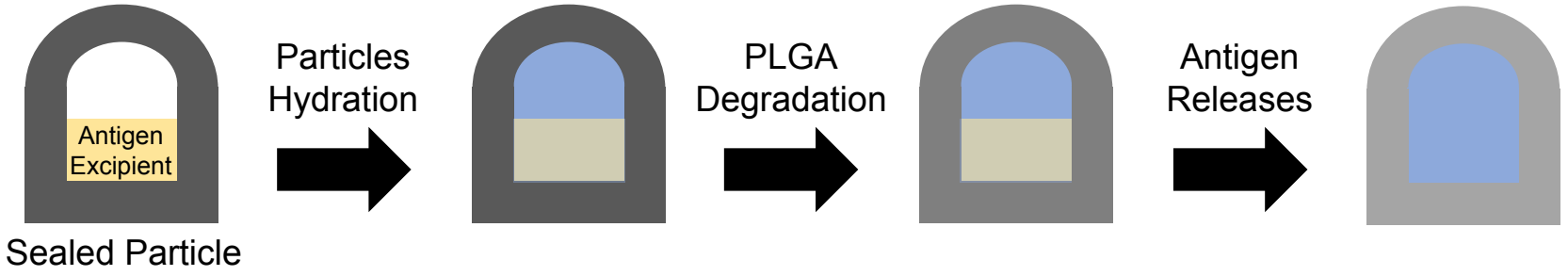

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- Combining top excipients results tunable release of >80% bioactive bevacizumab
- bPEI likely acts as a proton sponge limiting decrease in pH

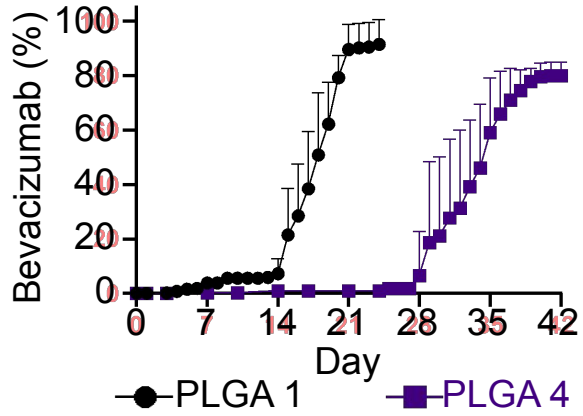
Bioactivity of bevacizumab in PLGA 1 (encapsulated and released)

	0	2	4	6	8	10	12	14	16
No Excipients	101	0							
1 µg bPEI	74	85	83	84	86	83	89	90	70
3 µg Sorb:MSG:$MgCl_2$	95	89	67	45	40	61	53	51	54
1 µg Sorb:MSG:$MgCl_2$	104	90	68	26	22	27	28	25	25
3 µg BSA	96	74	53	29	38	28	37	35	36
1 µg BSA	97	84	13	4	2	4	0		
6 µg Trehalose	94	19	1	0					

Day

Bioactive Bevacizumab (%)

Cumulative release bioactive bevacizumab

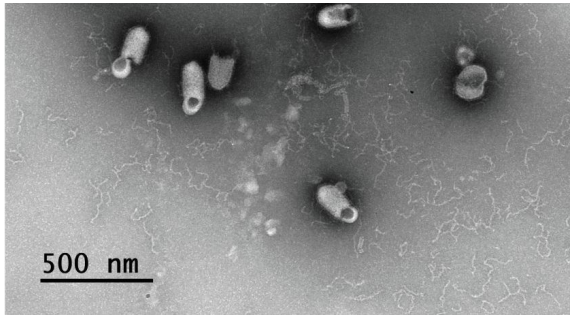


Ding, *Pharmaceutical Research* (2014)
Tzeng, J. Controlled Release (2016)

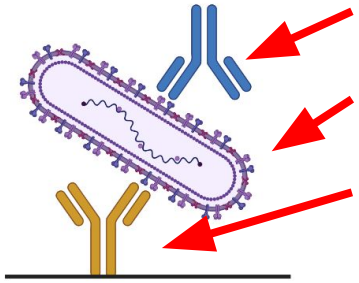
Stabilizing Rabies Vaccine Through Sealing and Release

- The rabies vaccine is an inactivated virus
- Rabies glycoprotein is the target of host neutralizing antibodies which prevent viral entry into host cells

TEM image of rabies vaccine



Sandwich ELISA



Callaway, *Science Advances* (2022)
Ng, *Cell Host Microbe* (2022)
Chabaud-Fiou, *Biologicals* (2017)
Gamoh, *Biologicals* (1996)

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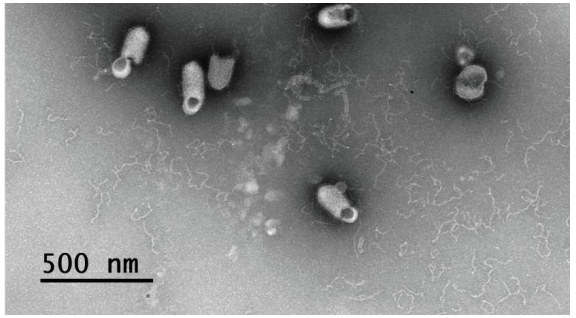

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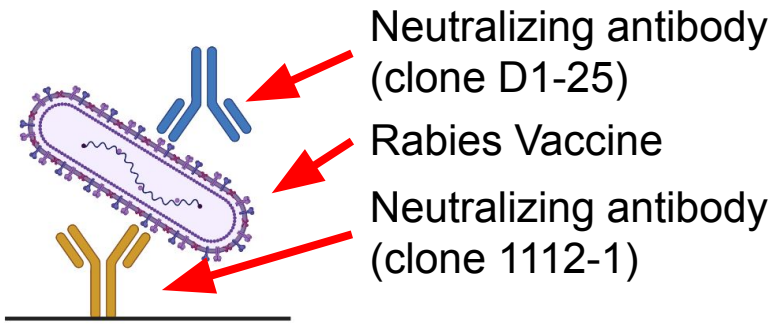
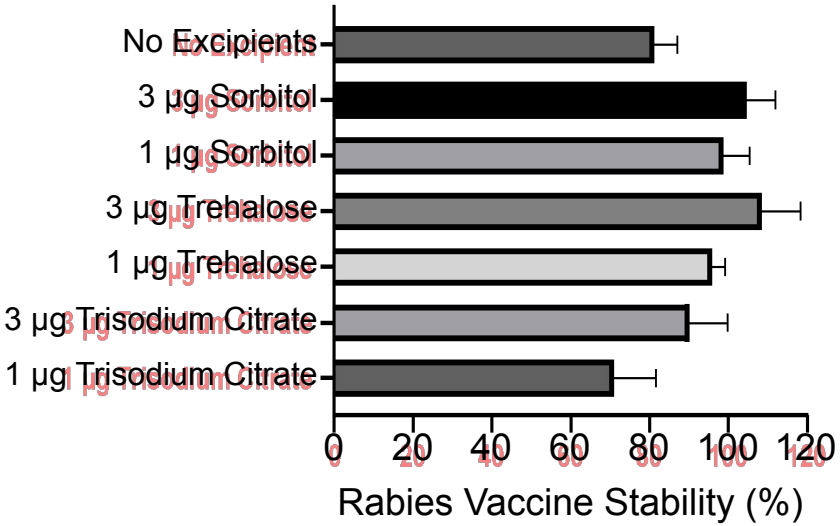


Stability of rabies vaccine after sealing in PLGA 1



Callaway, *Science Advances* (2022)
Ng, *Cell Host Microbe* (2022)
Chabaud-Fiou, *Biologicals* (2017)
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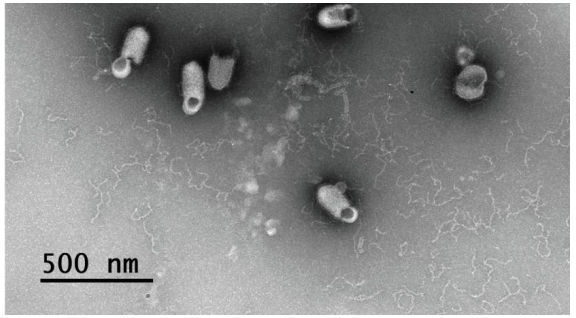
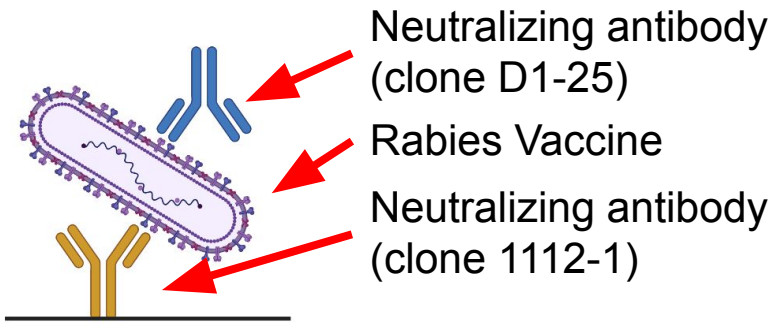
Stabilizing Rabies Vaccine Through Sealing and Release

- The rabies vaccine is an inactivated virus
- Rabies glycoprotein is the target of host neutralizing antibodies which prevent viral entry into host cells

TEM image of rabies vaccine



Sandwich ELISA



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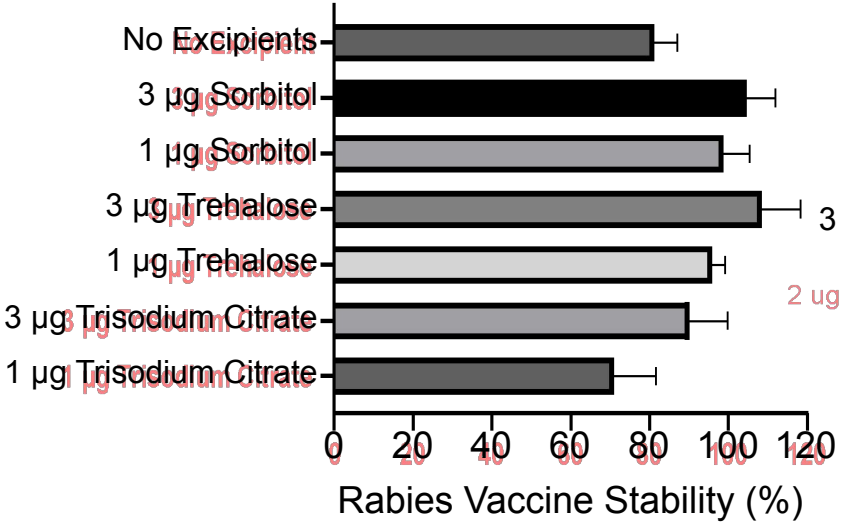
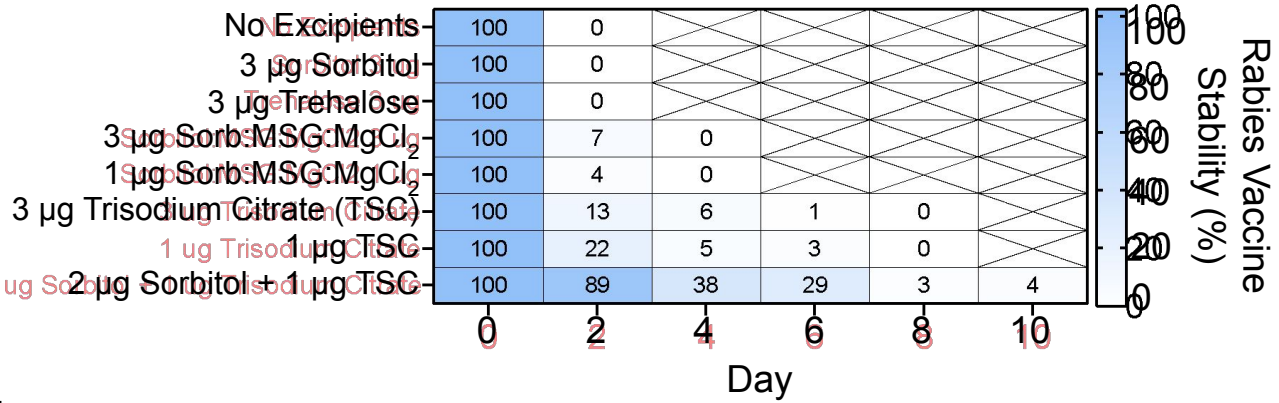


Stability of rabies vaccine prior to release in PLGA 1 (encapsulated and unencapsulated)



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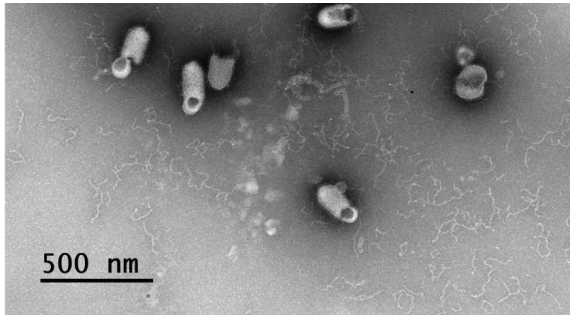

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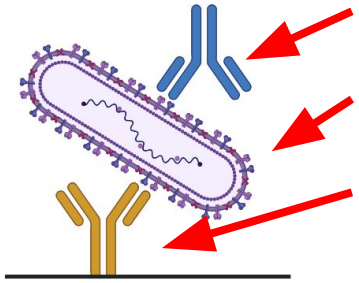

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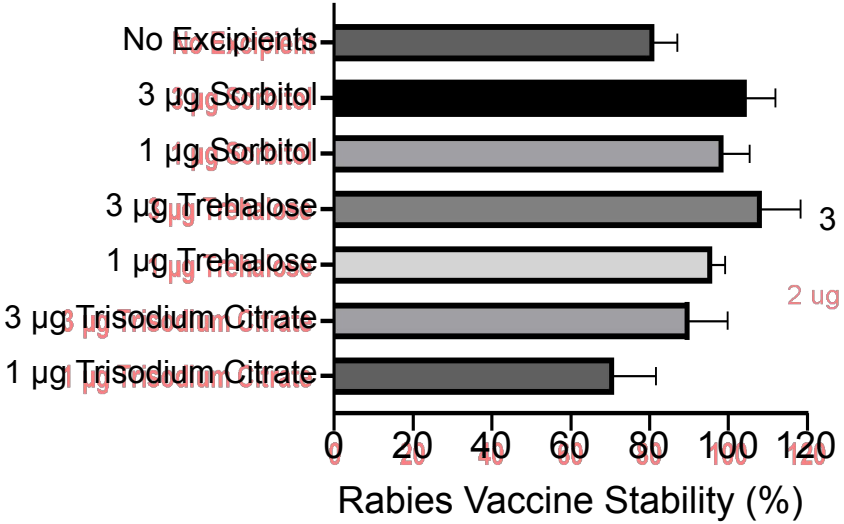

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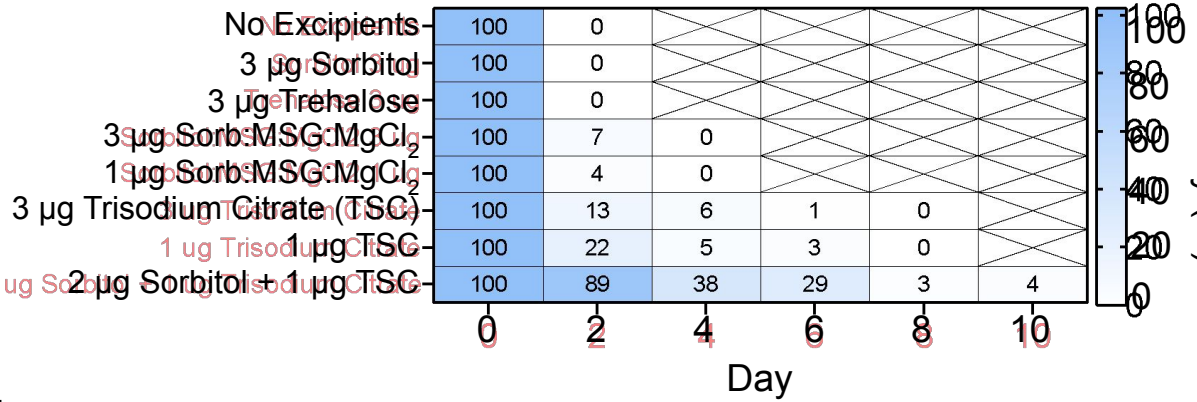
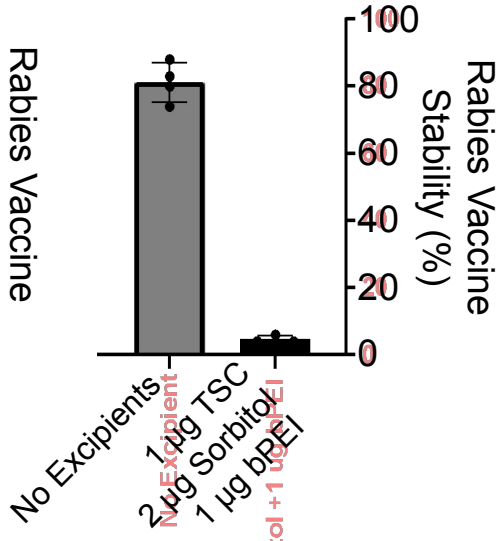


Vaccine stability after sealing in PLGA 1



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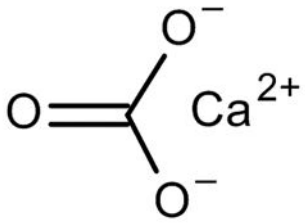
Stabilizing Inactivated Rabies Virus Through Sealing and Release

- To neutralize pH, we explored doping $CaCO_3$ into particle walls.
  - Only soluble in acidic conditions, acts as a buffer once in solution

$CaCO_3$



Undoped PLGA particles

Unsealed

Sealed

$CaCO_3$ Doped pLGA Particles

Unsealed

Sealed

Houchin, *J. Pharm. Sci.* (2008)

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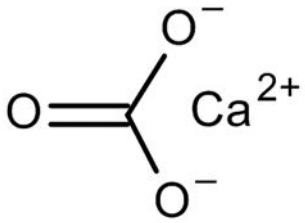

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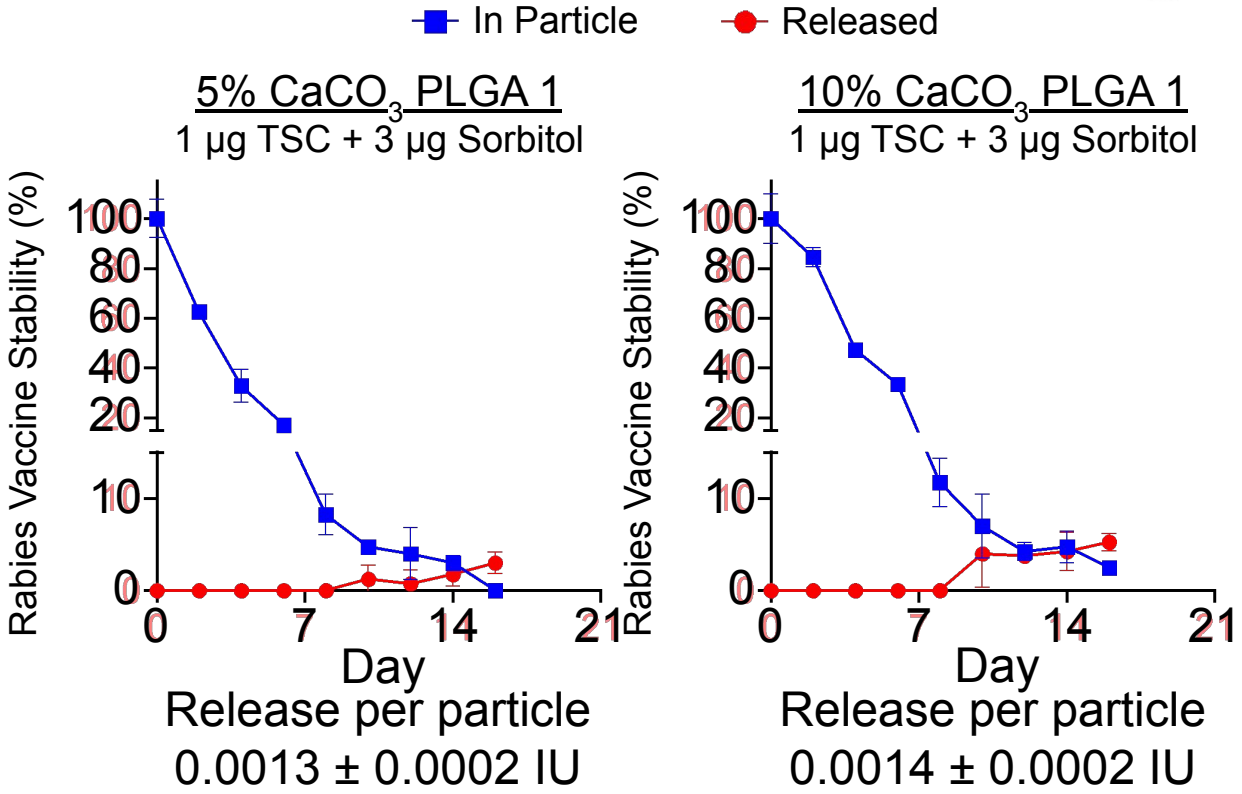

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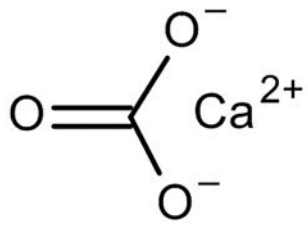

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Unsealed

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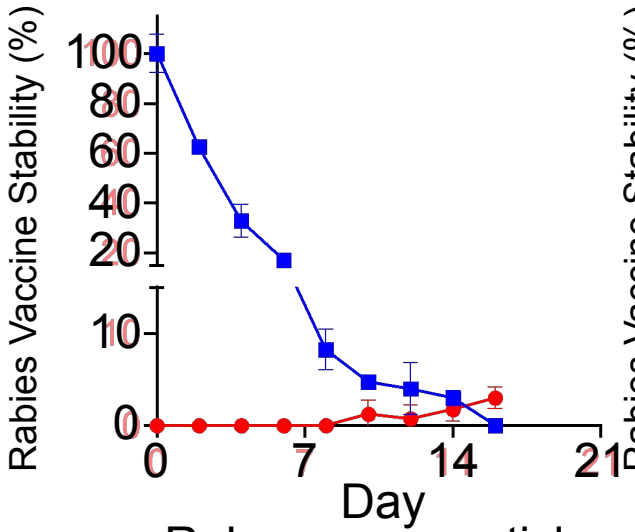
In Particle Released

5% $CaCO_3$ PLGA 1

1 µg TSC + 3 µg Sorbitol

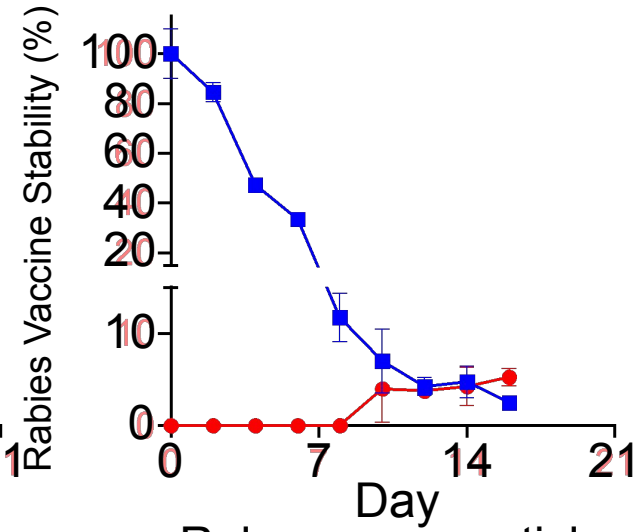


Release per particle

0.0013 ± 0.0002 IU

10% $CaCO_3$ PLGA 1

1 µg TSC + 3 µg Sorbitol



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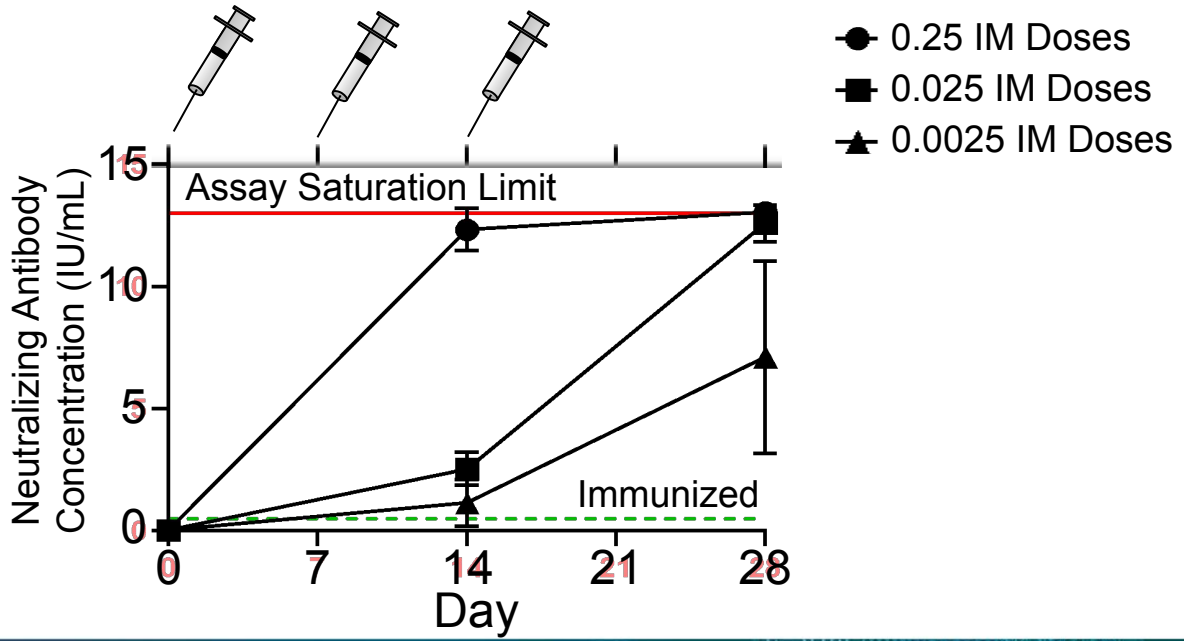
Dose finding study in Sprague-Dawley rats



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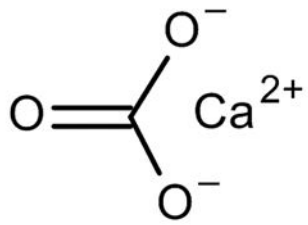

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Sealed

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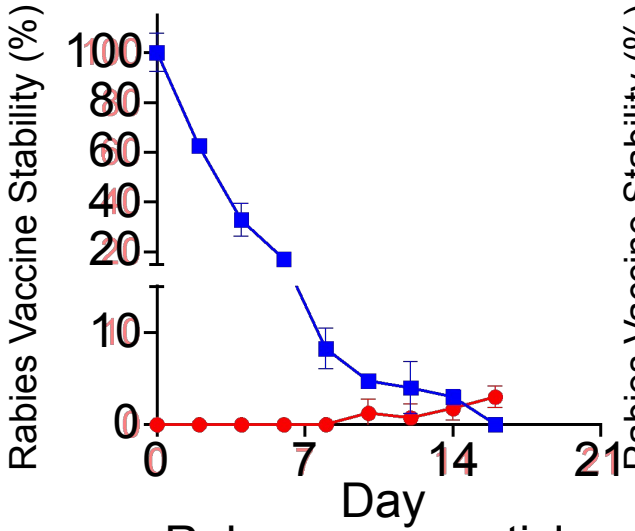

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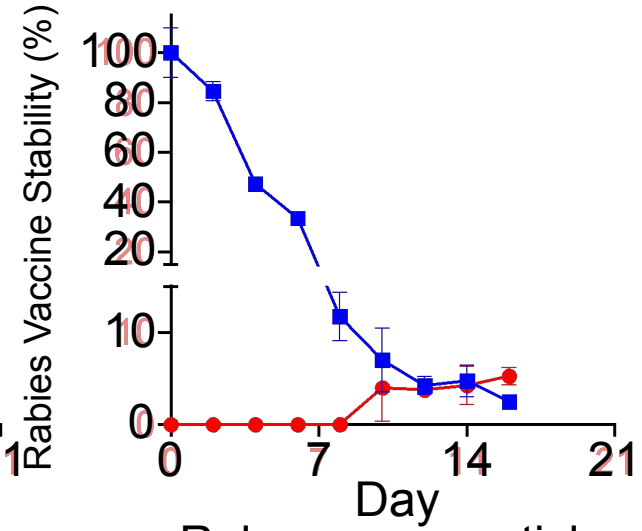

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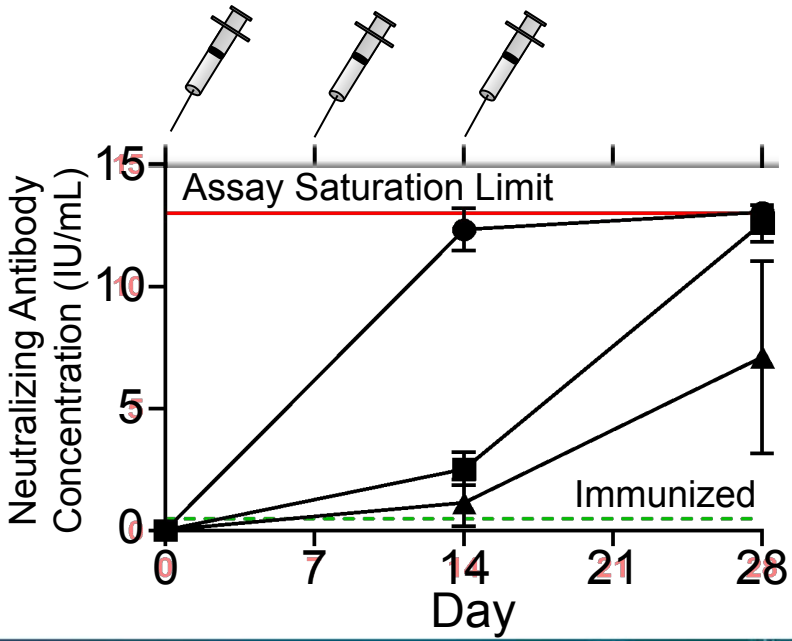

Recovery of 0.0014 IU per particle is a high enough dose to confer immunity!

Houchin, *J. Pharm. Sci.* (2008)

Conclusions

- PULSED microparticles exhibit pulsatile release that can be tuned based on the material properties of PLGA
- The loss of biologics stability during sealing and release can be prevented by co-loading with excipients

Future Work

- Continue exploring excipient formulations to increase release of the rabies vaccine in its stable form
- Explore alternative PLGA formulations to achieve clinically relevant release points
- Evaluate the immune response to vaccine released from PULSED microparticles

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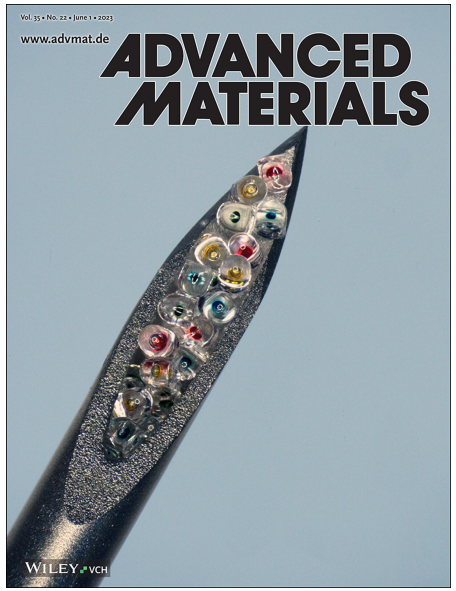
McHugh Lab

Published Work



A Scalable Platform for Fabricating Biodegradable Microparticles with Pulsatile Drug Release

Fabrication of Pulsatile Polymeric Microparticles Encapsulating Rabies Antigen

Questions?

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