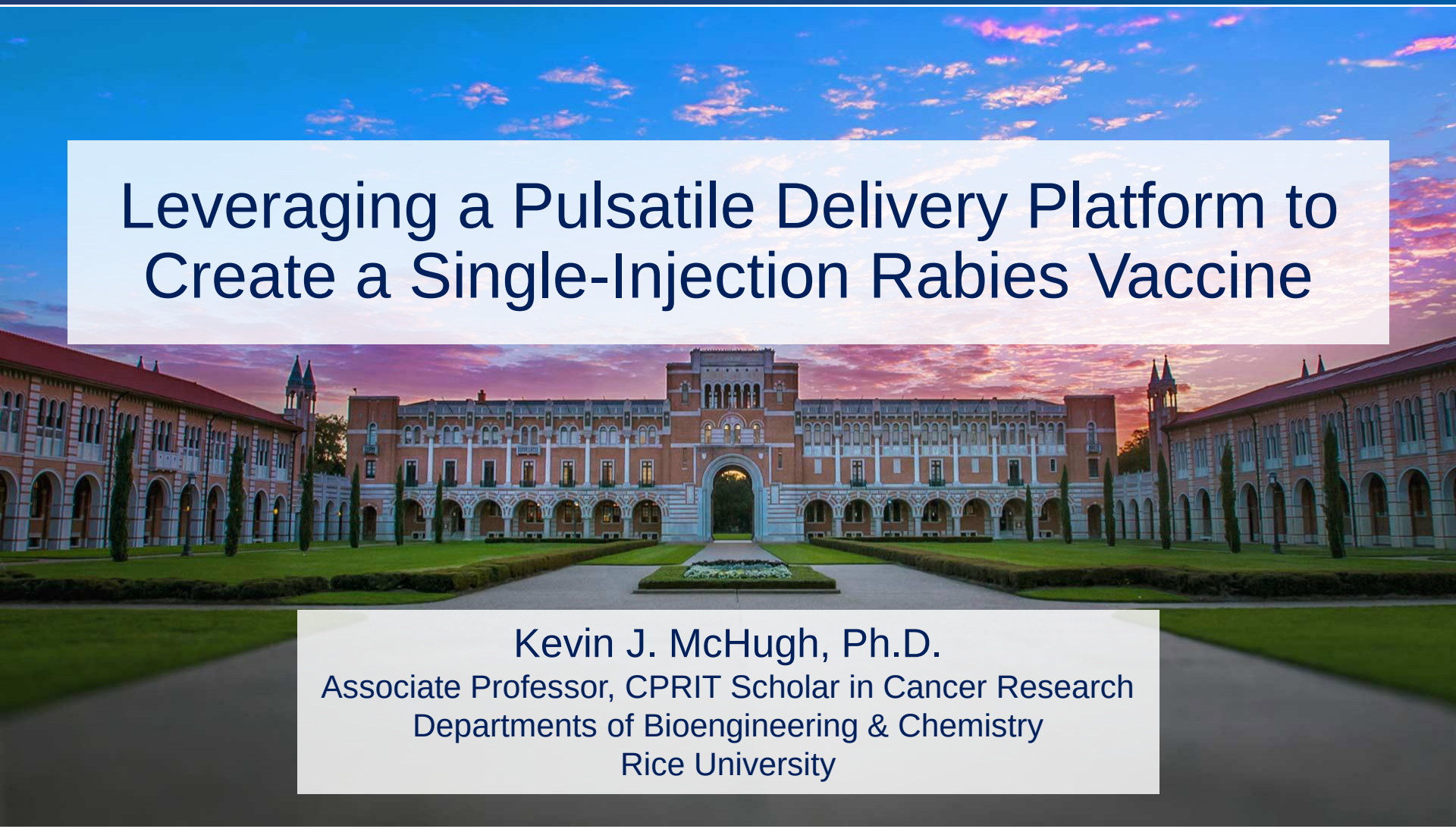
RICE UNIVERSITY
Department of Bioengineering



Leveraging a Pulsatile Delivery Platform to Create a Single-Injection Rabies Vaccine

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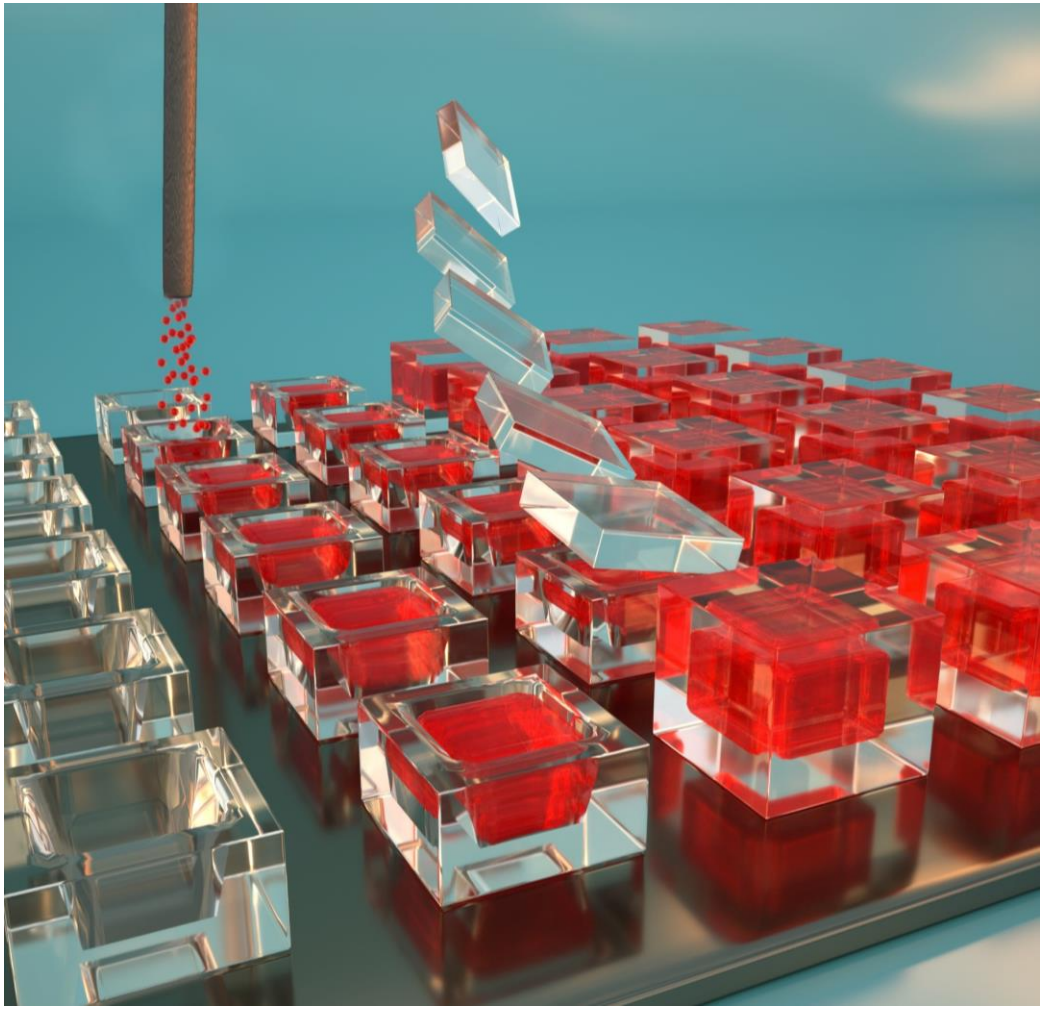
Philadelphia, PA
July 15, 2025

Microparticles Exhibiting Pulsatile Release



Vaccination

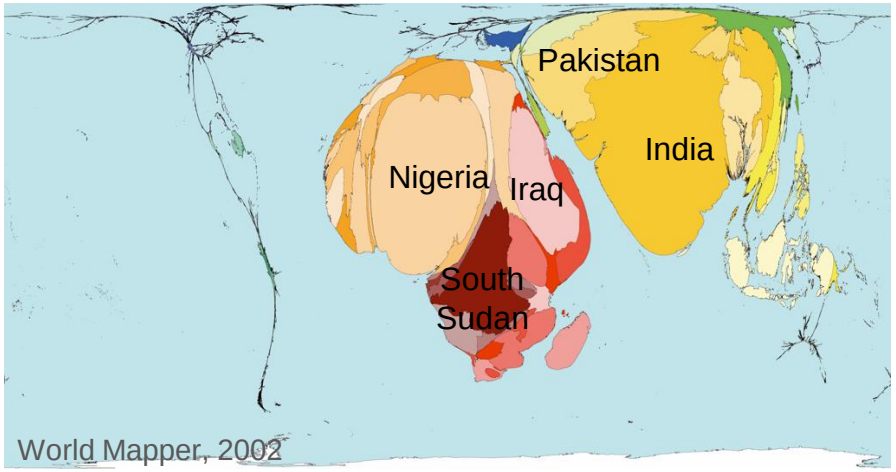
- Vaccination against infectious diseases is among the most effective and cost-effective healthcare developments
  - **An estimated 154 million deaths averted over the past half century[1]**
  - Every $1 spent on vaccination in low- and middle-income countries saves $52 downstream[2]
- Still, infectious diseases remain a leading cause of death worldwide
  - Responsible for **>14.4 million deaths annually[3]**
  - Also the leading cause of disability-adjusted live years (DALYs) lost worldwide[4]
- One major issue is the inability to distribute vaccines in LMICs
  - Complicated by the need for multiple doses administered over months
  - Vaccination coverage: 5% in 1974 → 86% in the 2010's → 81% now[5]
  - 19.5 million infants under-immunized each year → **1.7 million vaccine-preventable deaths**
- Another issue is the lack of potent vaccines
  - **We lack vaccines to prevent 12.7 million infectious deaths annually**
  - Some clinical vaccines are sub-optimal (e.g., Mosquirix), others don't exist
  - Antigen development and testing is difficult and costly

[1]Shattock AJ, et cl., *Lancet* 2024. [2]Sim SY, et al., *Health Aff* 2020;39:1343. [3]WHO. Noncommunicable diseases, 2023. [4]WHO. Global Health Estimates, 2016. WHO. [5]Immunization Coverage, 2023.

Infectious Disease

- The location of vaccine-preventable deaths occur underscore the challenges that lead to these deaths

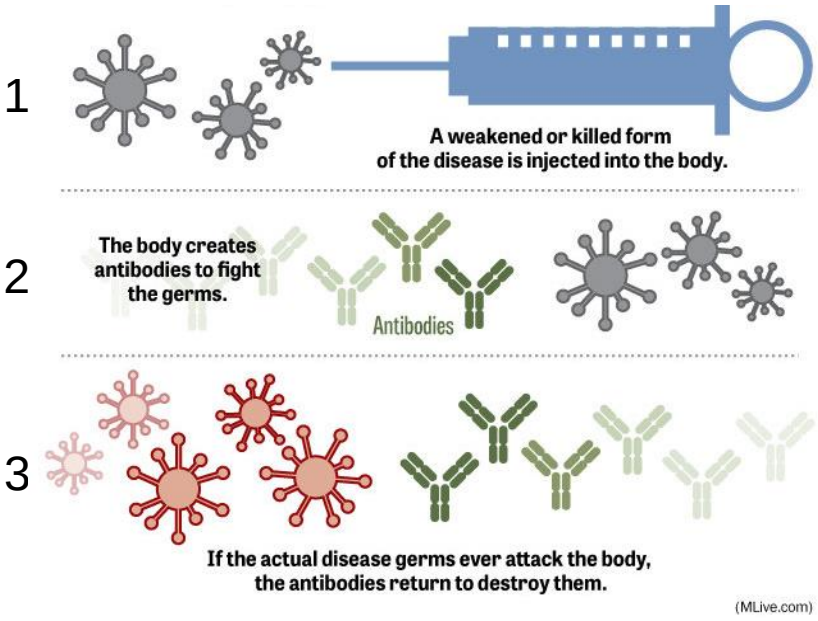
Vaccine-Preventable Deaths

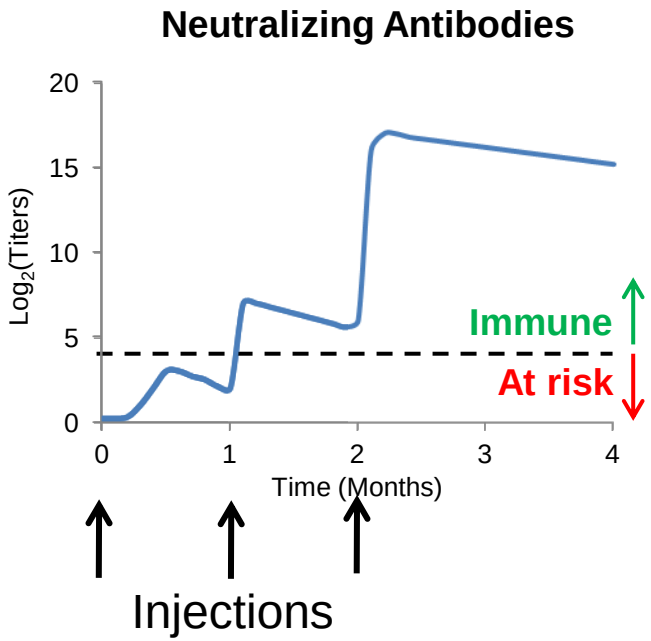


- A key issue is accessibility in low- and middle-income countries (LMICs)
- Barriers to vaccine adherence in LMICs
  - Limited financial resources/healthcare infrastructure (e.g., cold chain)
  - Limited patient access
  - Need for multiple injections
  - Missed opportunities to vaccinate

Immunization

- Immunity is conferred by administering a killed pathogen, weakened pathogen, a piece of a pathogen, or encoding a piece with mRNA
- One dose of vaccine is typically not sufficient to confer long-lived humoral immunity at the population level
- Administering additional doses train the immune system to expect future encounters with the pathogen

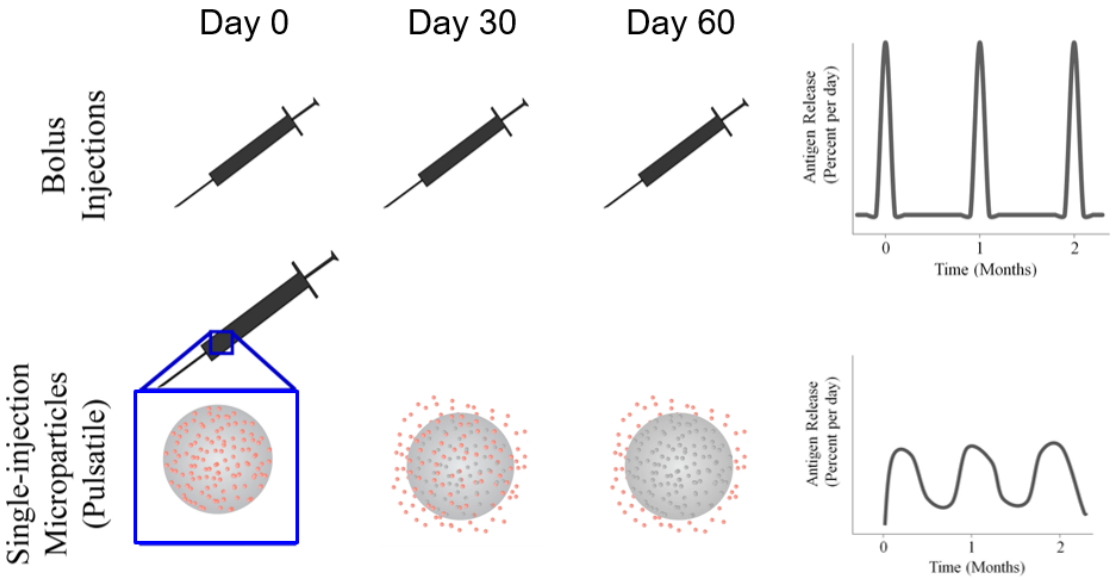




[1]CDC, Timing and Spacing of Immunobiologics, 2018. [2]World Health Organization, Immunization Coverage Fact Sheet, July 2017.

Single-Injection Vaccination

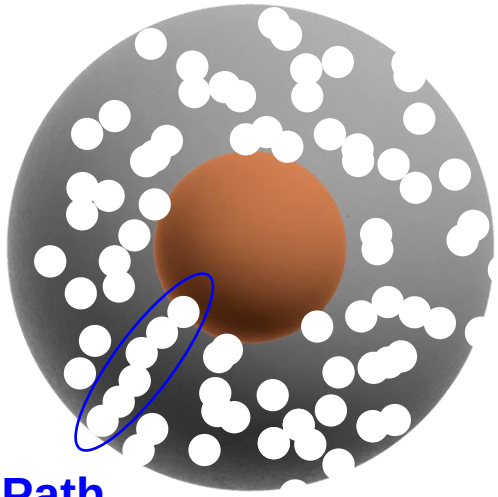
- Approach: Create a formulation that delivers antigen on a typical immunization schedule with only one injection
- Must be easy to use and affordable for the types of individuals who are currently undervaccinated
- Key features:
  - Biocompatible
  - Injectable
  - Biodegradable
  - Pulsatile Release



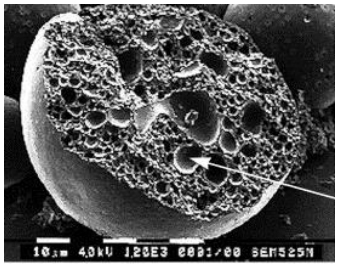
McHugh KJ, et al. *J Control Release* 2015;219:596.

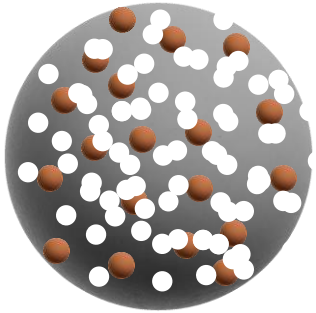
Current Controlled-Release Technology

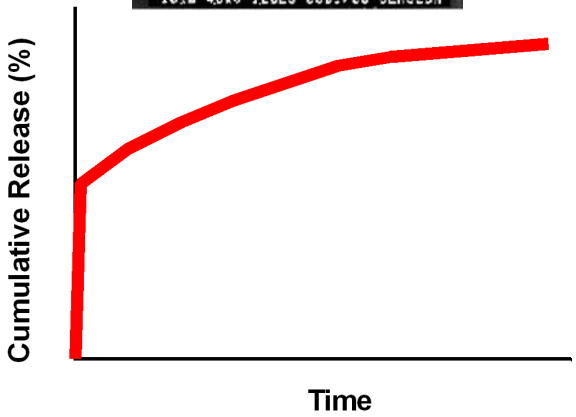
- Unfortunately, traditional microparticle fabrication methods do not yield the desired kinetics
- We hypothesized that microparticles with a single, consistently localized vaccine cavity would exhibit pulsatile release

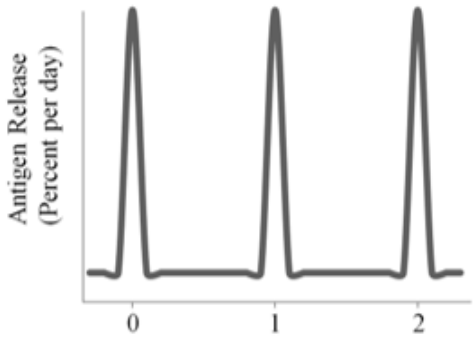


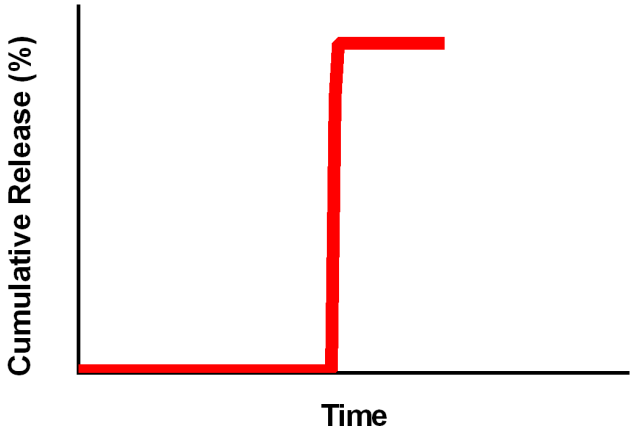
Release Path







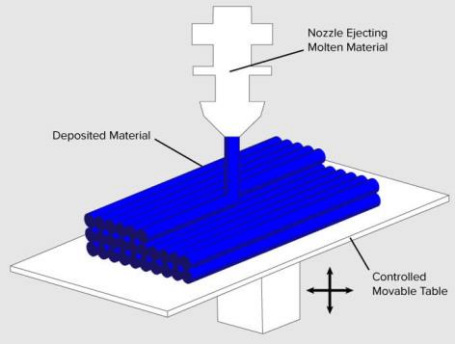


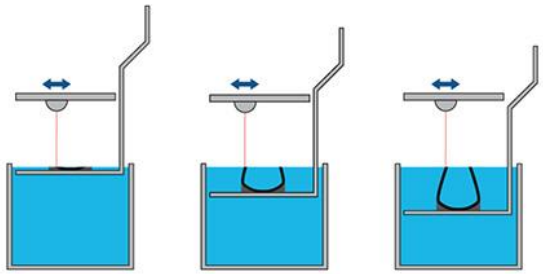


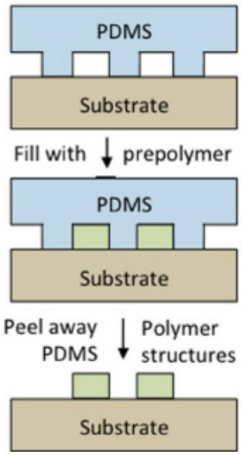
Cleland JL. Vaccine Design: The Subunit Antigen Approach, 1995.

Fabrication Method Selection

- Existing techniques:
  - Fused deposition modeling (3D printing) - resolution, material compatibility
  - Stereolithography (3D printing) - material compatibility, vaccine loading
  - Soft lithography - limited geometries possible (e.g., no internal cavity)
- Develop new approaches:
  - Stamped assembly of polymer layers (SEAL)
  - Particles Uniformly Liquified and Sealed to Encapsulate Drug (PULSED)



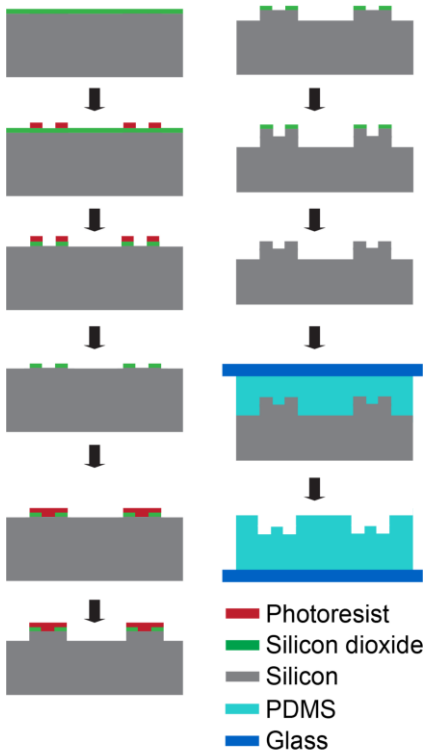




SEAL: Polymeric Shell Fabrication

Mold Fabrication

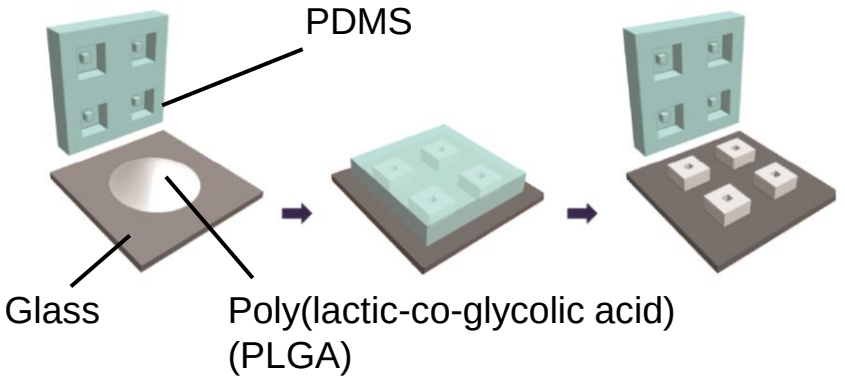
- Photolithography
- Deep reactive ion etching
- Buffered oxide etching



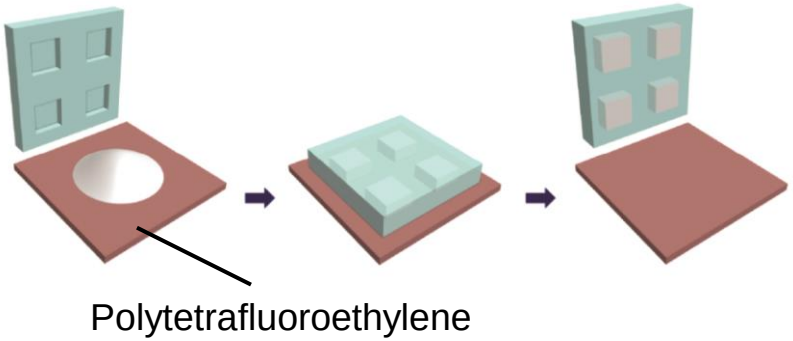
Particle Component Fabrication

- Soft lithography/hot embossing

Bases:



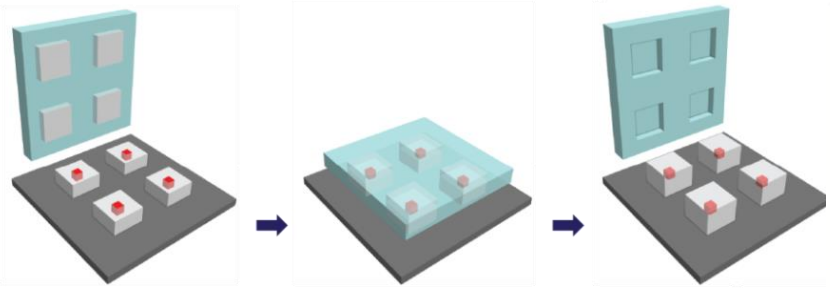
Caps:



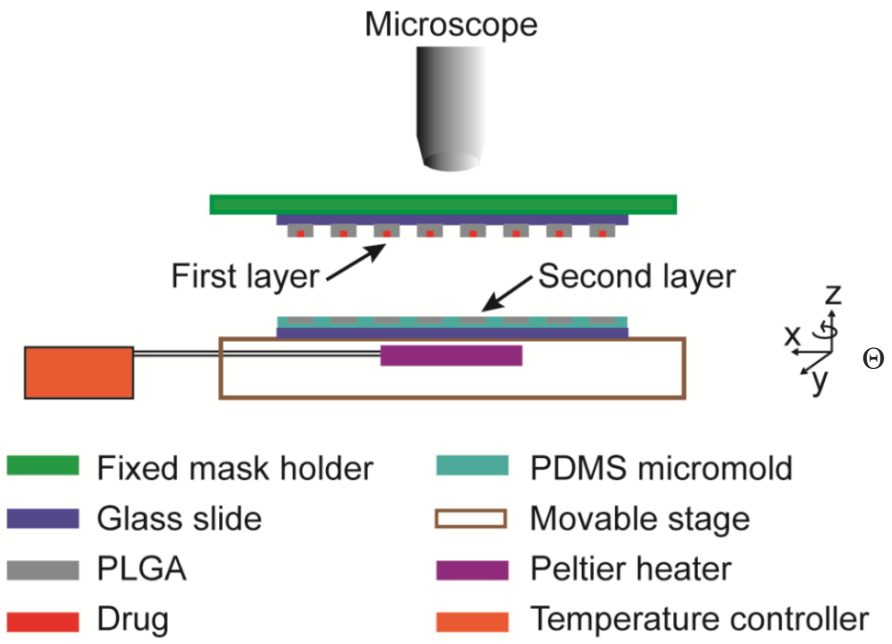
McHugh KJ, et al. *Science* 2017;357:1138.

SEAL: Microparticle Closure

- Cap and vaccine-filled base brought into contact and raised above their glass transition temperature (Tg) causing them to fuse
- Sealed components using a photomask aligner retrofitted with a Peltier heater



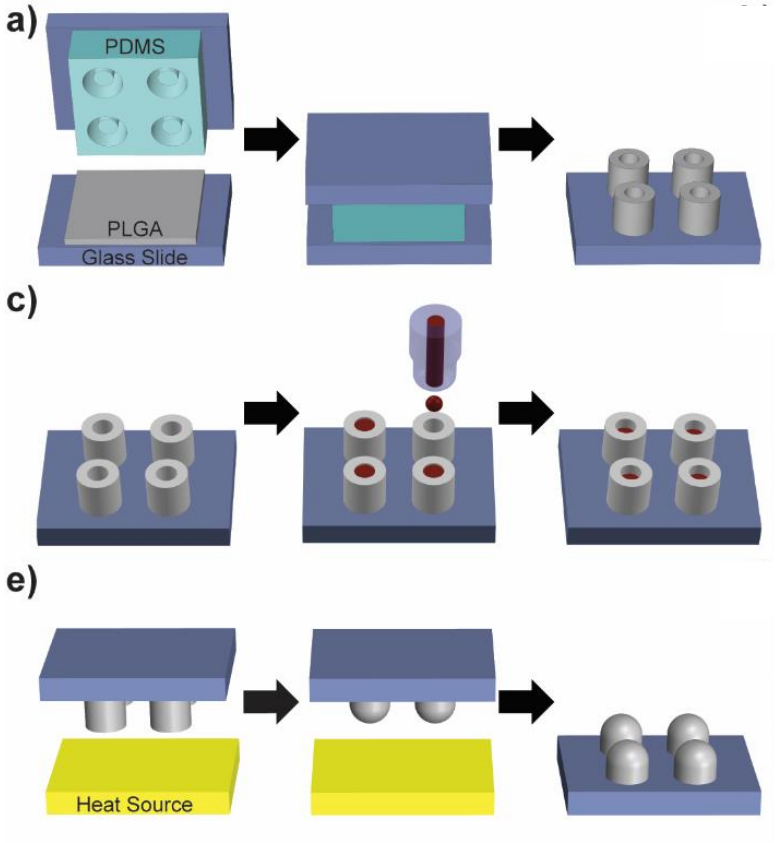
Polymer	T_g (°C)
PLGA 50:50, 19 kD, Acid	42
PLGA 50:50, 53 kD, Acid	43
PLGA 50:50, 75 kD, Ester	48
PLGA 85:15, 214 kD, Ester	53



McHugh KJ, et al. *Science* 2017;357:1138.

PULSED Fabrication Process

- One molded component, no alignment → Improved throughput and yield



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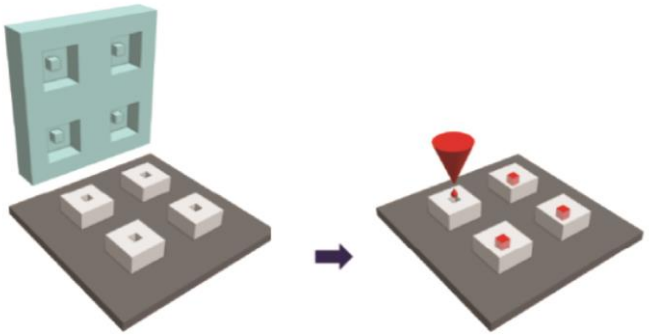
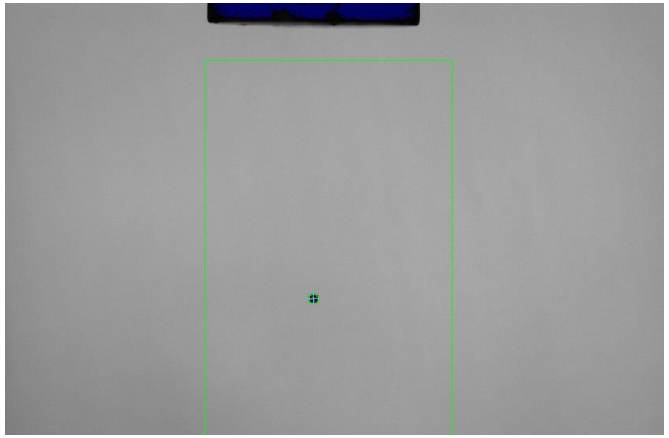
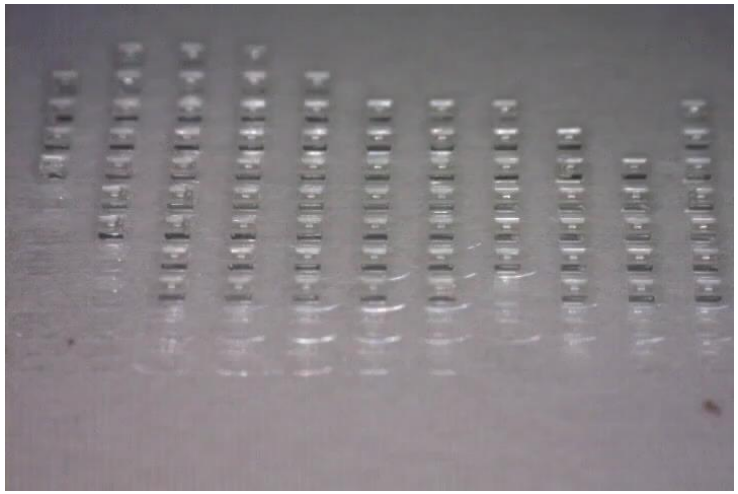
Robotic Particle Filling

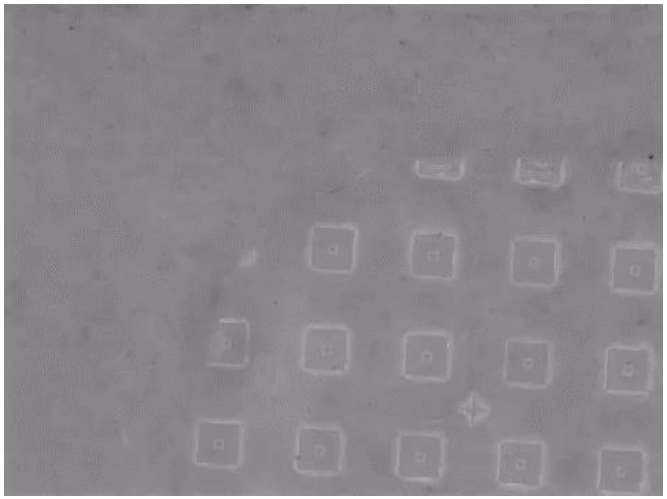
Using a piezoelectric dispensing robot to fill particles with a fluorescently labeled dextran (10 kDa)





Alexa Fluor 647-labeled dextran (10 kD)

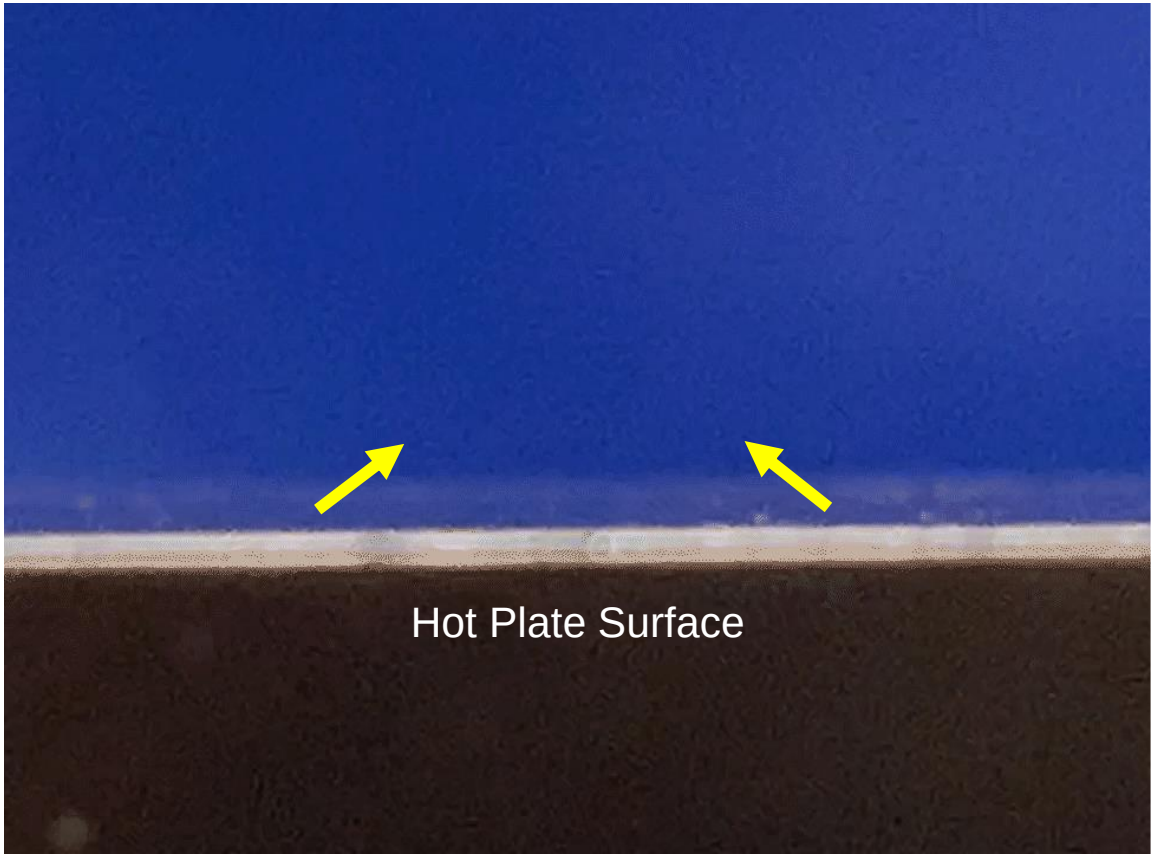


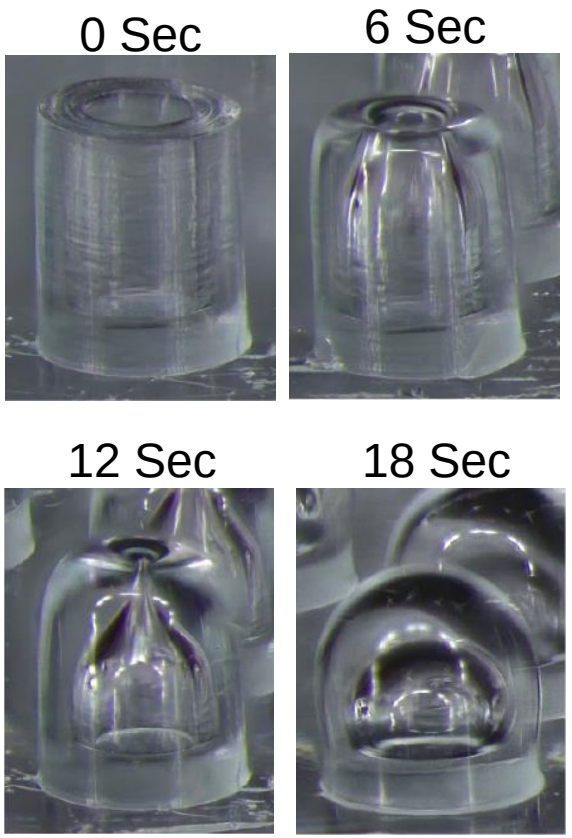


McHugh KJ, et al. *Science* 2017;357:1138.

Microparticle Sealing

- Developed a self-enveloping method that eliminates the need for the production and alignment of multiple components





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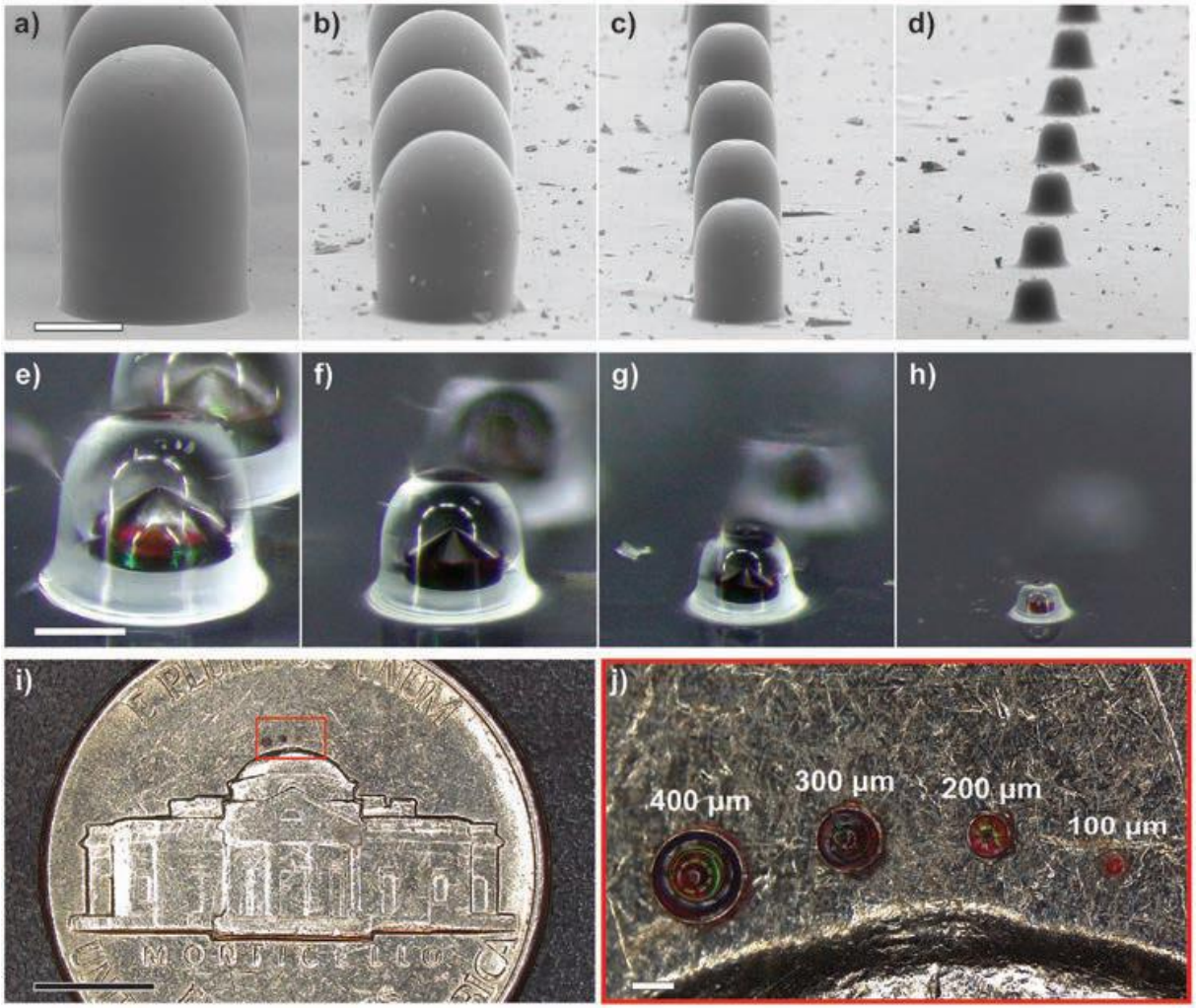
Particle Miniaturization



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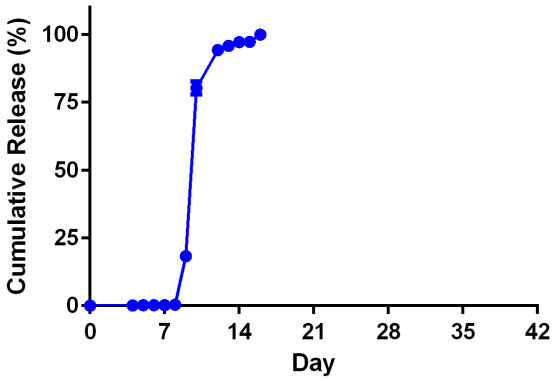
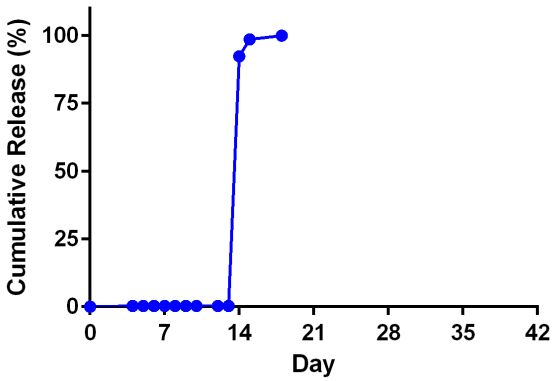
Pulsatile In Vitro Release Kinetics

Filled particles with Alexa Fluor 488-Dextran (10 kD) & incubated in PBS at 37°C

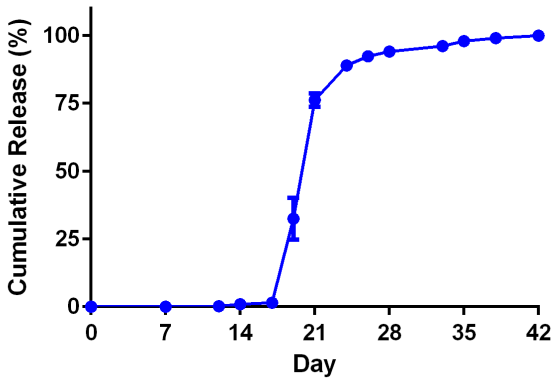
PLGA 50:50, 12 kD, Acid



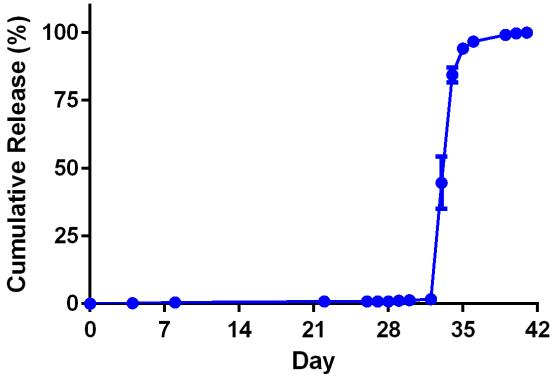
PLGA 50:50, 32 kD, Acid



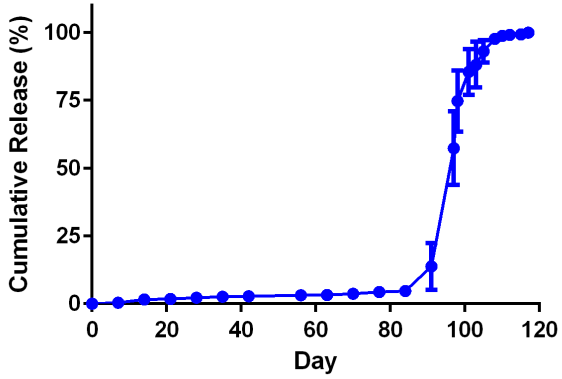
PLGA 50:50, 46 kD, Acid



PLGA 50:50, 61 kD, Ester



PLGA 75:25, 95 kD, Ester



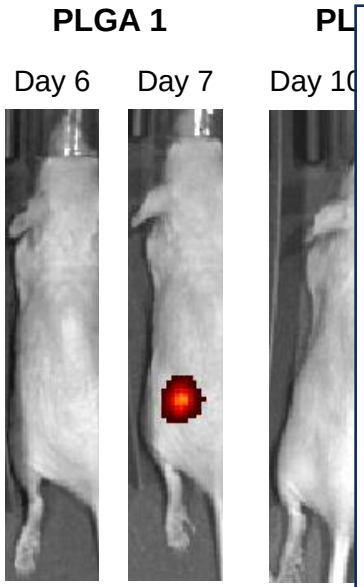
Release dependent on:

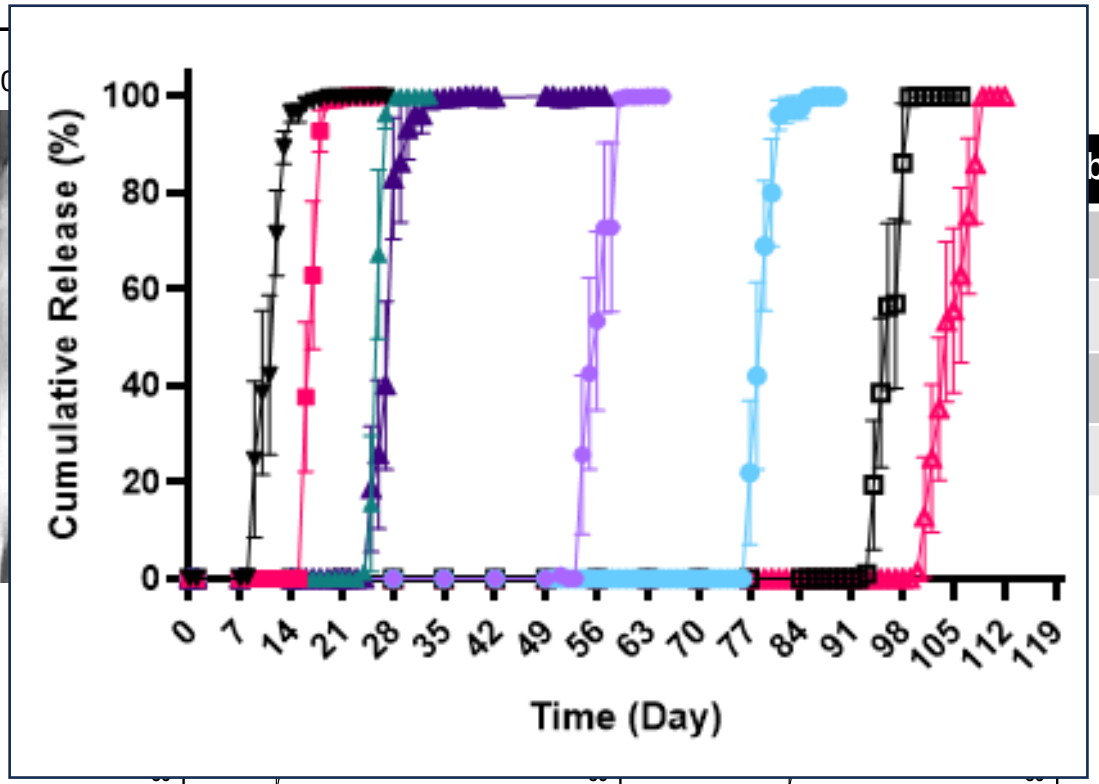
1. Molecular weight
2. End group
3. Copolymer ratio

Average ± SEM, n=10

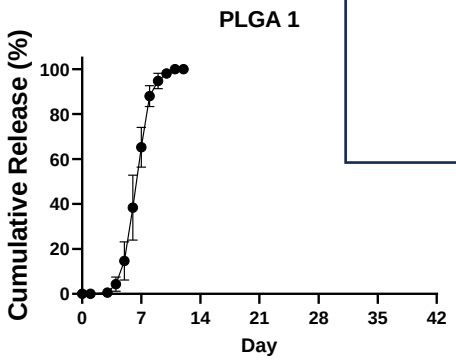
In Vivo Release

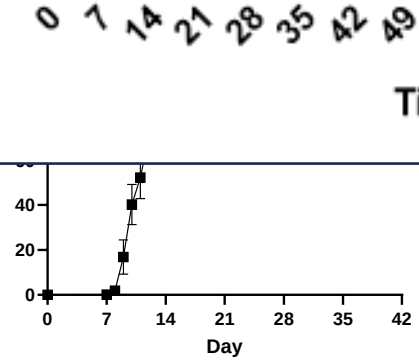
Injected one particle filled with Alexa Fluor 647-labeled dextran (10 kDa) into mice

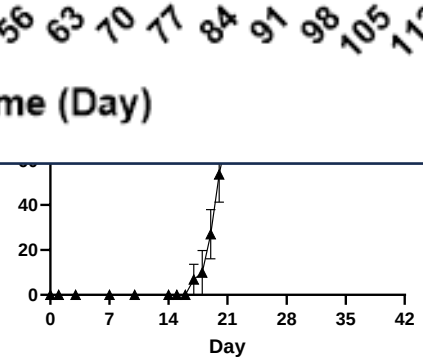


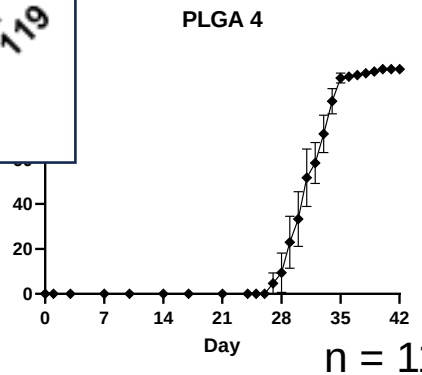


bel	Day of Release
	7 ± 1
	11 ± 2
	20 ± 2
	31 ± 2









n = 11-12

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Vaccination Model

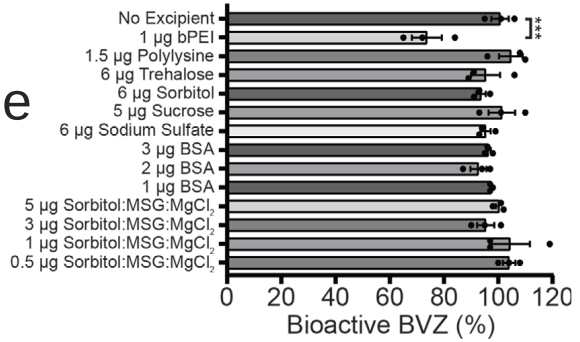
- Ovalbumin (OVA) encapsulated in core-shell particles expected to release after 9 and 41 days were injected subcutaneously into mice
- The injection of microparticles induced higher antibody titers than a two injections of soluble antigen → two-fold dose-sparing

McHugh KJ, et al. *Science* 2017;357:1138.

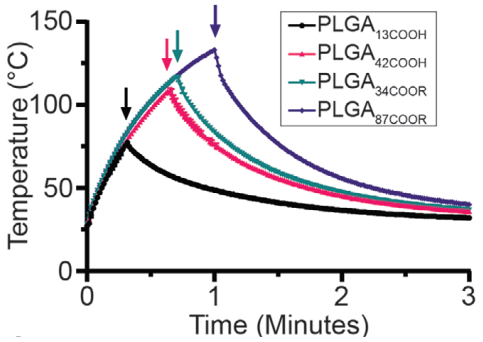
Encapsulated Protein Stability

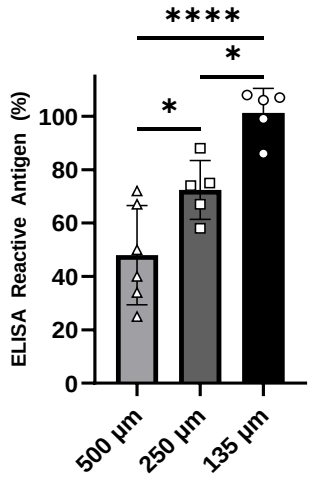
- Is heating harmful to the encapsulated protein?
- We tested this with Avastin, an antibody that binds to vascular endothelial growth factor, and HRP, an enzyme
- Avastin did not require any special treatment
- HRP was less thermostable; however, a loss of bioactivity could be mitigated using excipients or a modified sealing method

Avastin



Horseradish Peroxidase (HRP)





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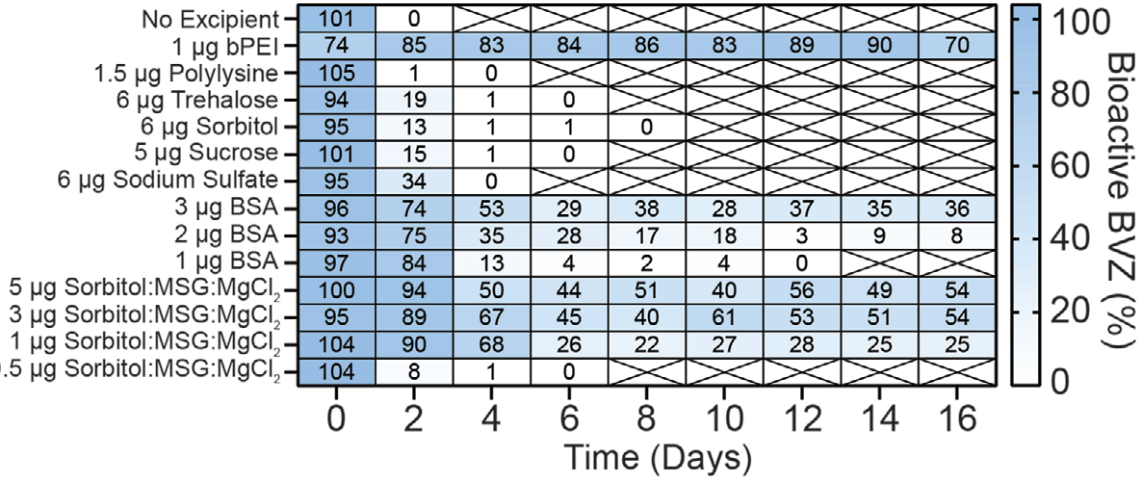
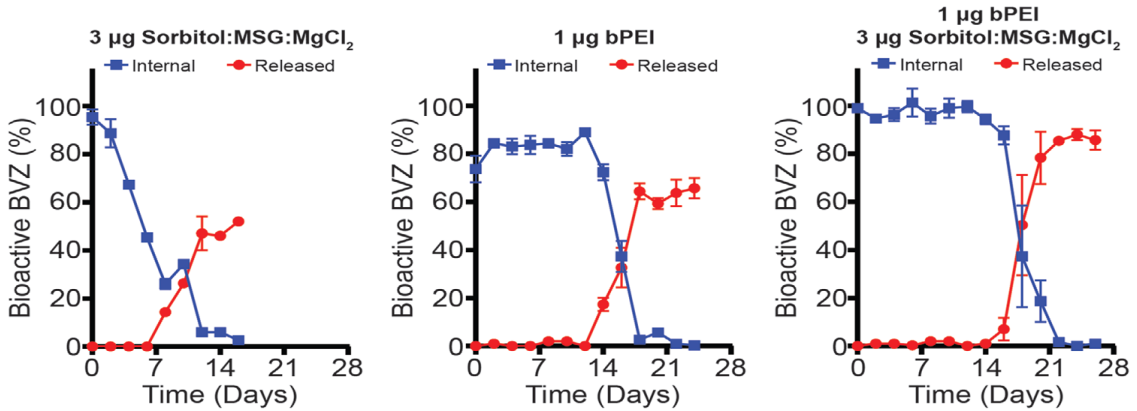
Protein Stability through Release

- Stabilizing Avastin for many days at 37 °C was more difficult
- Screened many excipient formulations, cutting particles open during release experiments to accelerate screening
- Ultimately, we identified excipients that enabled the release of 92 ± 9% recovery of bioactive Avastin with release centered around 18 days





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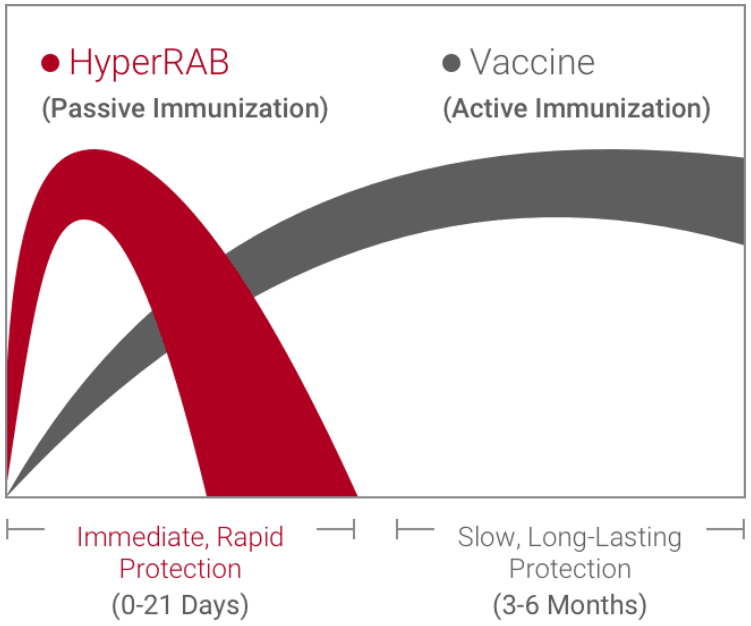
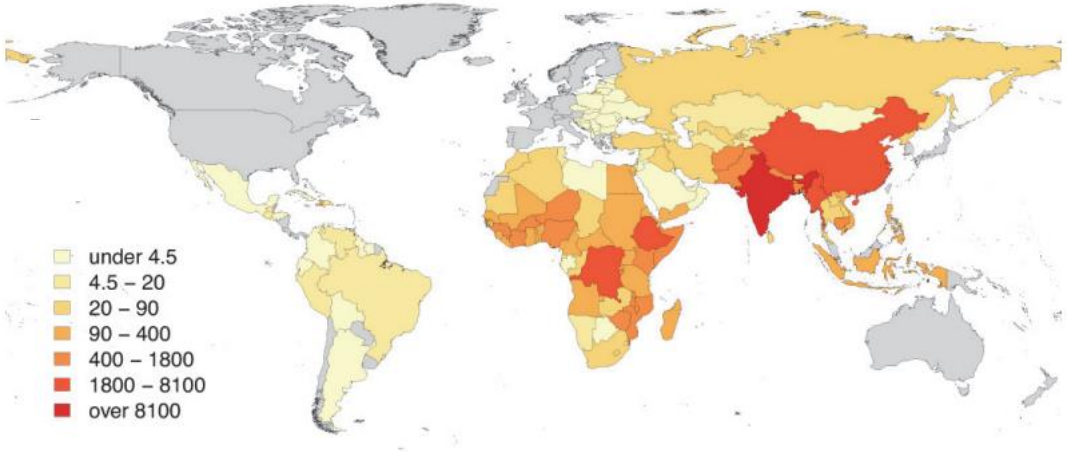
Rabies

- Rabies is the **deadliest disease** with a ~100% fatality rate w/o intervention
- Rabies kills 59,000 annually
- Rabies vaccine delivered after exposure alongside monoclonal antibodies
- Up to <u>four vaccine doses</u> are required → Accessibility issues

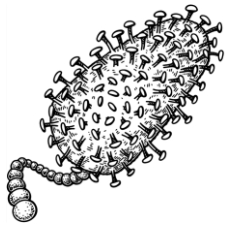


Annual Deaths Due to Rabies

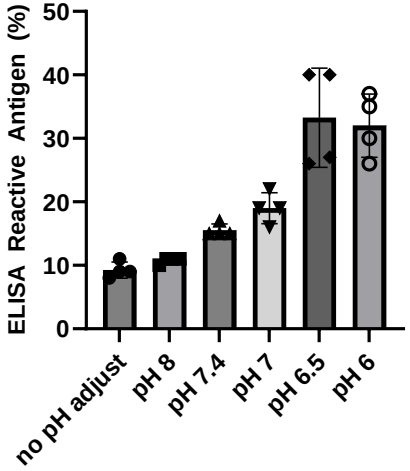


Hampson, et al., *PLOS Neglect Trop Dis* 2015.
Radhakrishnan, et al., *Trop Med Infect Dis* 2020.
Sudarshan, et al., *Int. J. Infect. Dis* 2007.

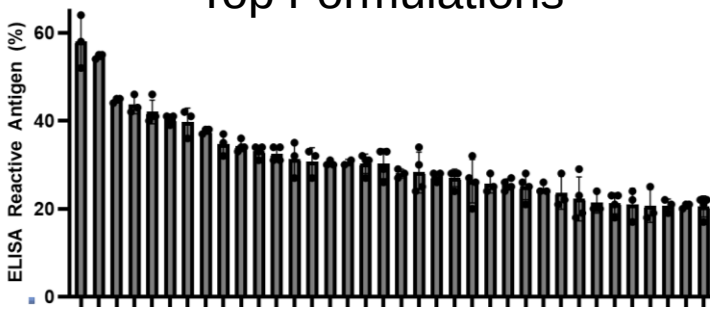
A Single-Injection Rabies Vaccine

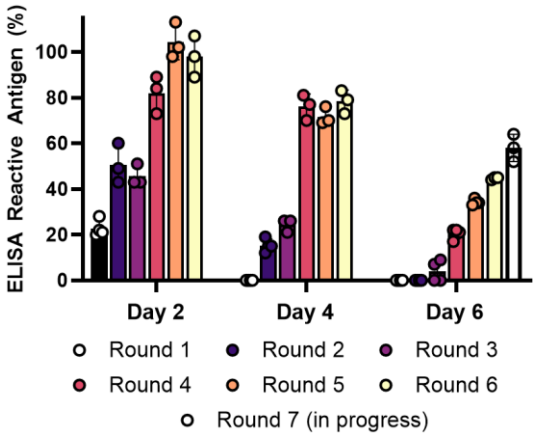


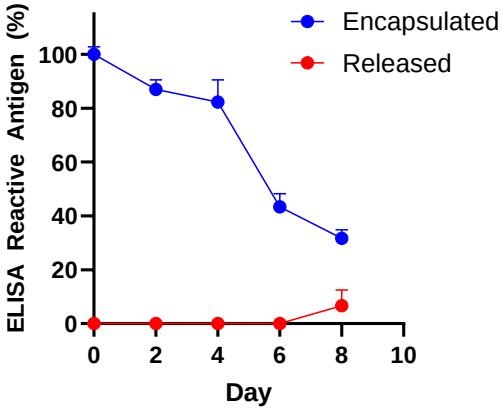
- Filling & sealing damage was easily mitigated, but maintaining stability through release is challenging
- After screening 352 excipient formulations *in vitro*
  - 68% had 0% stability after 6 days at 37°C in PBS
  - Recovery generally improved with each round of screening
- We are working on material-based stabilization strategies to avoid the need for extensive formulation



Top Formulations







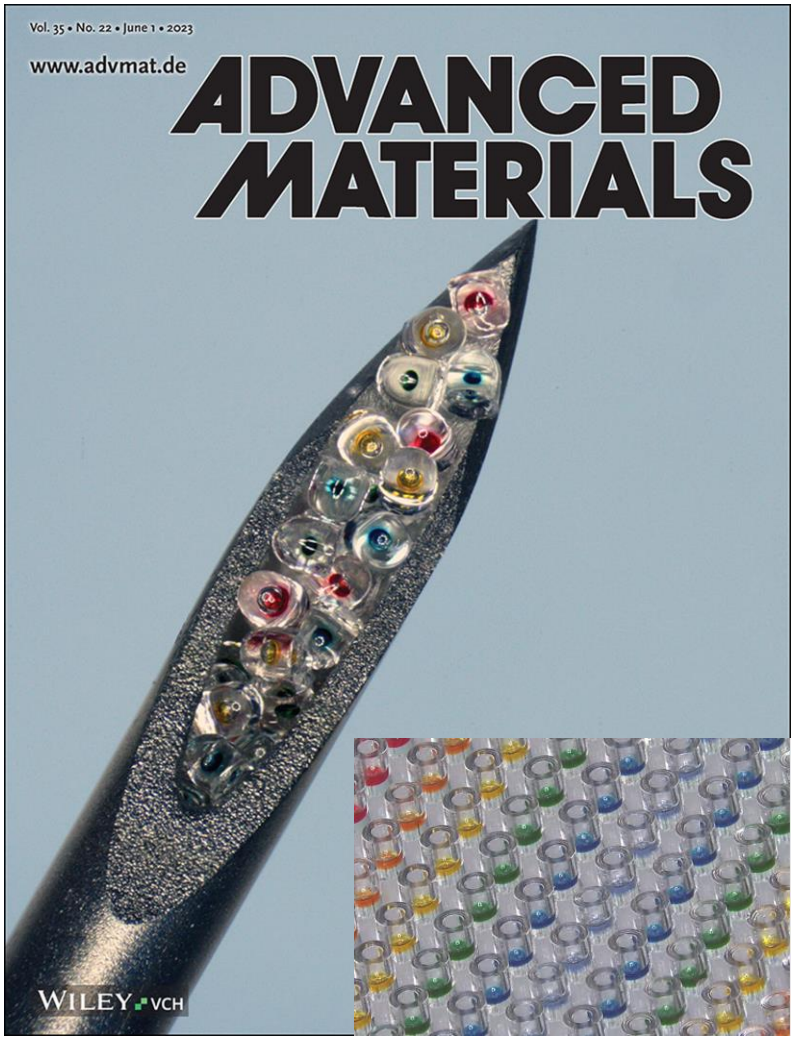
Summary & Future Work

Summary

- We can achieve pulsatile drug/vaccine release after a material-dependent delay
- With excipient incorporation, we can maintain the proper conformation/preserve the bioactivity of encapsulated protein payloads through release
- Releasing antigen from microparticles elicits a non-inferior and, in fact, dose-sparing response relative to multiple bolus injections

Future Work

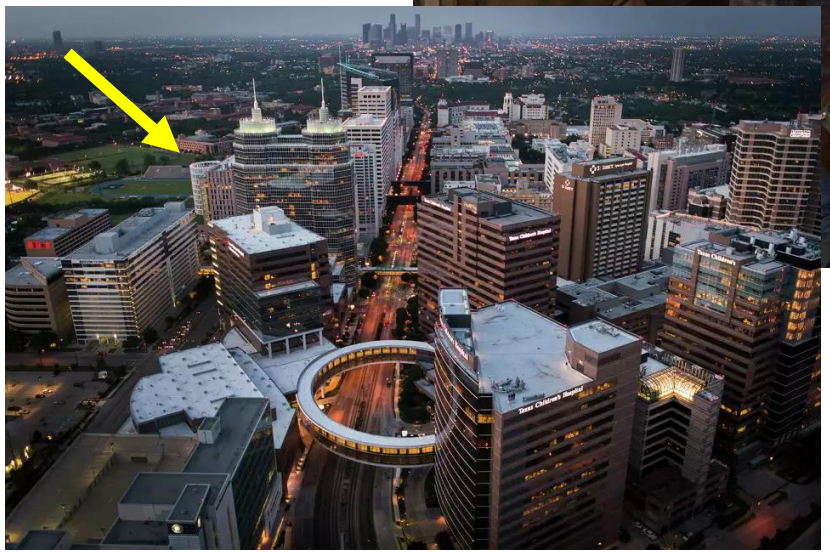
- Applying this technology for vaccination against infectious diseases and local cancer therapy
- Building out our release timing library
- Developing a universal material-based stabilization strategy
- Exploring alternative release kinetics to rescue failed vaccines



Graf TP, et al. *Adv Mater* 2023

Join Us!

We are looking to hire a postdoc & PhD students



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Team & Support

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- Katherine Chen
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- Gabby Lea
- Yosan Embafrash
- Krish Choksi
- Sam Wu
- Ruhi Rachakonda
- Miller Harris
- Bess Glenn
- Ananya Gimmy

Collaborators

- Jeffrey Hartgerink
- Zachary Ball
- Erin Seeley
- Michael Swierczynski
- Chris Pennington
- Carson Cole
- Many more!

NIH NIGMS

NANOCAN THERAPEUTICS CORPORATION

NIH National Institute of Allergy and Infectious Diseases

BILL & MELINDA GATES foundation

THE SONTAG FOUNDATION

CANCER PREVENTION & RESEARCH INSTITUTE OF TEXAS