Bison vaccination using bio-bullets

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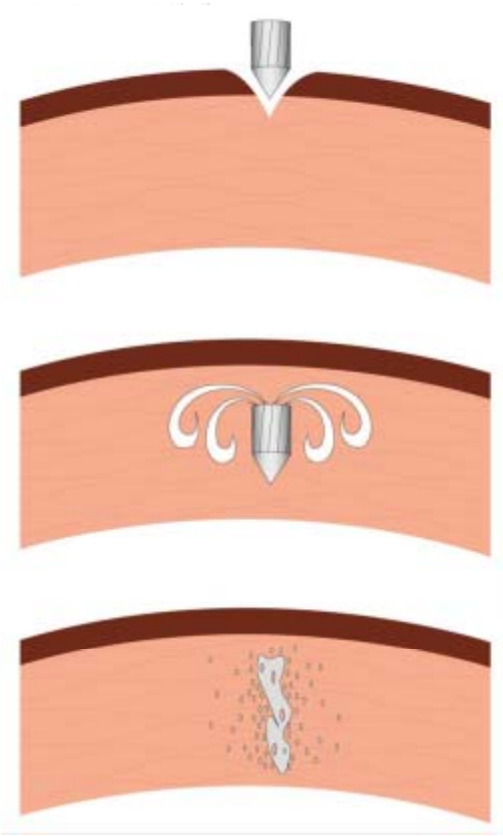
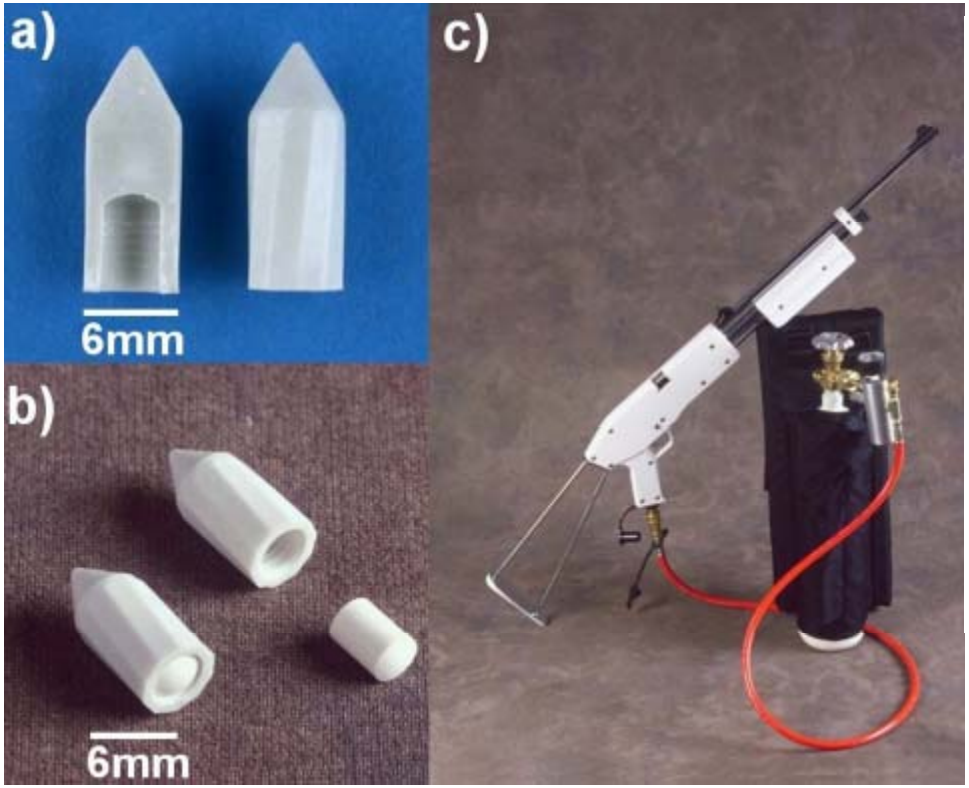
- ***SolidTech, OK*** **(R. Hansen)**

Current Biobullet Technology





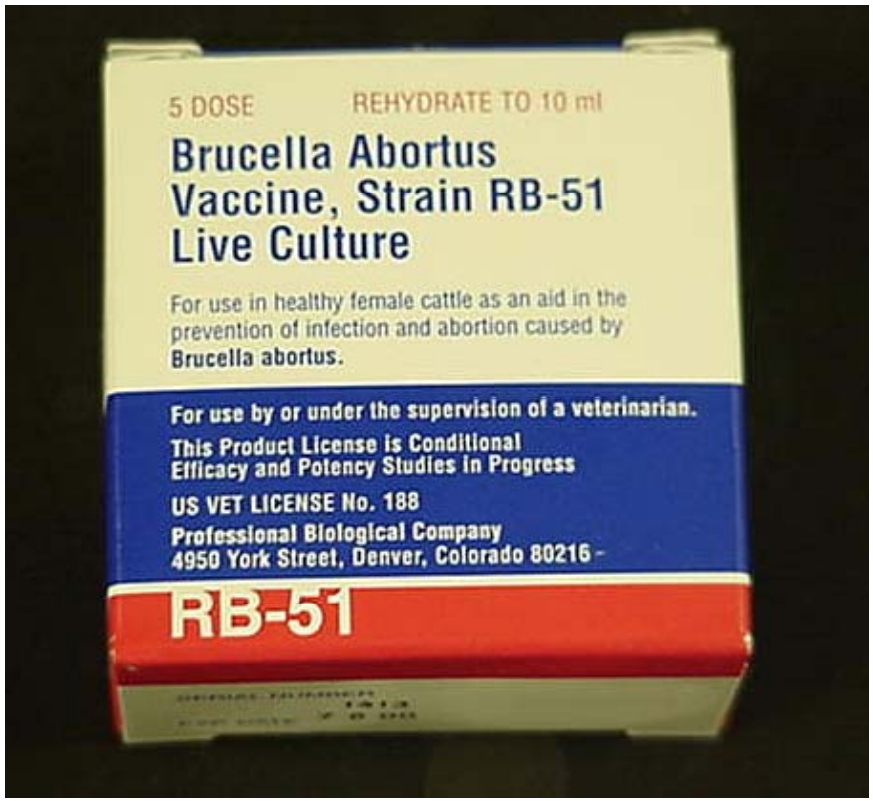
- Hydroxypropyl cellulose biobullets penetrate skin and lodge in muscle tissue when fired into target animal.
- Degradable bullets dissolve over time releasing therapeutic agents.

- Commercially available biobullets utilize medicinal pellets formed by compression loaded into the payload chamber.
- An air-rifle charged to ~1200 PSI accurately delivers biobullets to target animal at a range of ~ 30m.

Ideal Properties: Brucellosis Vaccine for Wildlife

- **Live vaccine persists for approximately 12 weeks to induce strong cell-mediated immune responses to protect host**
- **75-100 meter accuracy i.m.**
- **No adverse tissue localization/lesions**
- **Not impair diagnostics-based field strain brucella detection**
- **Ballistics compatible: "leave no trace", 100% biodegradable**
- **Administration does not disturb normal herd behavior**
- **Rapid, inexpensive, convenient formulation for field personnel**

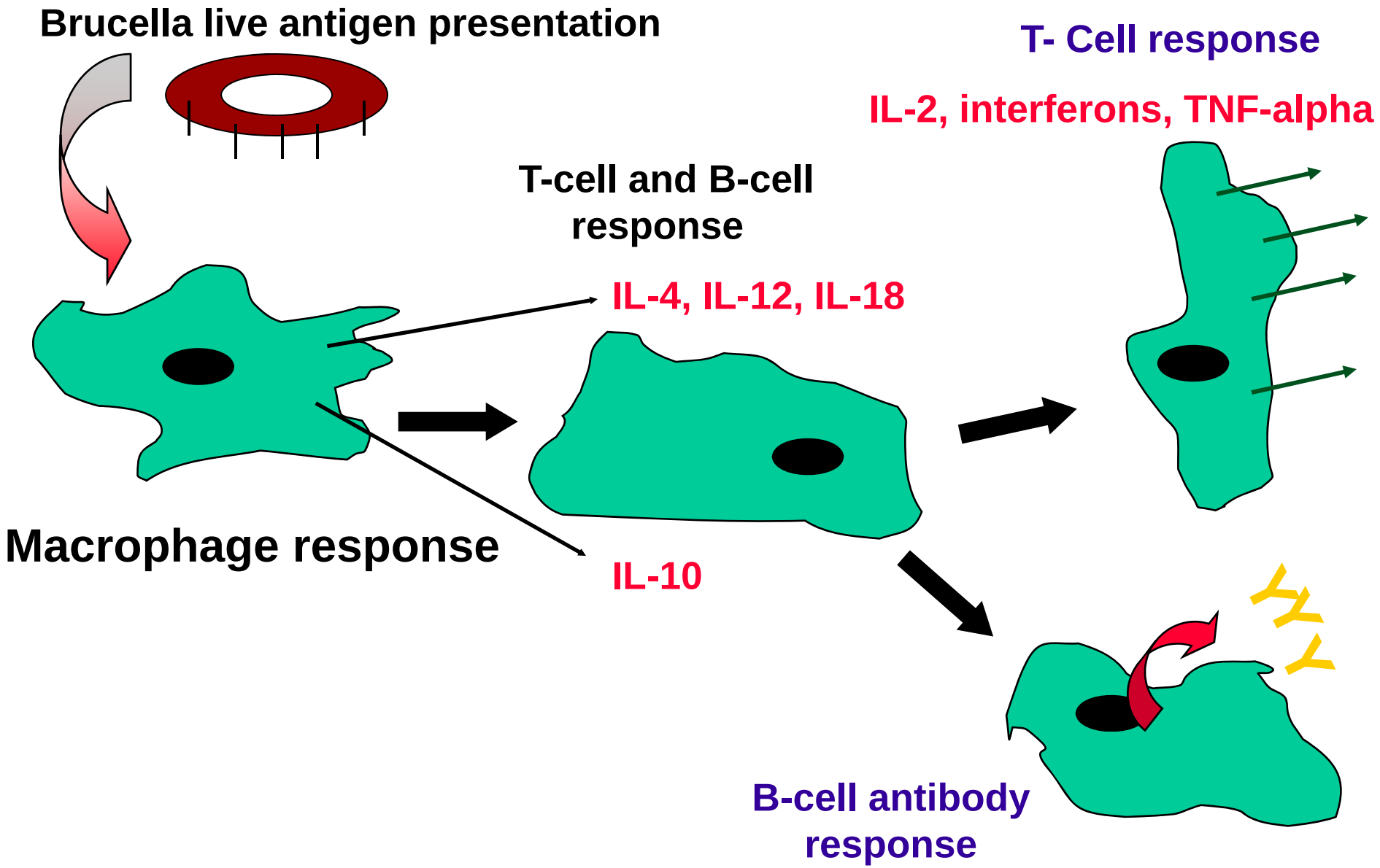
Commercial Strain: RB51 Live Brucella Vaccine



- **Official calfhood brucella vaccine for domestic cattle**
- **Calf-hood dosage:**
  ~ 10^{10} CFU i.m.
- **Does not induce serologic responses on brucellosis surveillance tests**
- **Multiple dosing possible**
- **Largely ineffective in elk**

How to deliver this live vaccine to wild animals at a distance of >50m?

Cell-Mediated Immune Response: Vaccine in host



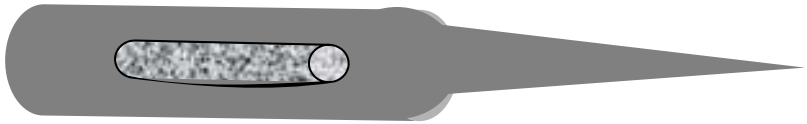
Bullet formulations: live vaccine payload fabrication

1. Live or DNA vaccine (payload) mixing with liquid gel precursor matrix loading on-site, on-demand

2. Incubate or photo-treat on-site to gel-encapsulate vaccine (payload) into bullet (seconds-minute)

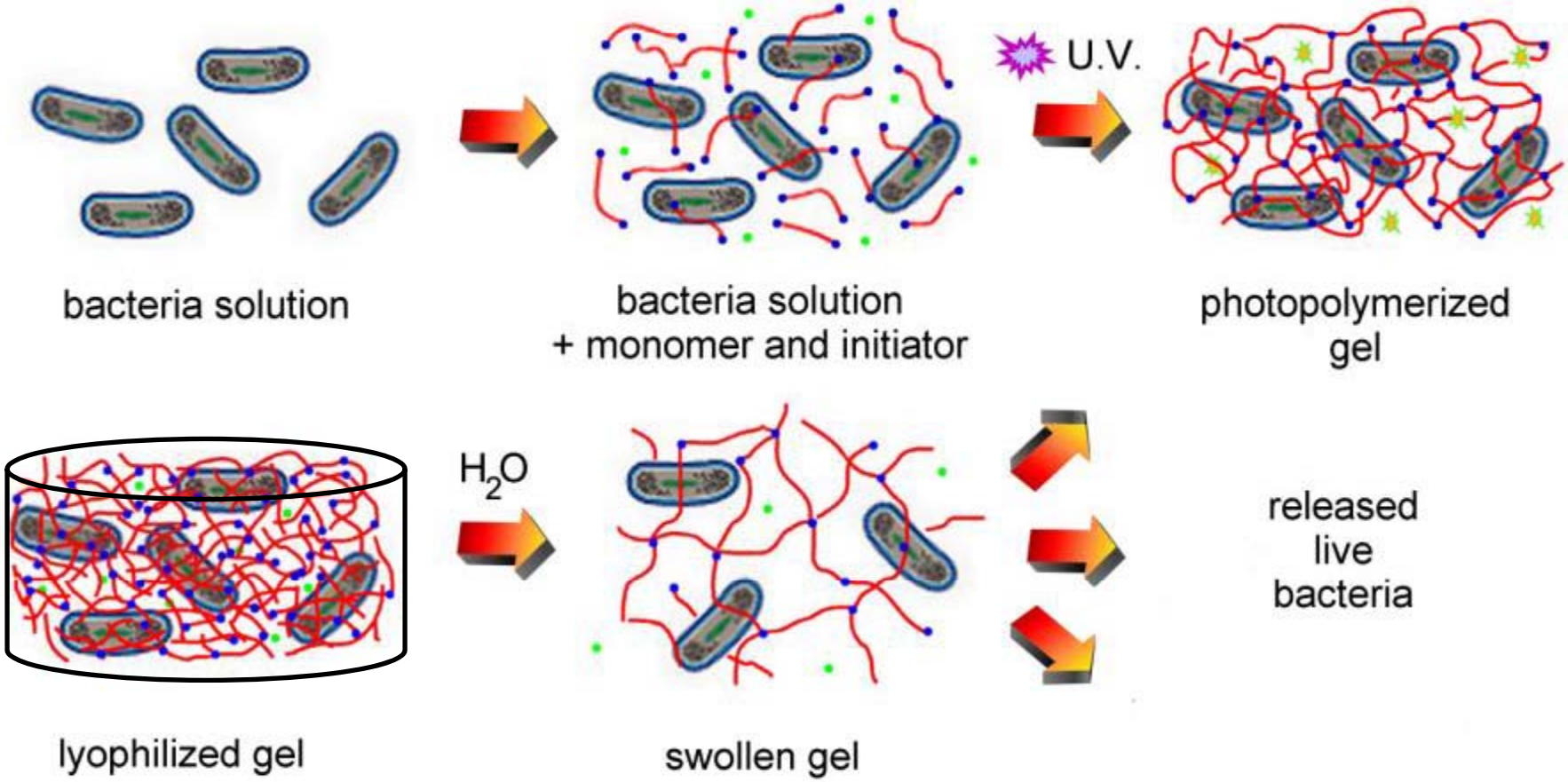
Bio-bullet blank vehicle (ballistics)

3. In situ gel-encapsulated vehicle with live/DNA vaccine (payload)



4. Payloaded bio-bullet vaccine vehicle for deployment

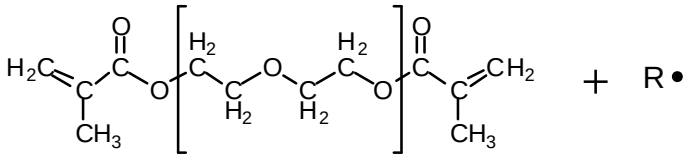
Polymer photo-encapsulation of vaccine: live RB51 bacteria in hydrogel carrrier



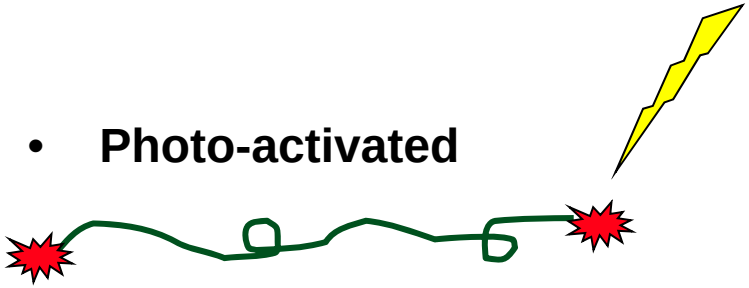
glassy, rigid **soft, elastic**

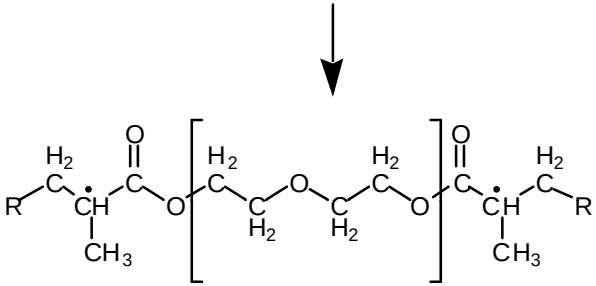
Scheme for PEG chemistry in hydrogel formation

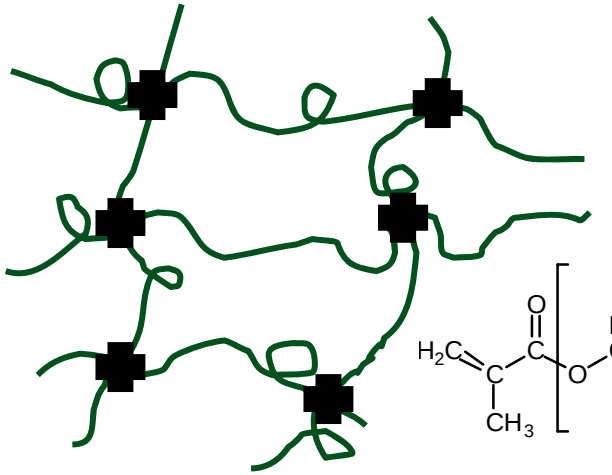
- **End functional PEG**

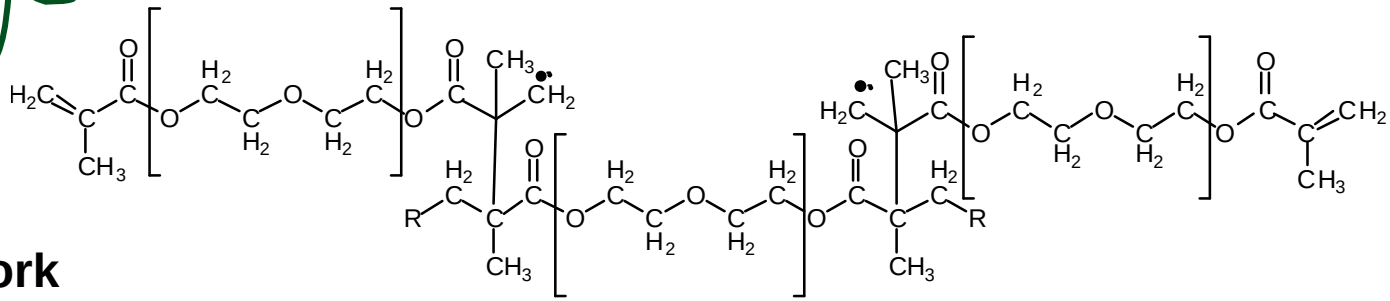


- **Photo-activated**



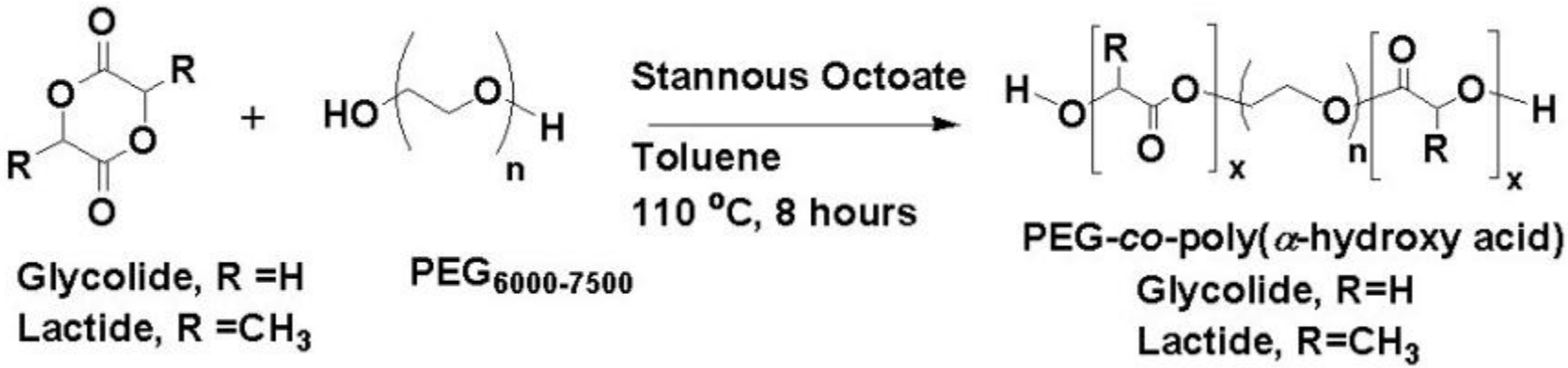






- **Polymer Network**

Degradable Endgroup Modification of PEG*



- **Degree of endgroup modification controlled by stoichiometric addition**
  - **5:1 molar ratio glycolide or lactide per mol PEG**
- **Endgroup modification confirmed by IR and ^{1}H NMR spectroscopy**
- **Yields > 85%**
- **Expected degradation properties:***
  - **PEG6000-co-poly(glycolide) $t_{1/2}$ = 2.5 days**
  - **PEG6000-co-poly(lactide) $t_{1/2}$ = 20 days**

* A.S. Sawhney, et al. *Macromolecules* **1993**, *26*, 581-587; Y.J. Du et al *Macromolecules* **1995**, *28*, 2124-2132; Y.M. Chung et al *Biomacromolecules* **2002**, *3*, 511-516; D.K. Han et al. *Macromolecules* **1997**, *30*, 6077-6083; S. Lu et al *Macromolecules* **2000**, *33*, 2509-2515

Photopolymerization of PEG Macromers

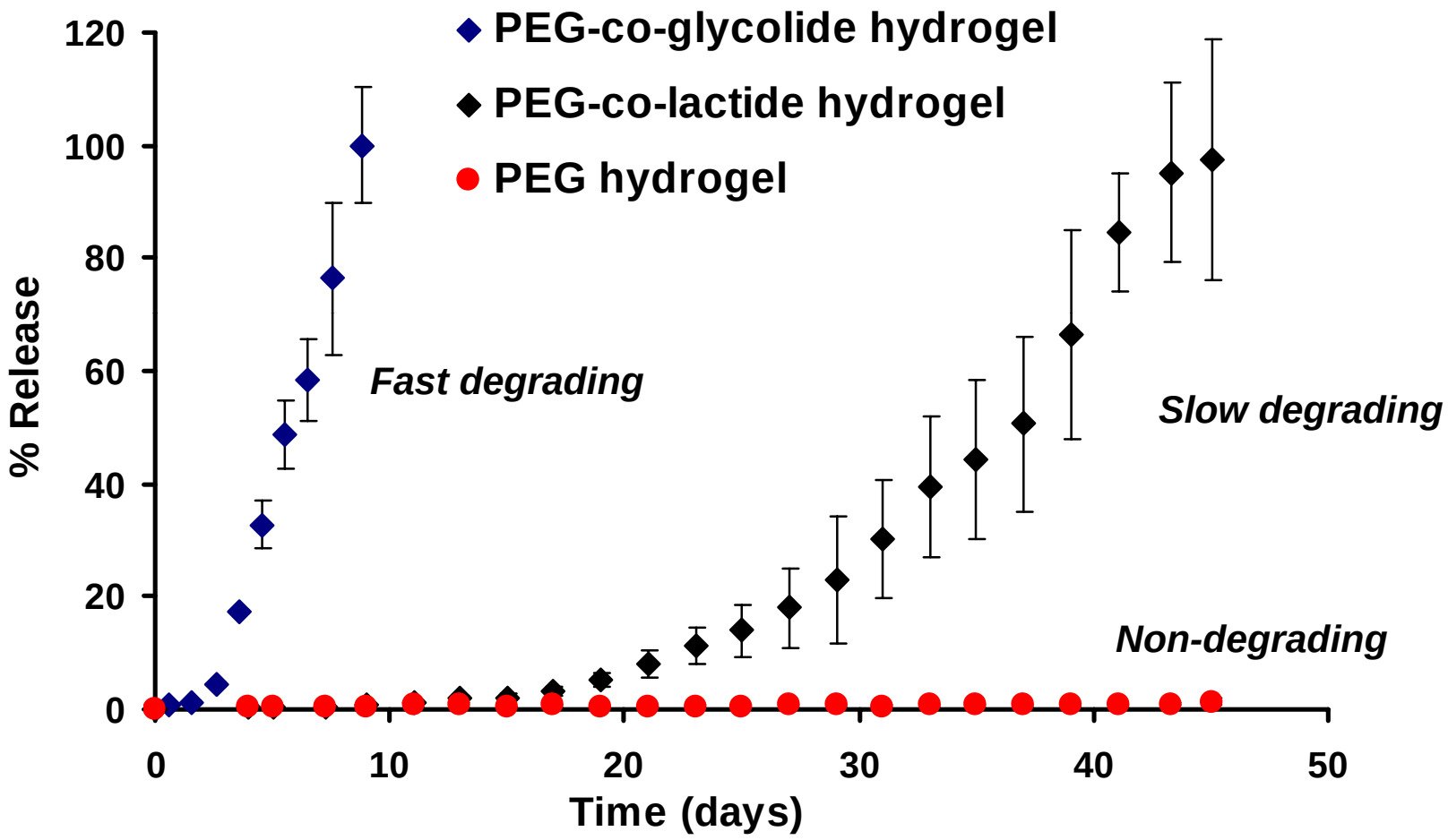
- 35% w/w PEG macromer solution in saline
- Irgacure 184 photo-initiator (Commercial)
- 90s exposure to UV light
  - low pressure Hg lamp, 20 mW/cm^2
  - possible from 12V outlet in vehicle in field

Liquid before photo-polymerization

Gel after photo-polymerization

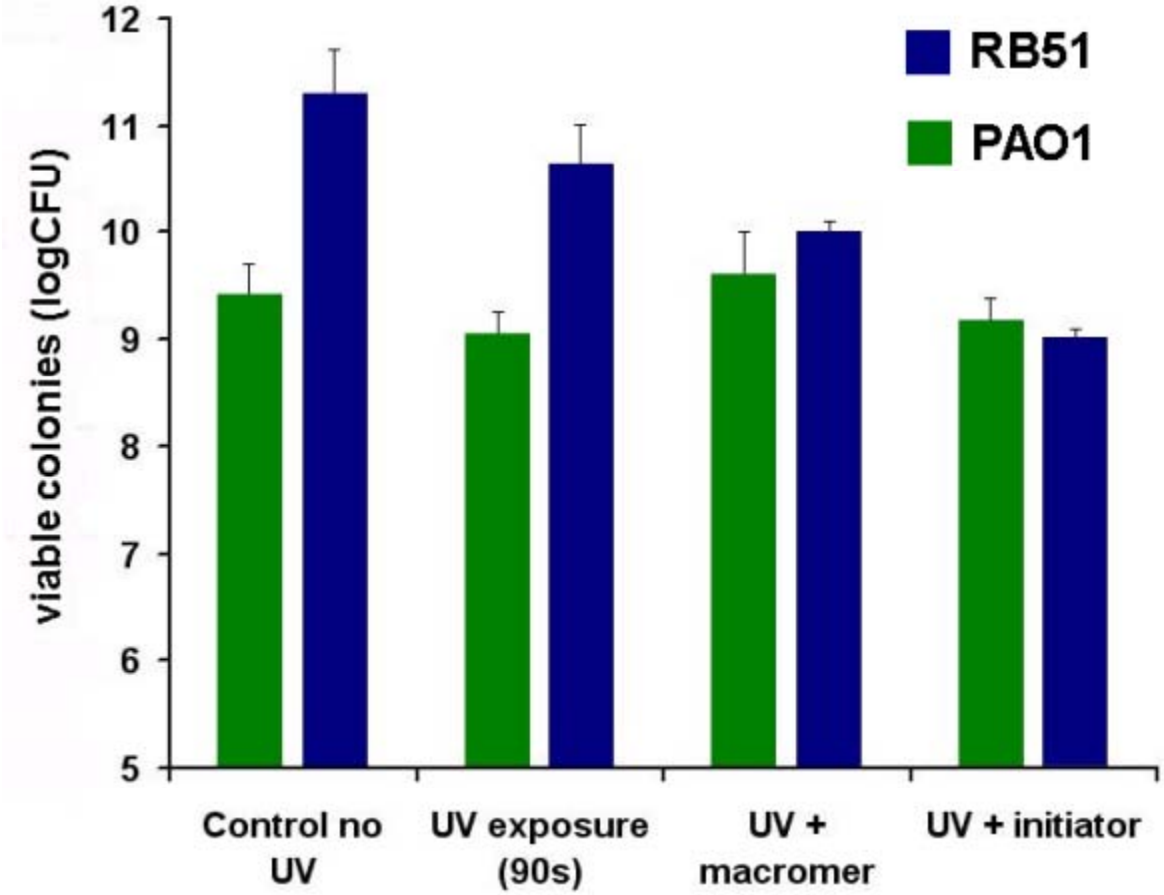
viscous solution $\xrightarrow[\text{~ 1 minute}]{\textit{UV light}}$ **rigid gel**

Release of 1μm microspheres from PEG hydrogels



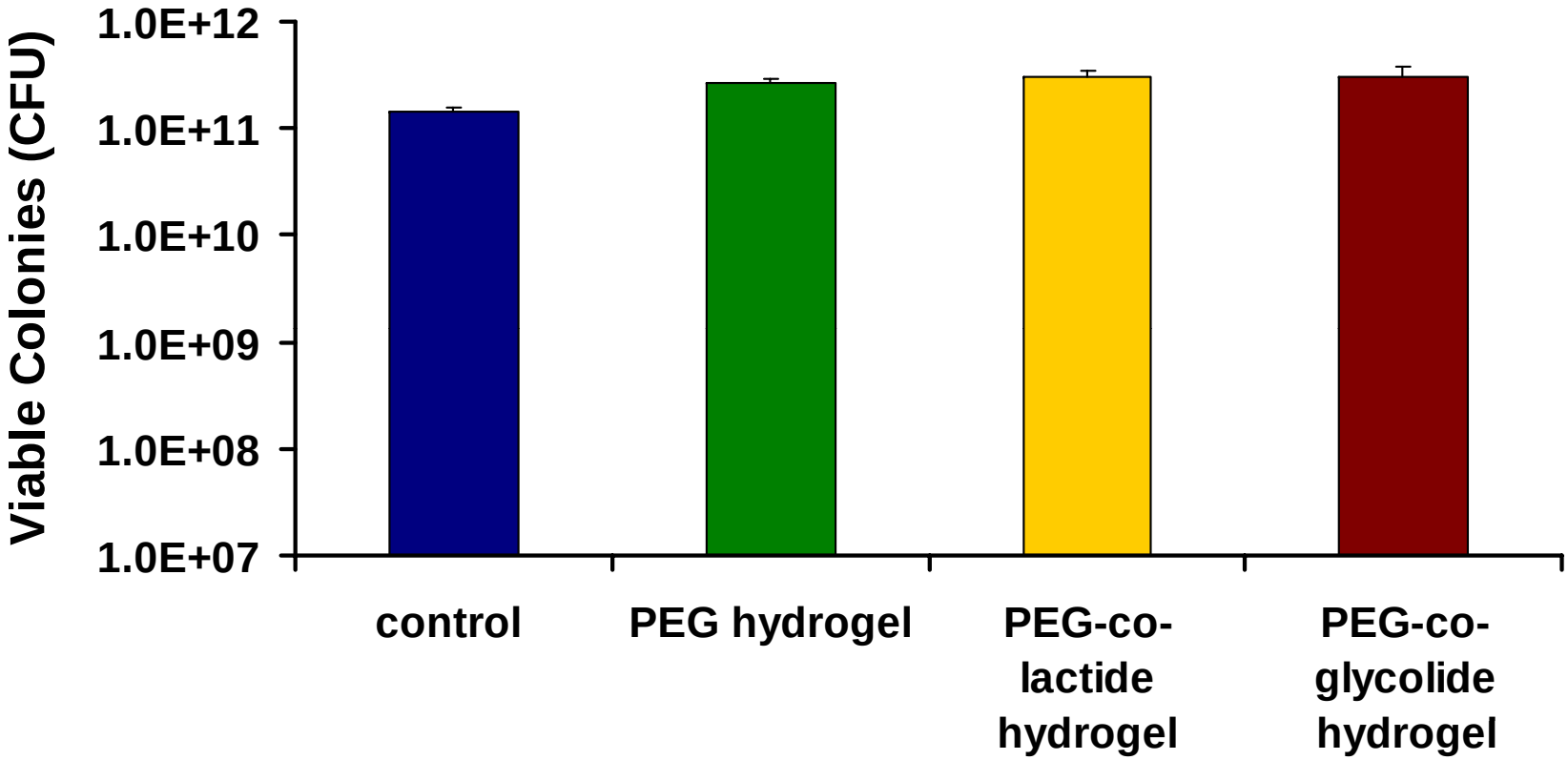
- Endgroup chemistries on PEG macromers can be used to control degradation and release of vaccine cargo

Viability of bacteria exposed to hydrogel processing procedure



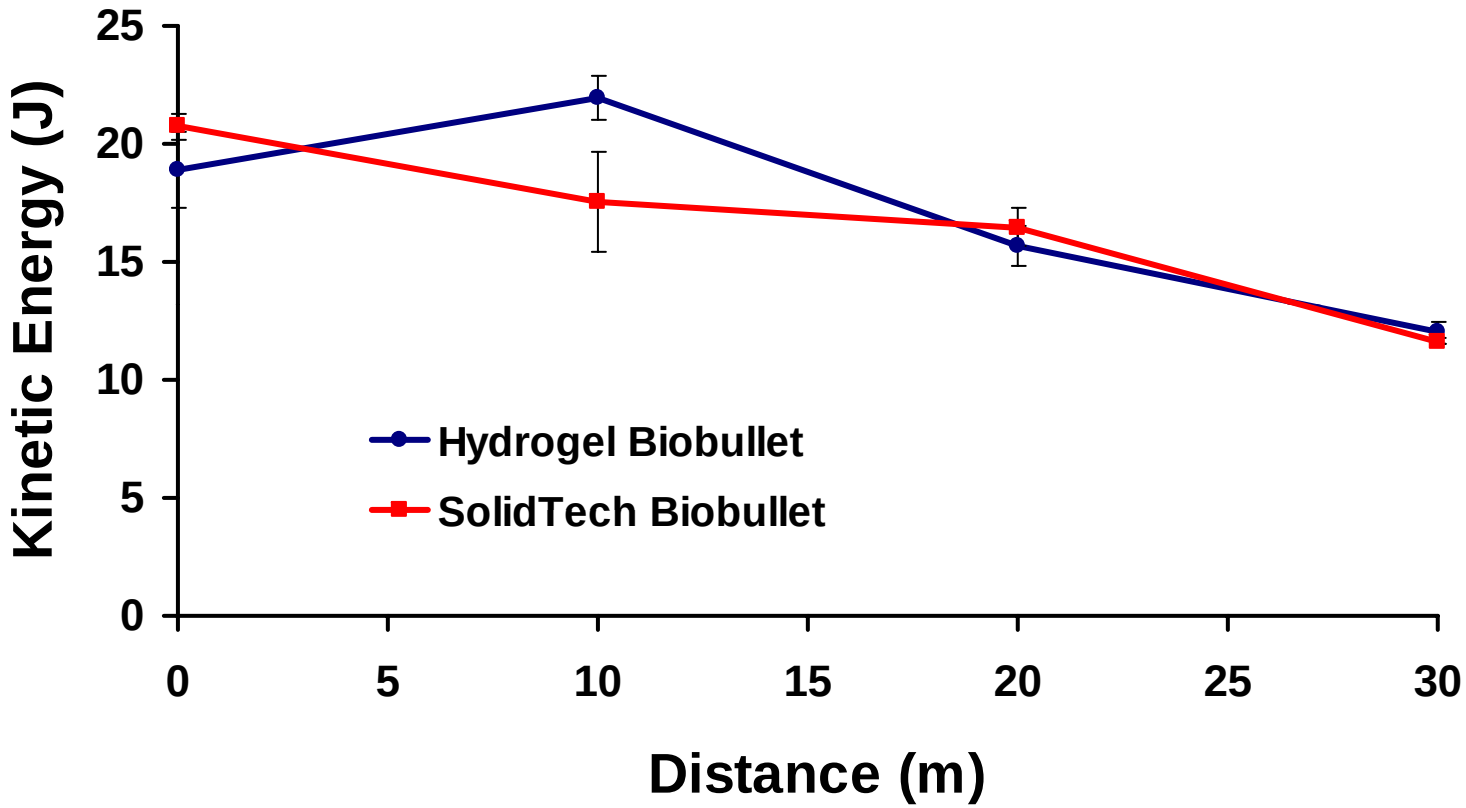
- **RB51 loses viability after exposure to hydrogel processing conditions when no hydrogel is formed.**
- **Viability loss may decrease when the reactive macromers scavenge generated free radicals.**

RB51 viability after hydrogel encapsulation



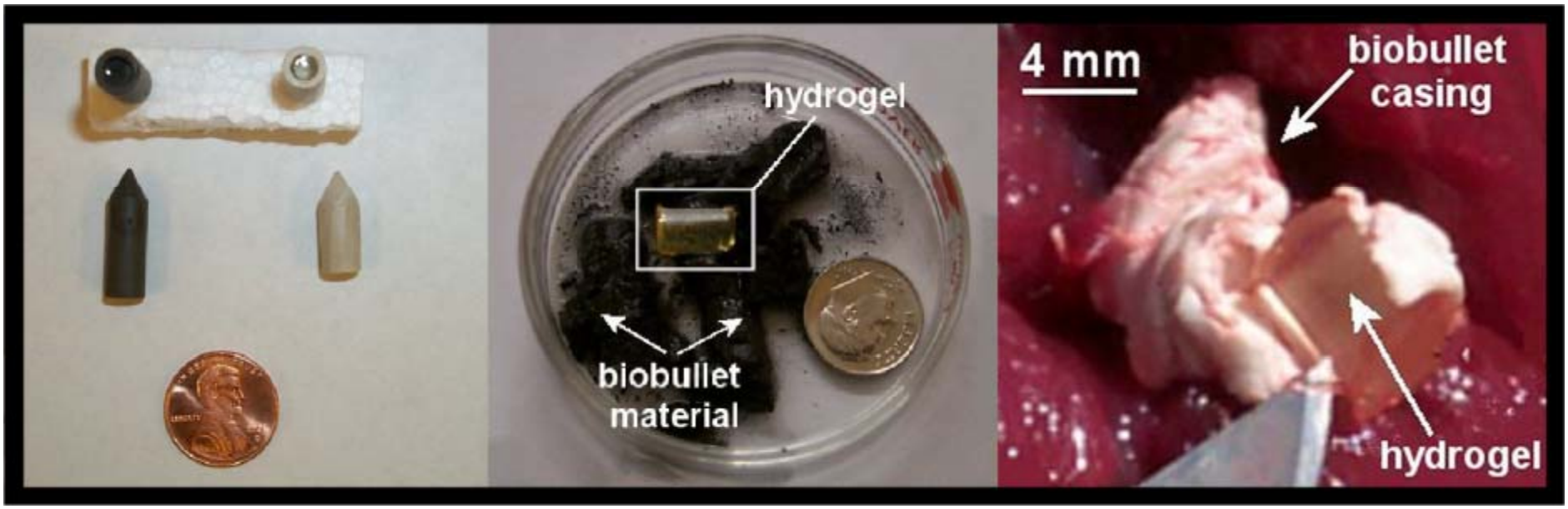
- RB51 exhibits excellent viability after PEG encapsulation
- Endgroup chemistries do not effect vaccine viability

Hydrogel-loaded biobullet ballistic performance



- Hydrogel-loaded and factory-loaded biobullets perform similarly
- Similar downfield energy should produce similar penetration of commercial bullets (~ 10cm i.m.).

Hydrogel-loaded biobullets



Biobullets with PEG gels polymerized in payload chambers.

Hydrogel-loaded biobullet placed in buffer for 24 hrs.

Hydrogel-loaded biobullet recovered from elk 24 hrs after entry.

- Hydrogels readily formed in biobullets upon end-on photo-polymerization
- Hydrogels remain intact inside biobullet when fired into live animals.

Hydrogel Biobullet Vaccine Carrier Studies in Bison

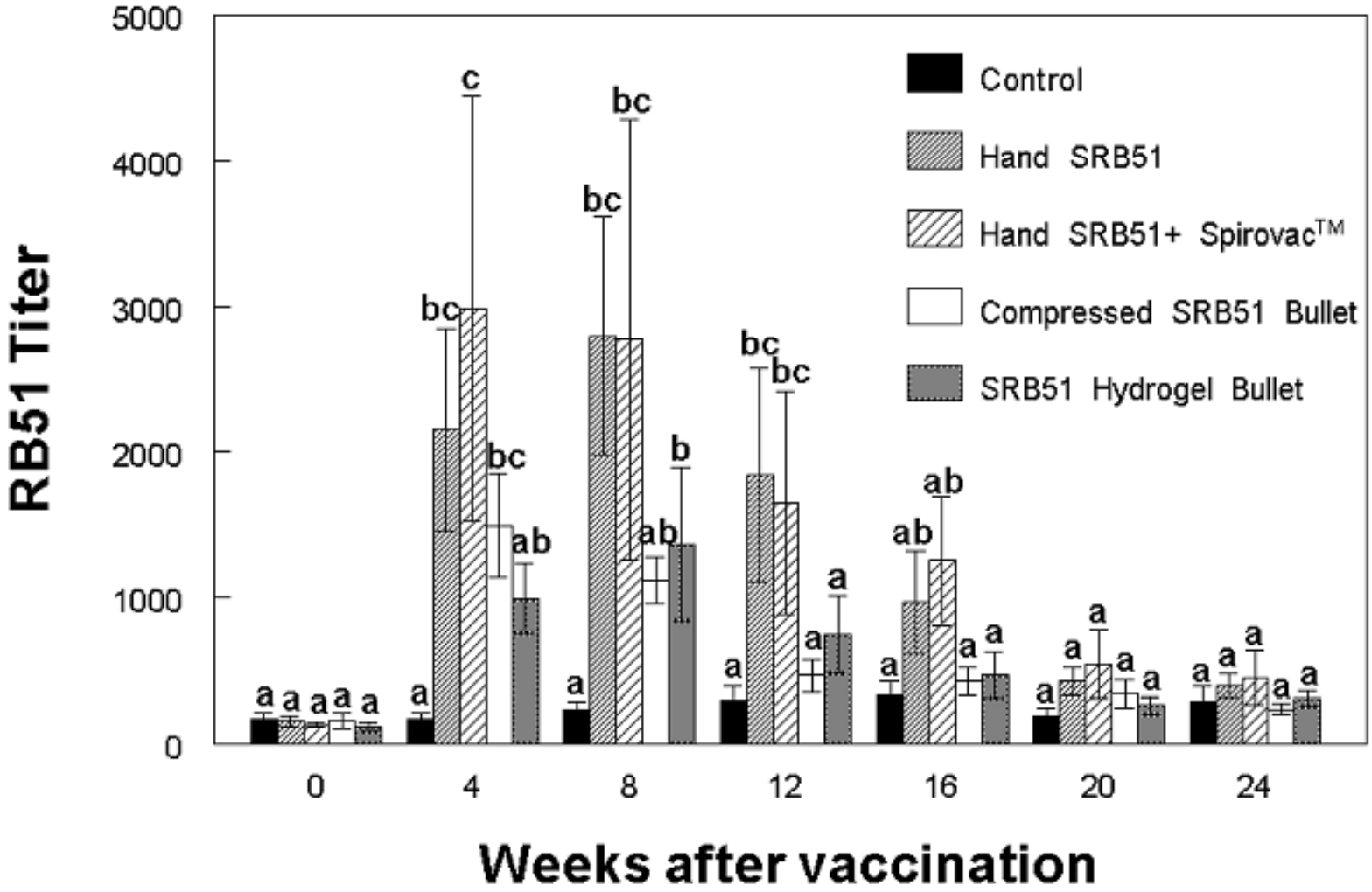
- **Collaborative work with USDA labs, Ames, IA (S. Olsen)**

- **4 bison trial groups treated with RB51 encapsulated in:**
  1. **PEG-co-glycolide degradable hydrogels**
  2. **PEG-co-lactide degradable hydrogels**
  3. **Unmodified PEG non-degrading hydrogels**
  4. **Compressed hydroxypropyl cellulose pellet**

- **All samples compared to RB51 hand vaccination (Control)**
  ~ 3.3 x10^{10} CFU dose

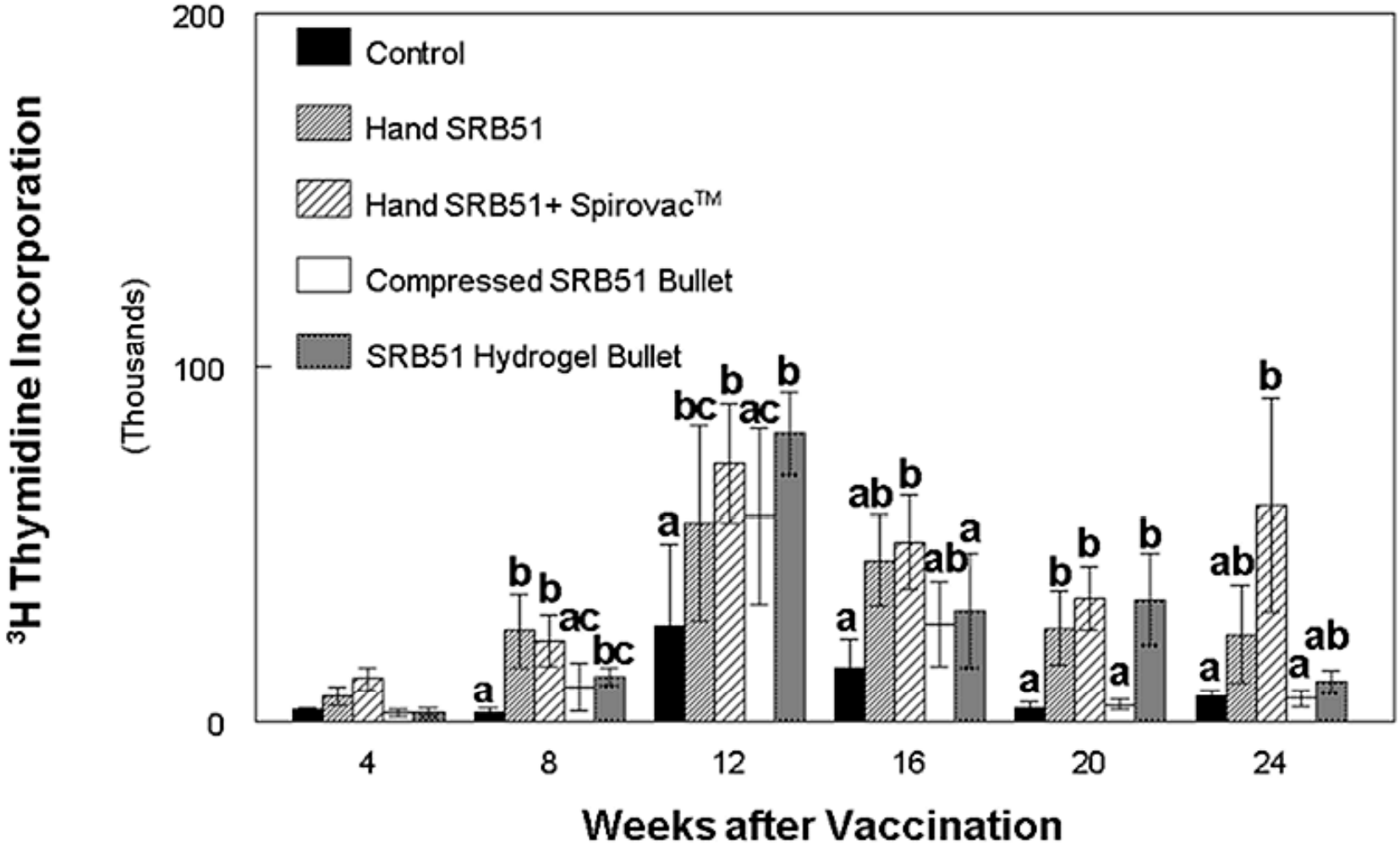
- **Initial empirical conclusion: biobullet vaccine protects >70% abortion in penned bison.**

Bison antibody production: hand vs. hydrogel vaccines



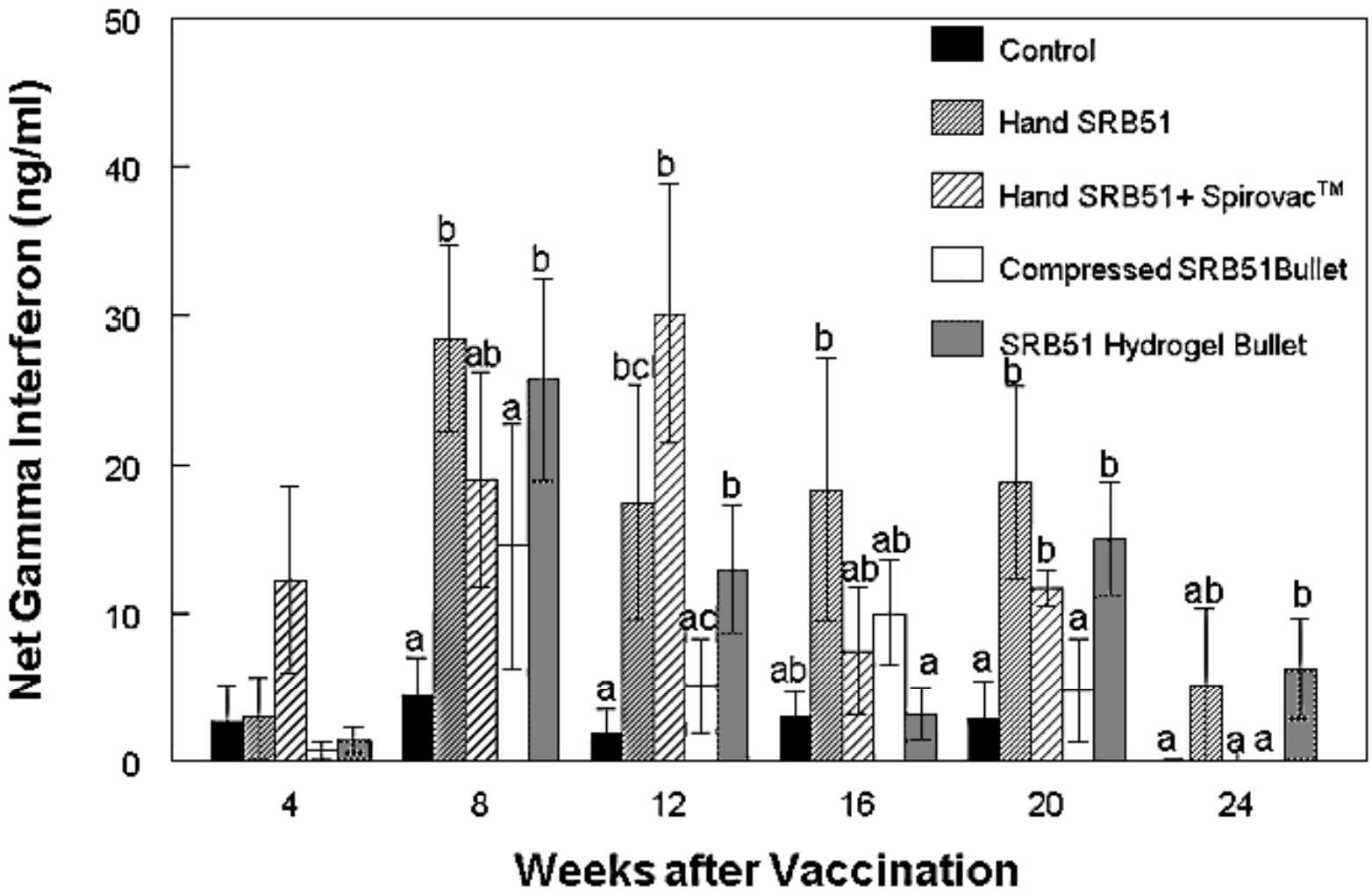
- 4-8 week peak in antibody stimulation; similar for all

Vaccinated T-cell stimulation: hand vs. hydrogel



- **12-week peak in T-cell stimulation; similar for all**

Bison interferon production: hand vs. hydrogel



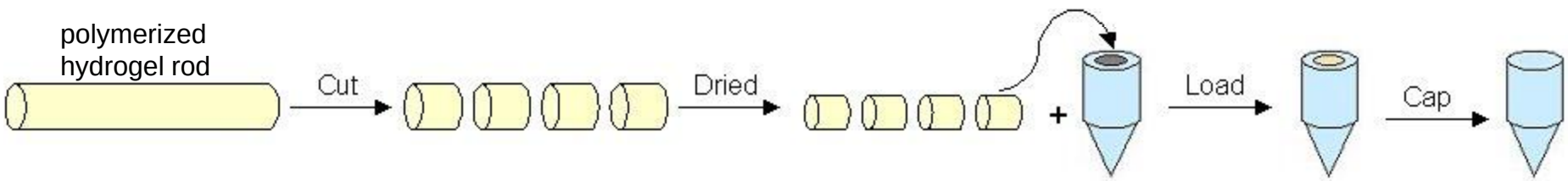
- biobullet interferon production prolonged and superior

Lyophilized vaccine-loaded hydrogel biobullets

- Hydrogels encapsulate and effectively deliver live vaccine

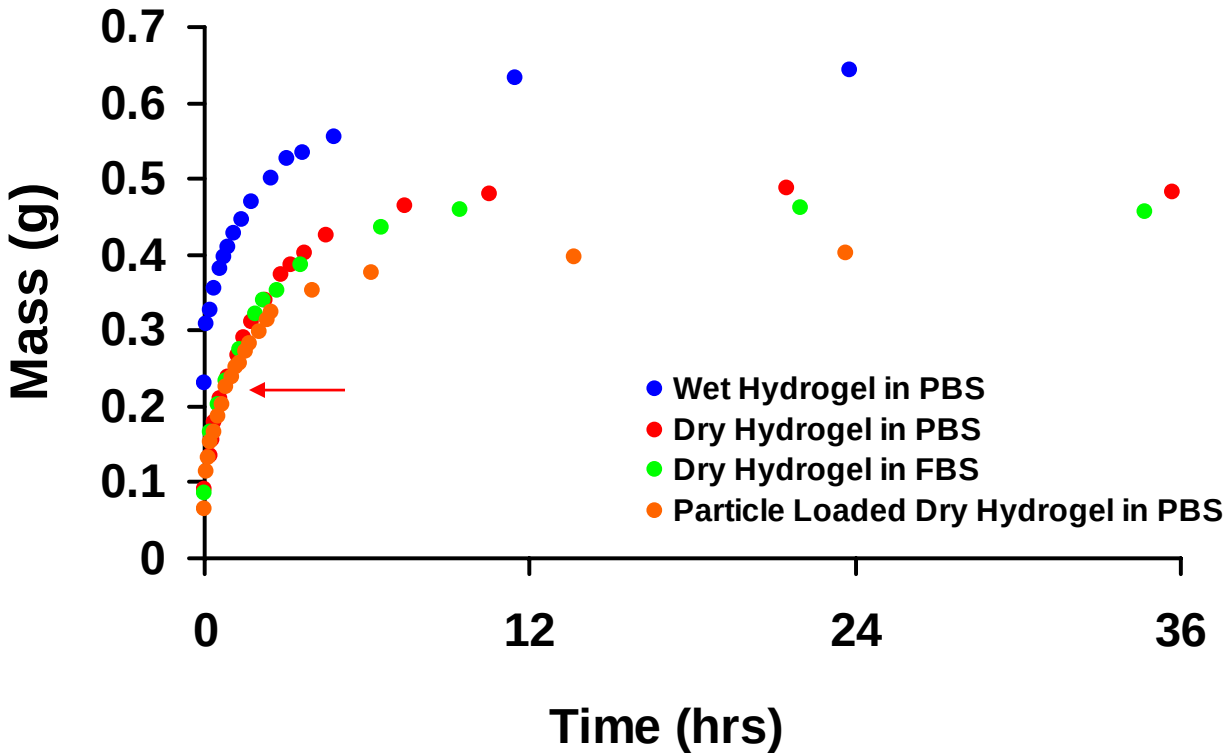
- Wet gel-loaded bullets dry out over time (affecting ballistics), and soften the biobullet casing. Storage problematic

<u>Proposal:</u> lyophilized (freeze-dried) hydrogel formulations could result in a more versatile biobullet



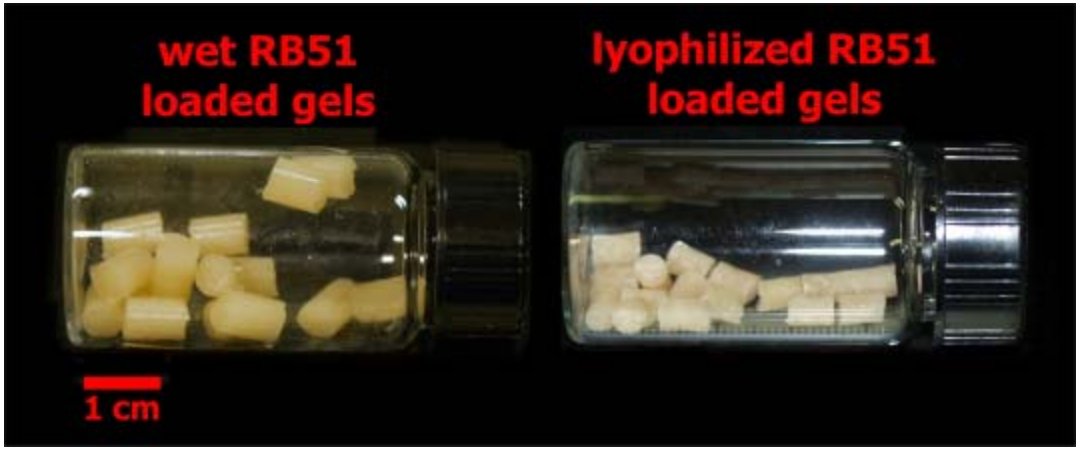
endcap of solvent-welded bullet polymer

Rehydration swelling of dried PEG hydrogels

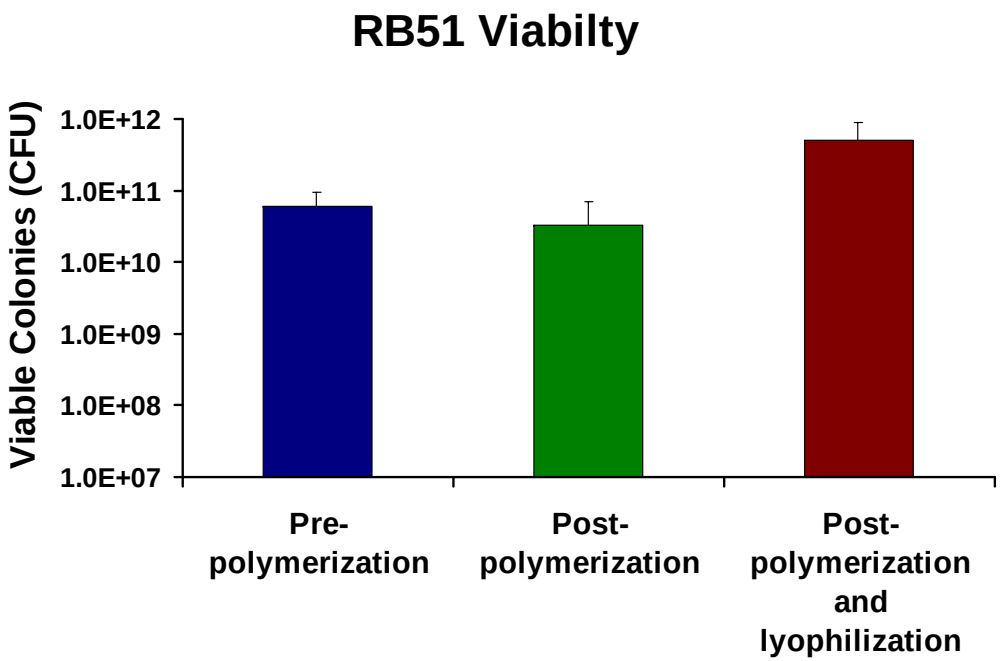


- Dried hydrogel rapidly rehydrates to original wet mass in ~ 1 hr (arrow).
- Swelling rates and maximum swelling are equal in buffer and serum.
- Dried hydrogels do not attain same swelling as wet hydrogels.
- Encapsulated particles do not affect swelling rate, but slightly decrease maximum swelling.

RB51 vaccine encapsulated in dried hydrogels

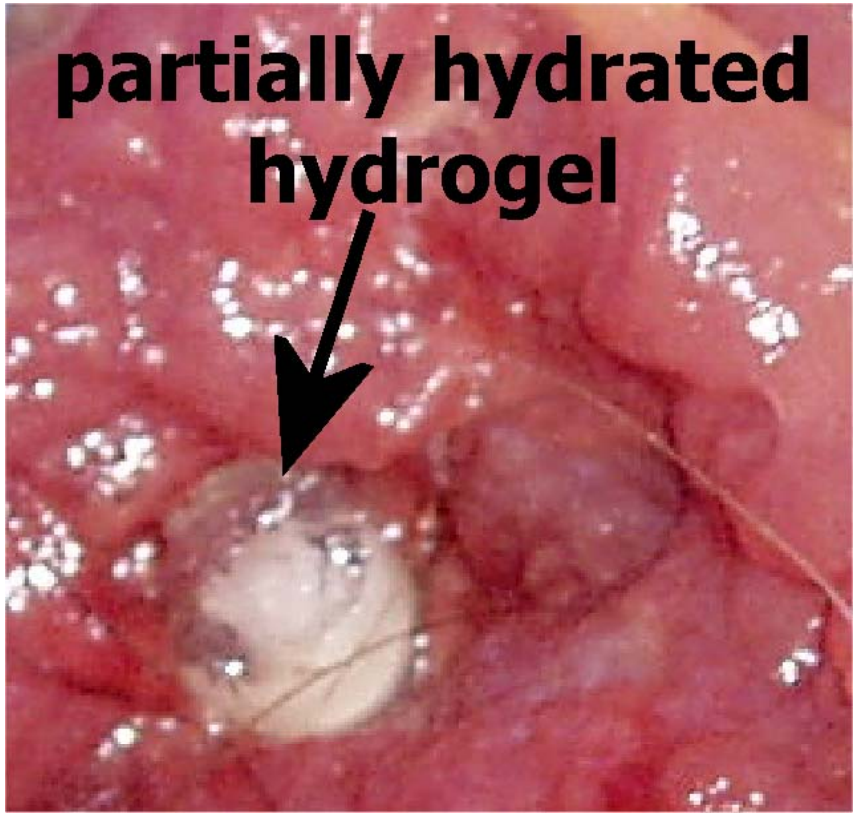


- Lyophilization of RB51 loaded gels results in dense, rigid pellets



- No drastic loss in vaccine viability observed after processing

Dry hydrogel swells in tissue post-ballistic delivery



Hydrogel recovered from horse hindquarter tissue. This gel penetrated 5 cm into the muscle bed.

Recovered hydrogel after 1 hr in horse muscle (gel is >50% rehydrated).

Conclusions: Ballistic Delivery of Live Vaccine

- **Unique delivery constraints: leave-no-trace, wild animals**
- **Live microbial vaccine viability preserved in hydrated and dessicated 100% degradable gel vehicles**
- **Ballistics requirements for Yellowstone Park delivery mandate successfully fulfilled**
- **Disease protection using live vaccine in bison cohorts confirmed**

***Publications*:**

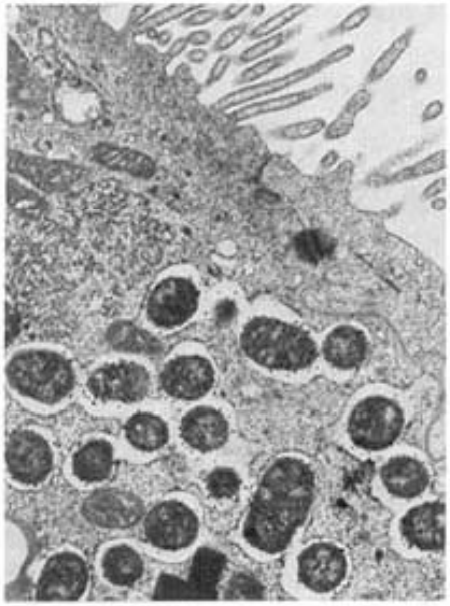
R.J. Christie, D.A. Findley, S. Olsen, M. Dunfee, R.A. Hansen, D.W.Grainger, "A degradable ballistic delivery system for Brucella live vaccine", Vaccine, (2006).

R.J. Christie, D.A. Findley, , S. Olsen, R.A. Hansen, D.W. Grainger, "Immunological markers in bison from ballistically delivered Brucella live vaccine", Vaccine, (2006).

R.J. Christie, E. Kaiser, S. Olsen, D.W. Grainger, "Viability comparisons of wet versus lyophilized photo-polymerized Brucella live vaccine gel preparations", unpublished.

B. Abortus: infection and transmission

- ***B. abortus*: infectious sporous bacterium found in reproductive organs and mammary gland tissues, lymph nodes in ruminant animals, specifically maintained in *membranes and fluids surrounding the developing fetus*.**



***B. abortus*-infected epithelium**

- **During late pregnancy, bacteria replicate in specific fetal epithelial cells, *causing abortion*.**

Transmission of *B. abortus* results from*:*

- **Massive *B. abortus* in placental fluids and genital exudates from the aborting female.**
- **Strong chemoattractant effects of expelled fetal membranes - other animals ingest this material.**
- **Bacteria infect through mucous membranes of eye, nose, or oral cavity, pass epithelial barriers in tonsils, oronasal lymphoid tissues, and tear ducts, enter lymphatic system and bloodstream. Taken into white blood cells, spores persist to ultimately grow in the fetal epithelium.**

Cheville, N. F. , D. R. McCullough, L. R. Paulson. **Brucellosis in the Greater Yellowstone Area.** National Research Council, Washington, D.C. 1998

Brucella in U.S. Wildlife

- **Brucellosis: infected wildlife populations of bison, elk, and extremely low incidence in black and grizzly bears, coyotes, wolves (probably), beaver, and likely many other small mammals (extent unknown).**

- **Approximately 40-50% of bison in Yellowstone Park test positive for brucellosis using standard serology tests (late 1990's data).**

- **1-3% of elk in northern Yellowstone Park show brucellosis.**

- **Elk from the southern portion of Yellowstone associate with elk feedgrounds in Wyoming and Idaho, so test-positive rate is much higher: 15-35% depending on feed ground.**

- **Bison in the Jackson bison herd have tested as high as 77% positive (27 of 35 bison tested in 1989 in the National Elk Refuge)**

- **Infected wildlife spread brucella to domestic cattle populations.**

Wildlife species of interest for Brucellosis Ballistic Vaccination

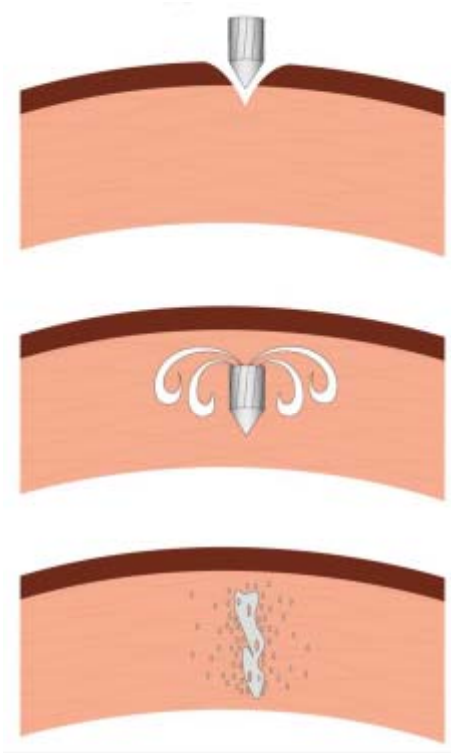
- B. abortus in bison
- *B. abortus* in elk
- *B. suis* bv4 in reindeer

5000 bison

130,000 elk

Vaccinate these animals using ballistics delivery at a distance?

Opportunities for ballistic delivery of live vaccine to wildlife:



- **brucellosis**
- **immuno-contraceptives (coyotes, deer)**
- **chronic wasting disease (CWD)??**

- R.J. Christie, D.A. Findley, S. Olsen, M. Dunfee, R.A. Hansen, D.W.Grainger, “A degradable ballistic delivery system for *Brucella* live vaccine”, *Vaccine,* (2006).

- R.J. Christie, D.A. Findley, , S. Olsen, R.A. Hansen, D.W. Grainger, “Immunological markers in bison from ballistically delivered *Brucella* live vaccine”, *Vaccine,* (2006).

- R.J. Christie, E. Kaiser, S. Olsen, D.W. Grainger, “Viability comparisons of wet versus lyophilized photo-polymerized *Brucella* live vaccine gel preparations”, *unpublished.*