An *In Vitro* System to Model Specific Events Occurring at a Subcutaneous or Intraocular Injection Site

Randy Mrsny

UNIVERSITY OF BATH

Conflict of Interest Statement

The *in vitro* model described in this presentation has been licensed to Sirius Analytical who are in the process of commercializing an instrument. The University of Bath and its employees stand to profit from the sale of these instruments.

Release Criteria - Current State of Affairs

Lot release for drug delivery systems utilize a set of criteria that defined formulation performance

- Pulmonary delivery – plume geometry, particle size, dose delivered
- Oral delivery – capsule/tablet dissolution time and conditions, dose delivered
- Transdermal patches – dose release rate, etc.

- Liquid formulations for Subcutaneous (SC), intramuscular (IM), intraocular (IO) injection – visual inspection (solution clarity), sterility, level of endotoxin/ pyrogens; no criteria for dose performance upon administration

Potential Issues for Injection of Liquids

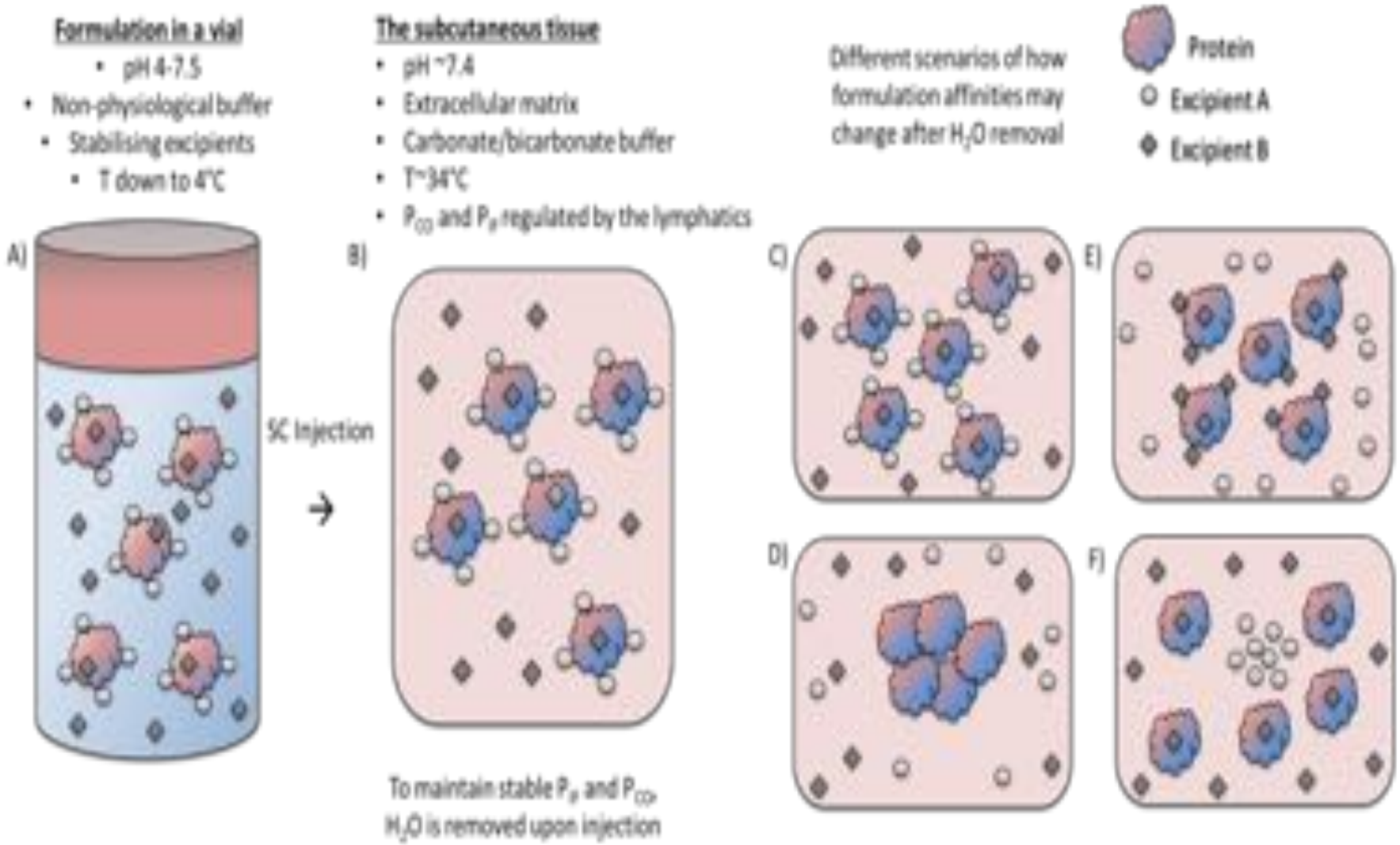
Current formulations are designed to

- Keep protein therapeutic stable in a vial for >2 years
- Minimize injection volume (high concentration)
- Minimize pain upon injection

Proteins can be stressed after injection by

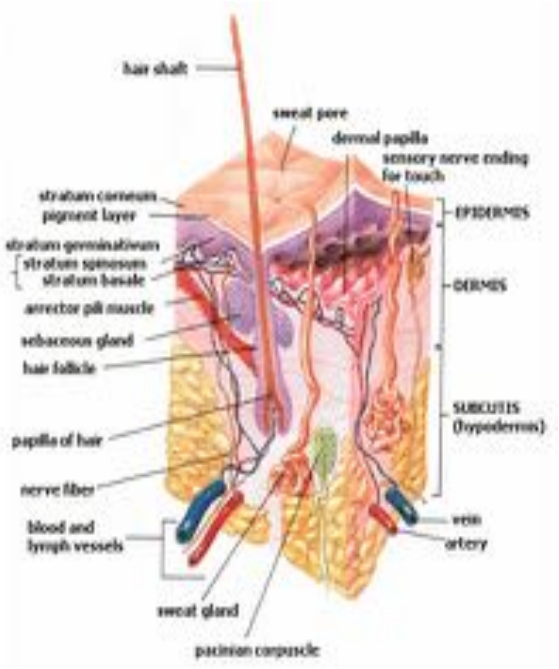
- Transition from formulation to homeostatic conditions
    - Physical stress due to change in pH
    - Transition through pI?
    - Change in concentration of stabilizing agents
    - Alteration in stabilizing agent concentration
    - Altered interactions with stabilizing agents

Possible Events After Injection



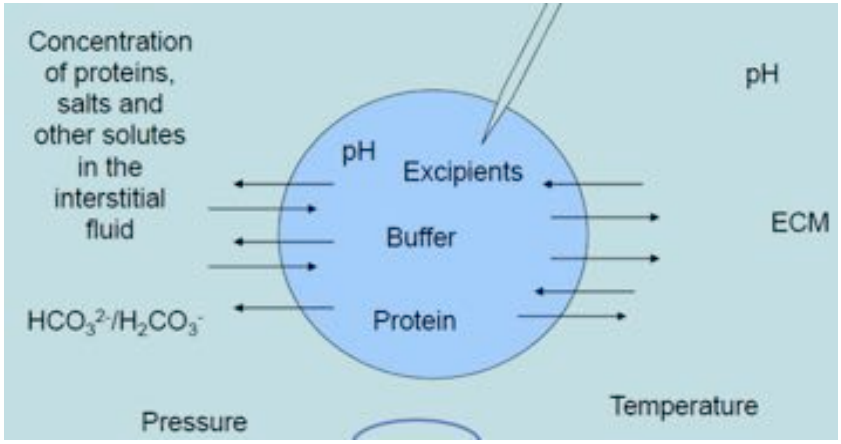
What We Know

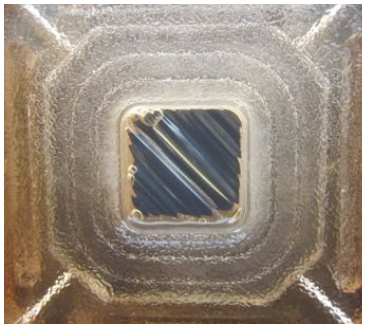
- Site to site and patient to patient variability is seen for bioavailability (%BA) outcomes.
- Some differences may be caused by pathological events and outcomes.
- No animal model correlates to (%BA) observed in man; %BA of human epoetin-β is 80% in dogs, 76% in rats, and 70% in mice but only 20–36% in man; interferon-α has 42% BA in dogs but 80% in man; recent work for mAbs with minipigs was not better.
- Conditions/characteristics of physical and chemical environments of the SC space are species specific.
- Insolubility/precipitation/binding upon injection can lead to cellular responses and clearance.

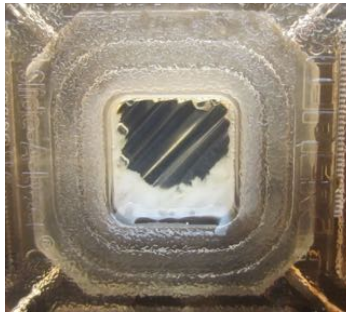


What We Need

- A tractable *in vitro* model to examine the potential impact of specific, individual post-injection events.
- A dynamic system that emulates approximate time & conditions for post-injection transitions.

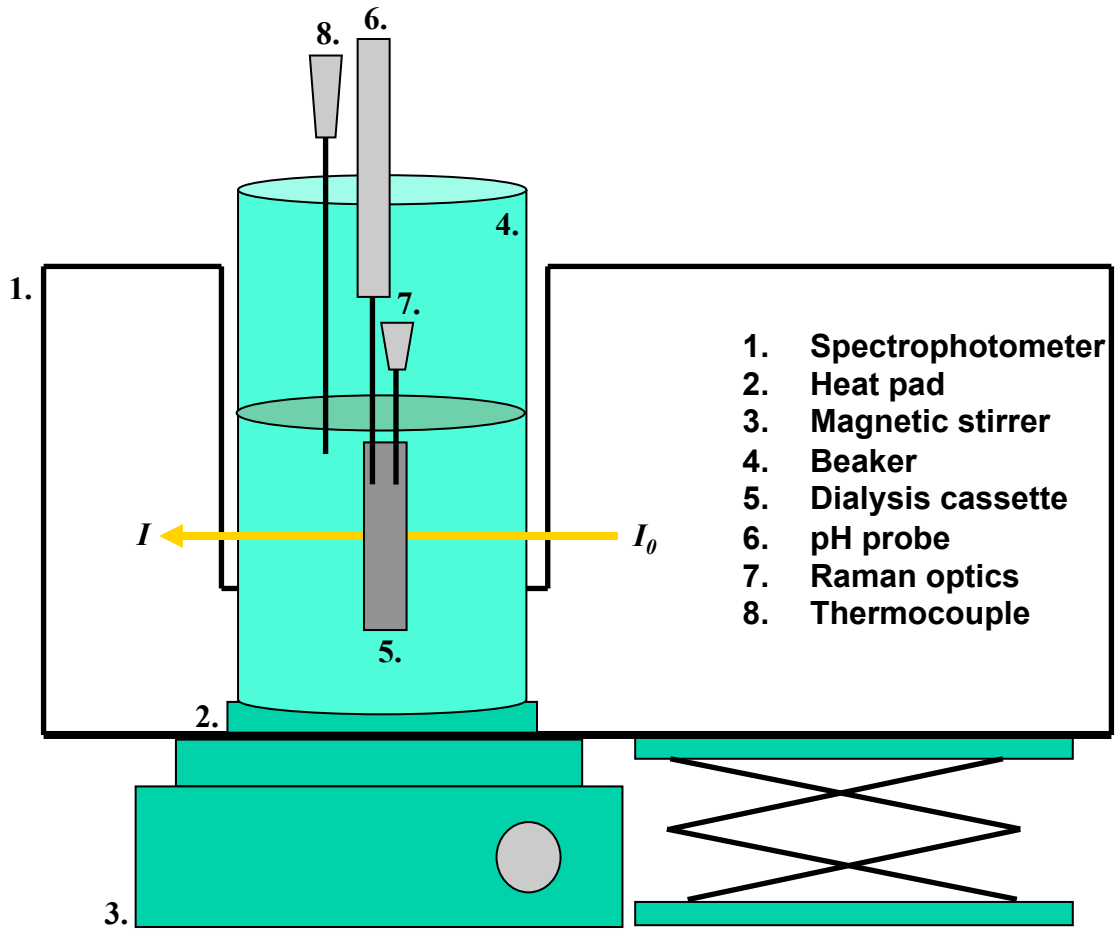


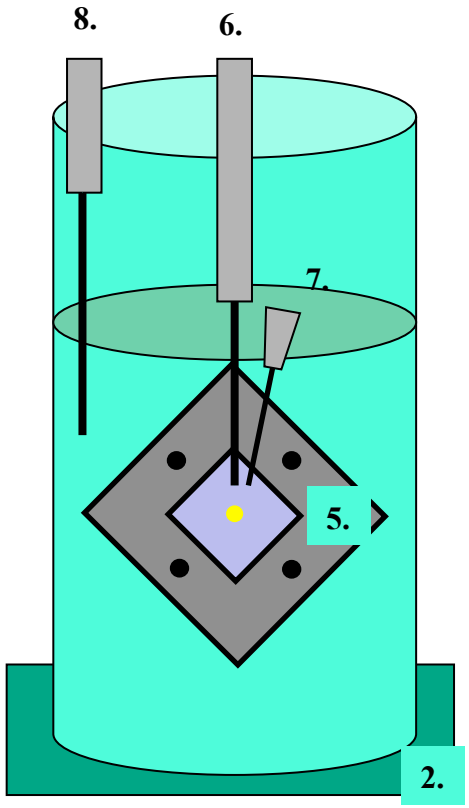




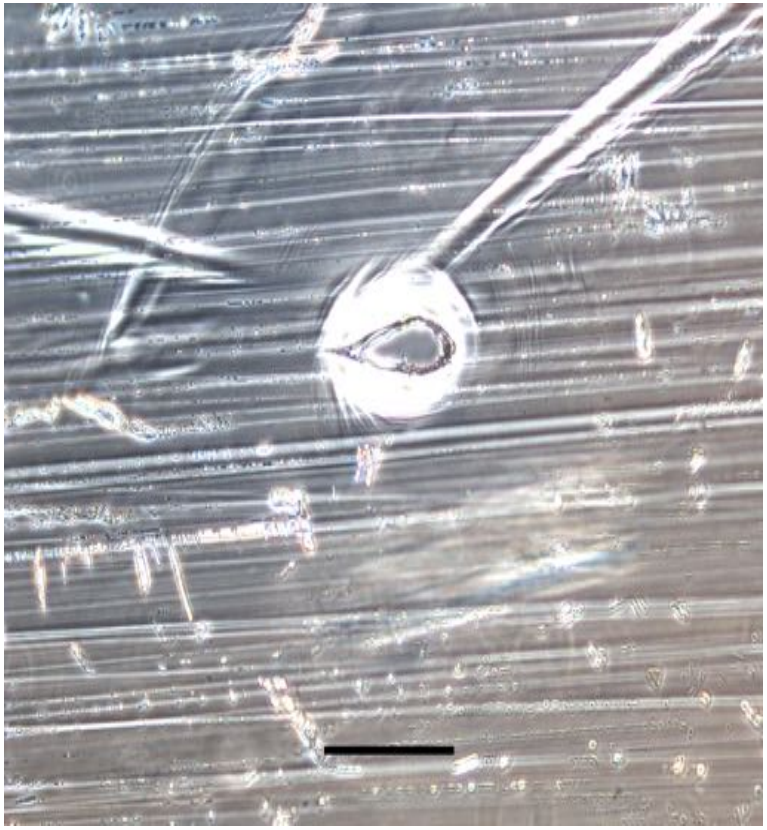
What We Did – Simple but Effective

A dialysis-based system emulates transition events.
Injection site environment can be monitored in real time.

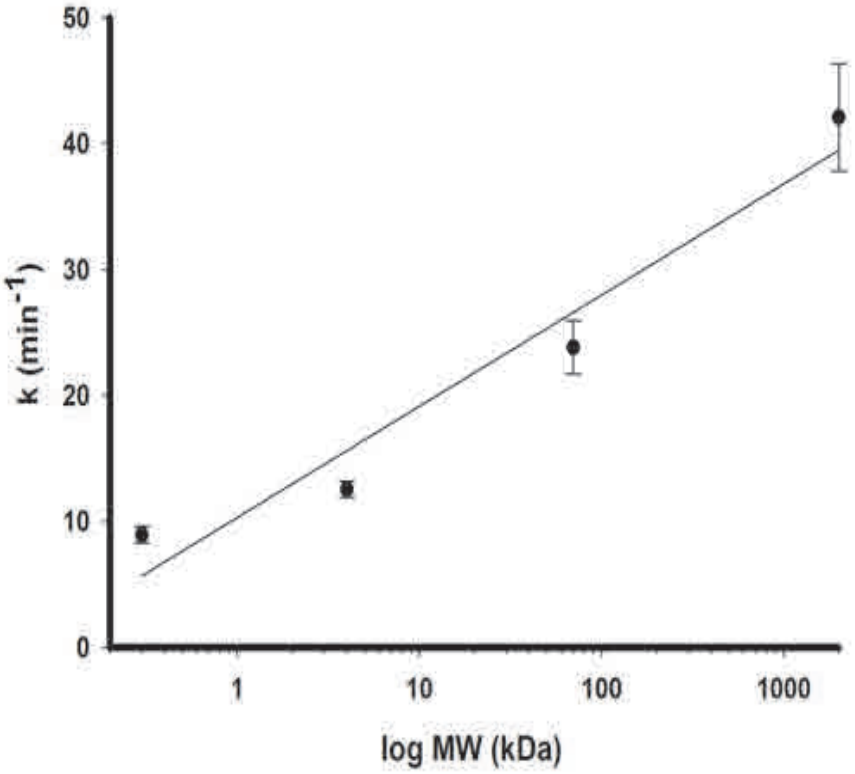




Injection Chamber Validation



Dermarolller-induced defect in 14 kDa MWCO dialysis membrane

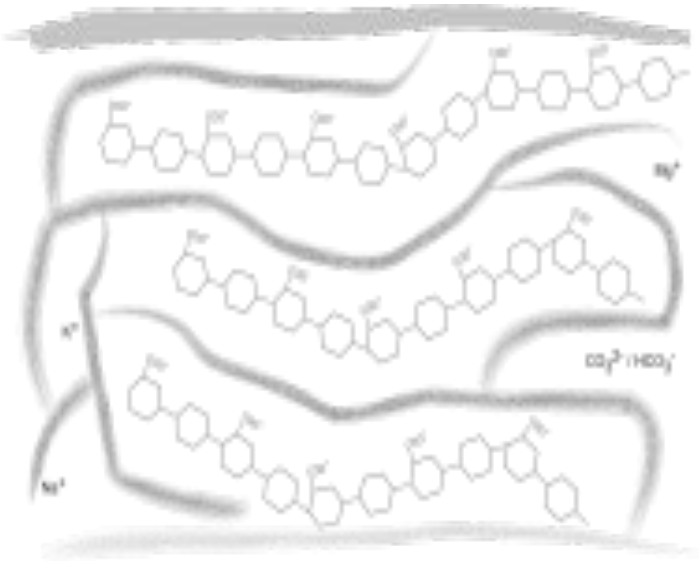


Permeability rates from injection chamber

The Extracellular Matrix (ECM)

Composed of an interlocking hydrogel

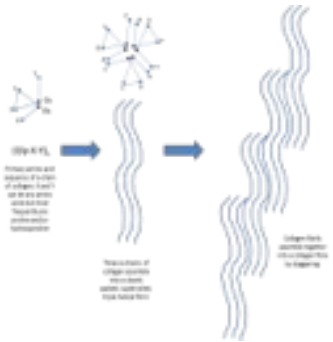
- Glycosaminoglycans
- Hyaluronic acid
- Collagens
- Fibronectin
- Elastin
- Laminins



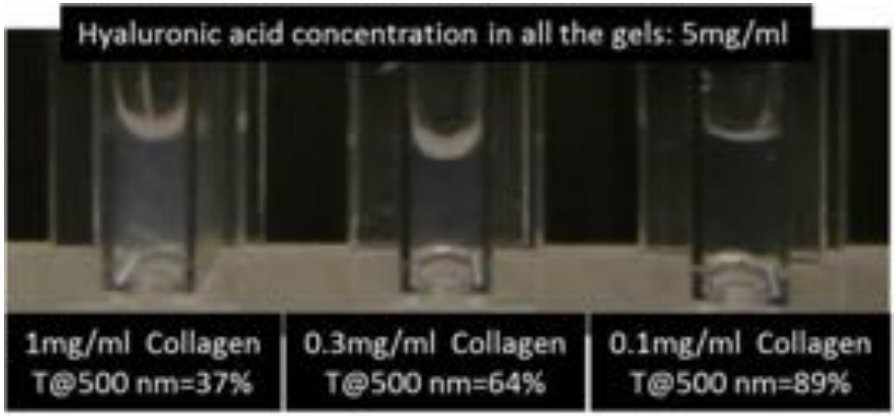
Presents a net negative charge

- Can trap and store growth factors

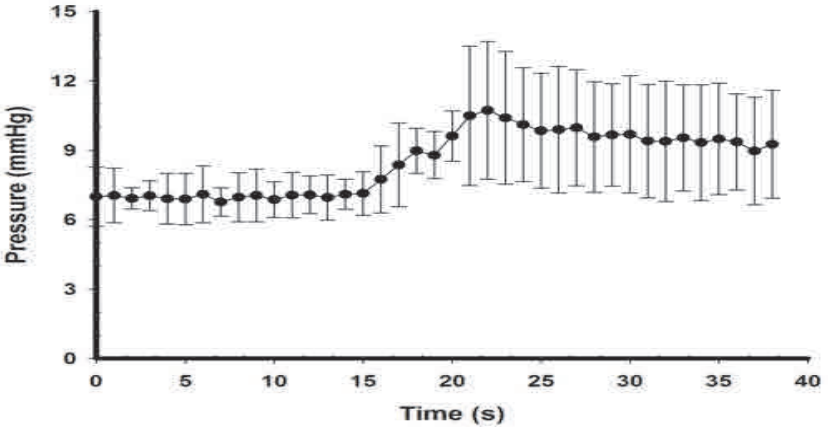
Establishes a hydration reservoir



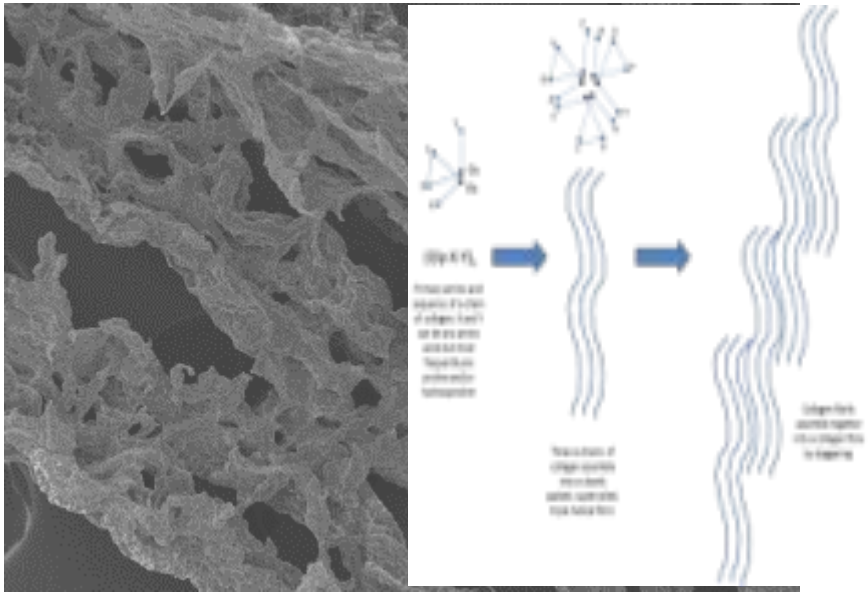
ECM Components Validation



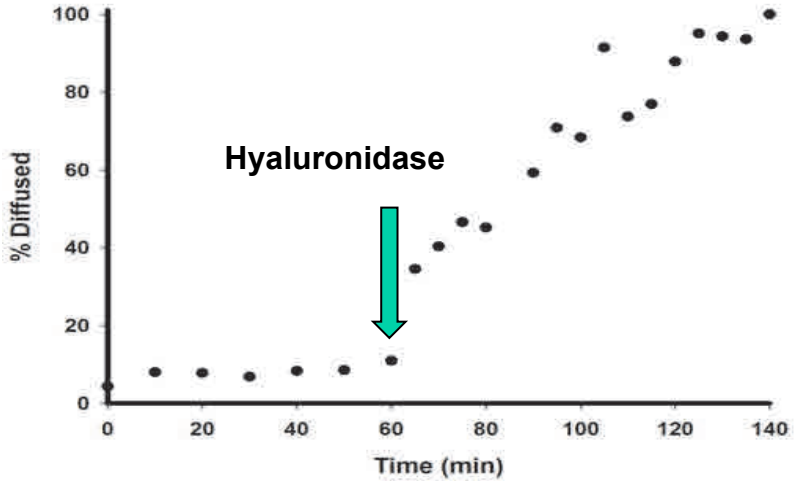
Optically clear matrix conditions



Pressure changes upon injection

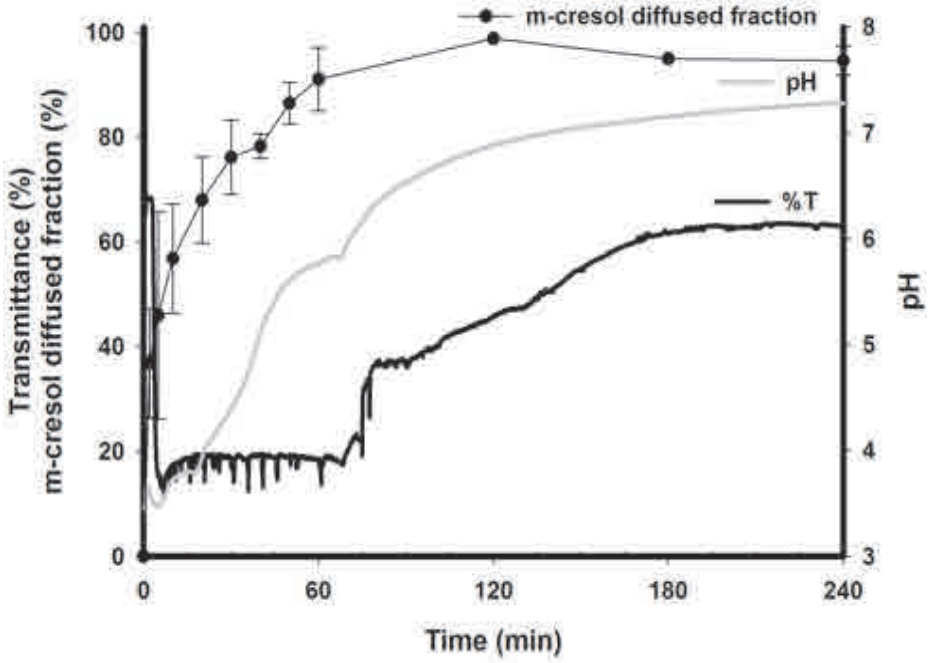


Appropriate matrix structure



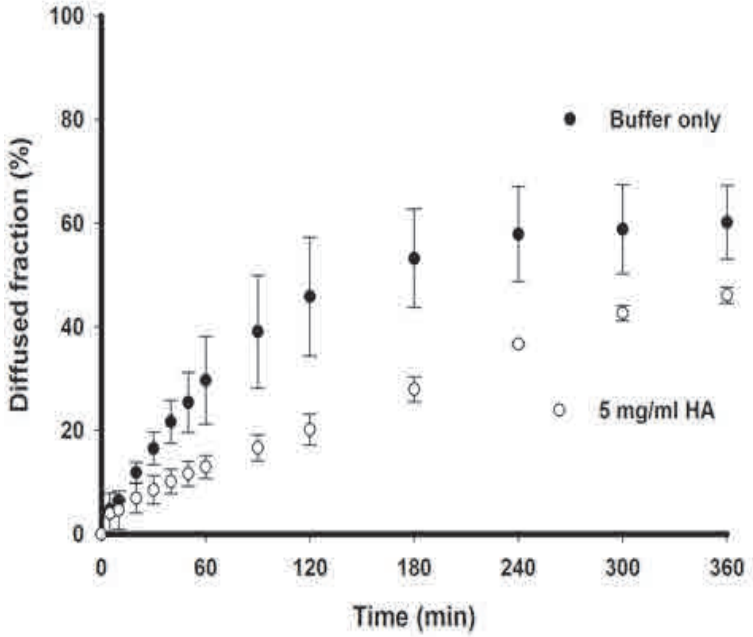
Loss of HA from injection chamber

Insulin Formulation Outcomes



Composite graph of real-time changes in pH, percent light transmission (%T) and extent of excipient observed in the infinite sink for a slow-release insulin formulation

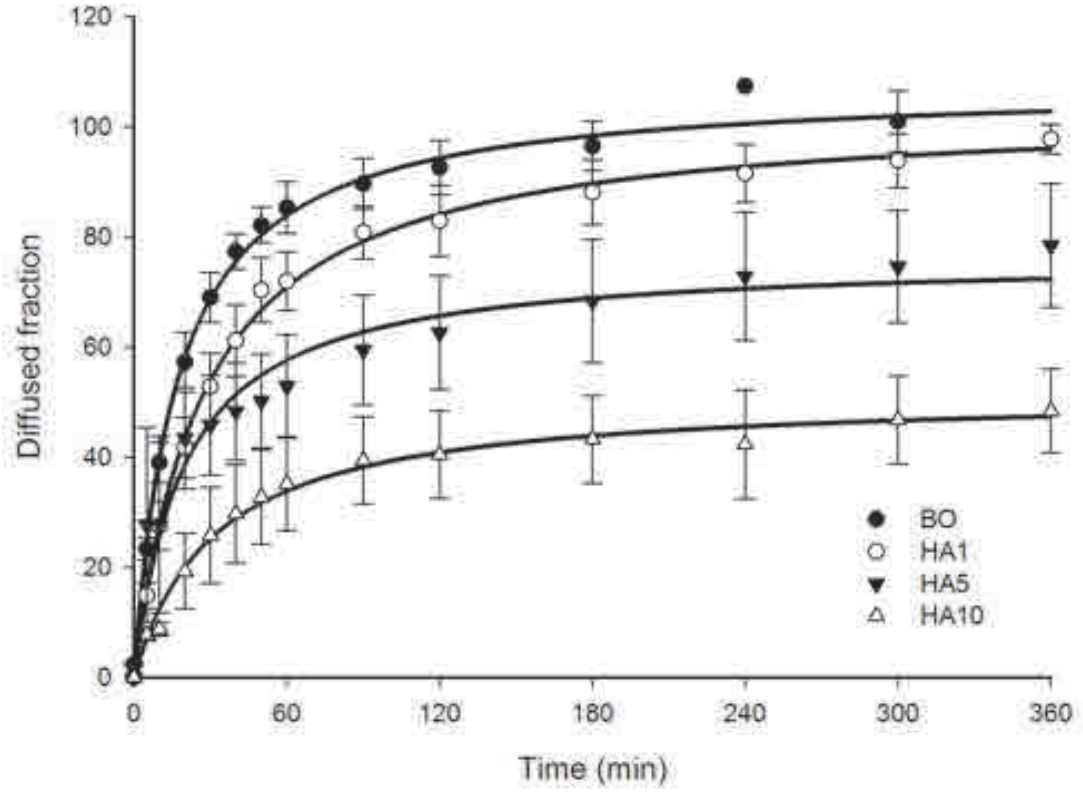
Rate, but not the extent, of insulin release from the injection chamber into the infinite sink is dependent upon ECM elements

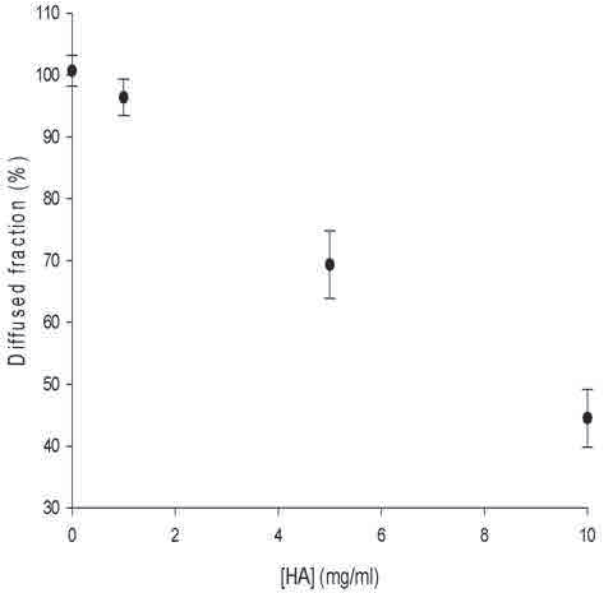


Four mAbs Obtained from Genentech

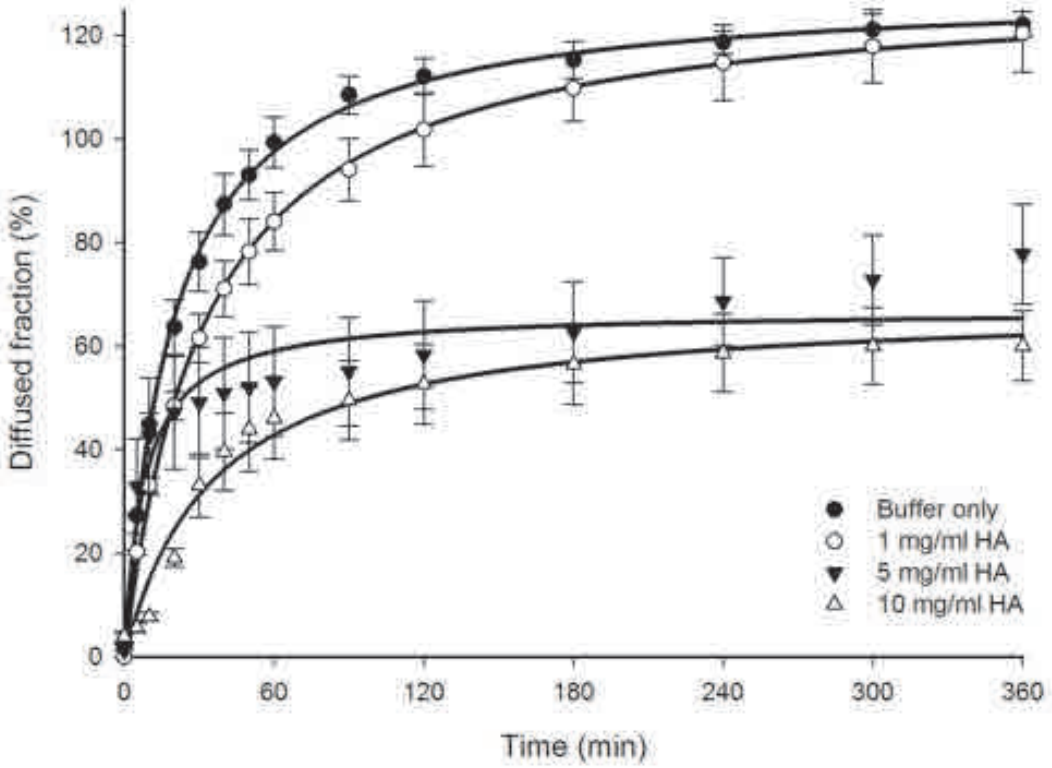
Name	[a]Isoelectric point (pI)	[b]Charge at pH 7.4	Concentration in mg/mL	Formulation and pH	Viscosity in centipoise
mAb T	9.1	+13	150	200 mM Arginine-based buffer, pH 5.5	5
mAb F	8.7	+9.0	150	200 mM Arginine-based buffer, pH 5.5	12
mAb 2	7.6	+1.0	125	30 mM Histidine-based buffer, pH 6.0	80
mAb L	6.1	-5.3	125	30 mM Histidine-based buffer, pH 5.7	7

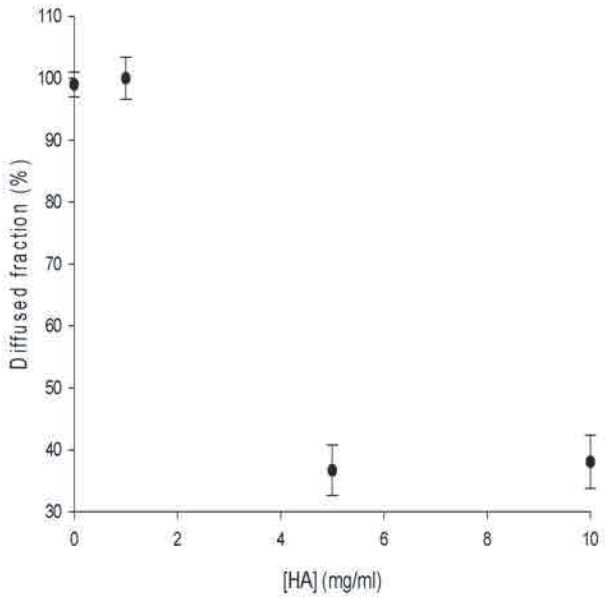
HA interactions for mAb T



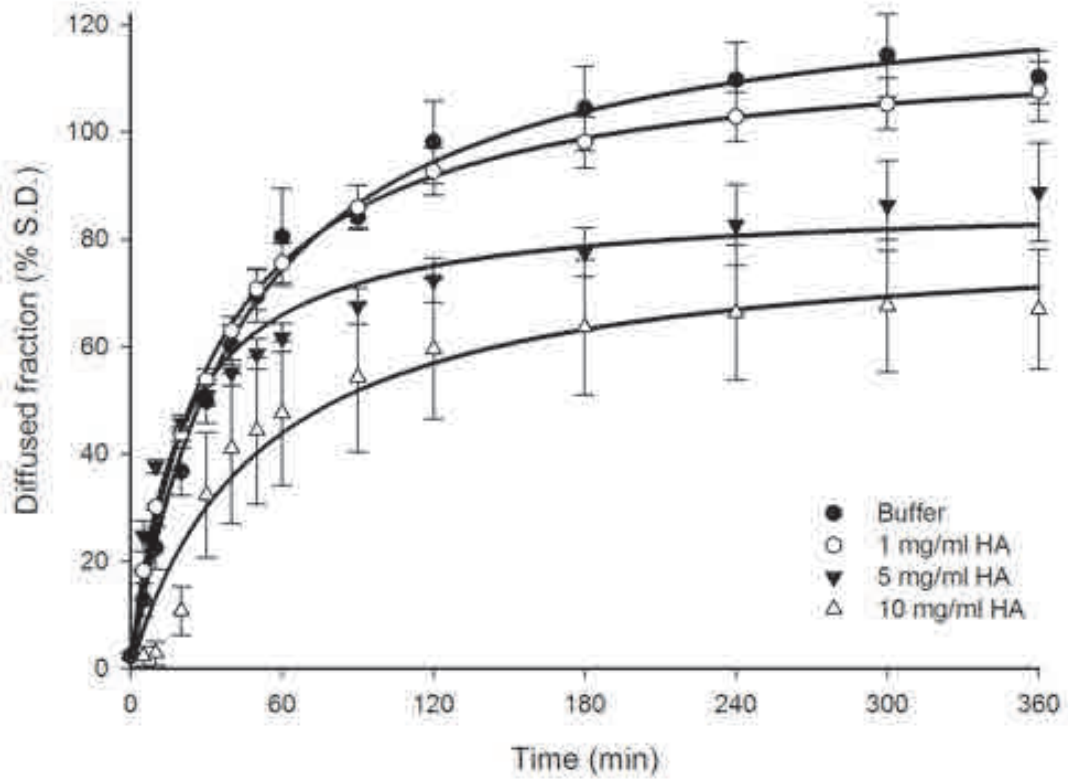


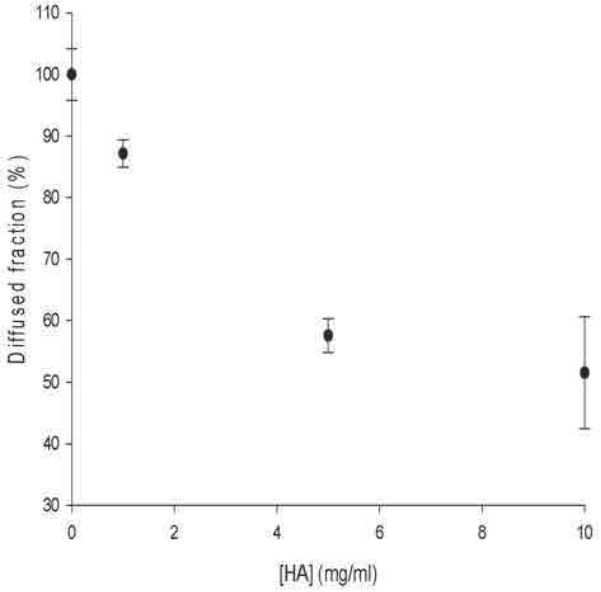
HA interactions for mAb F



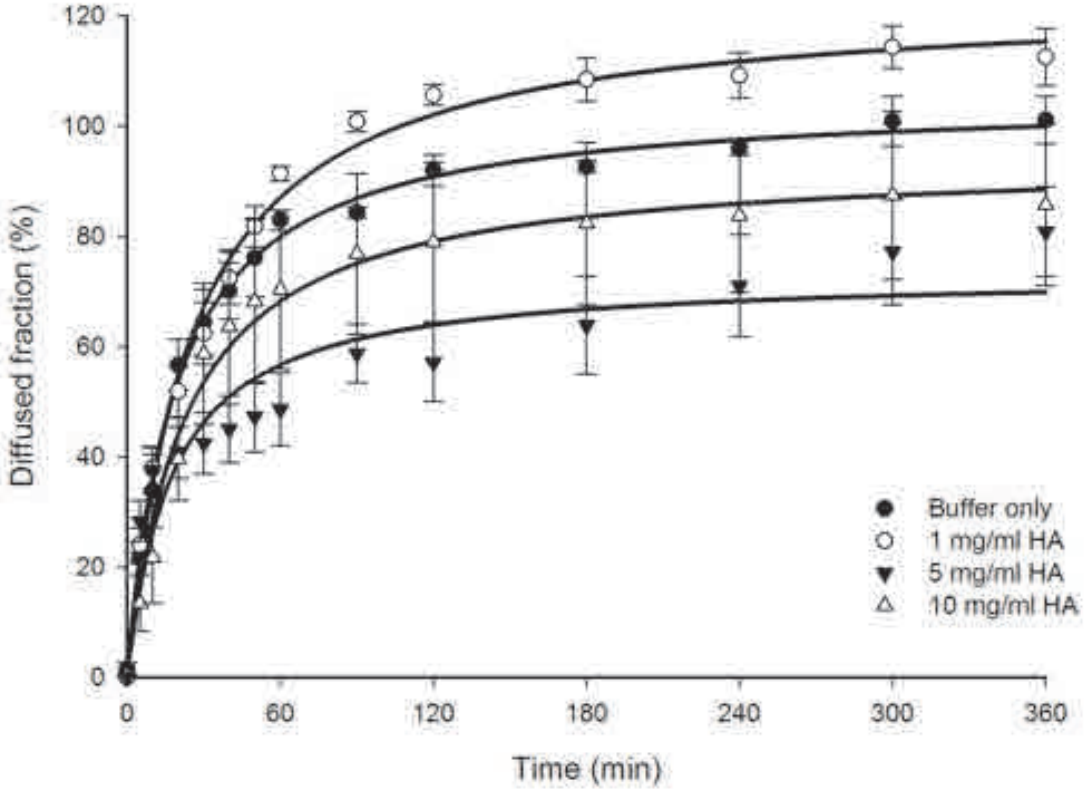


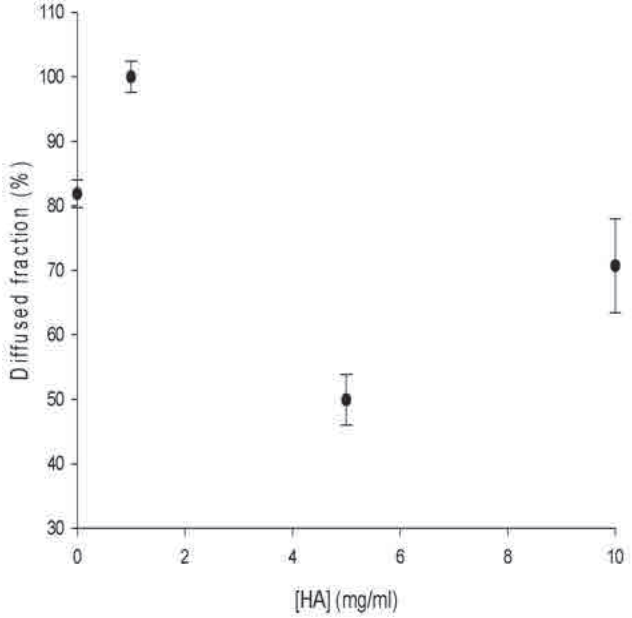
HA interactions for mAb 2



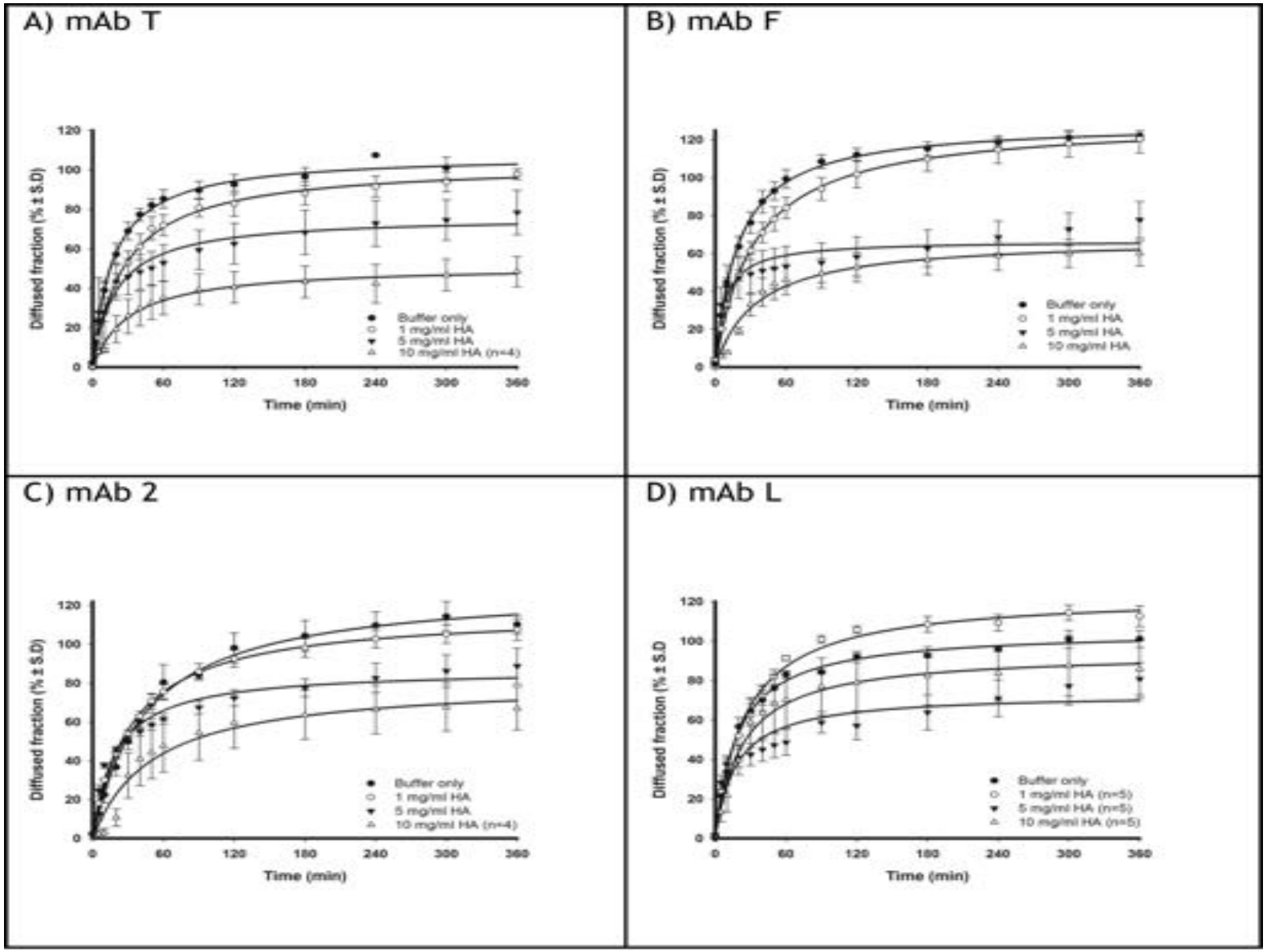


HA interactions for Ab L

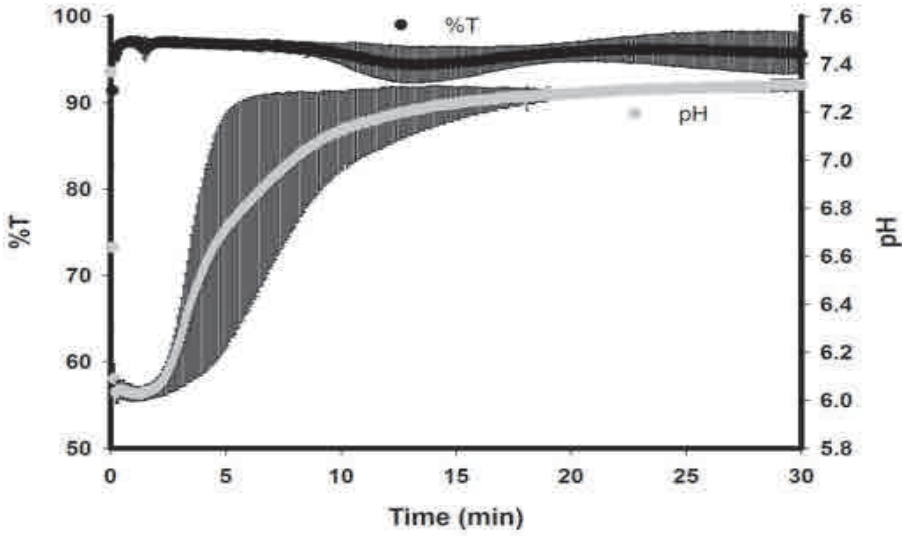




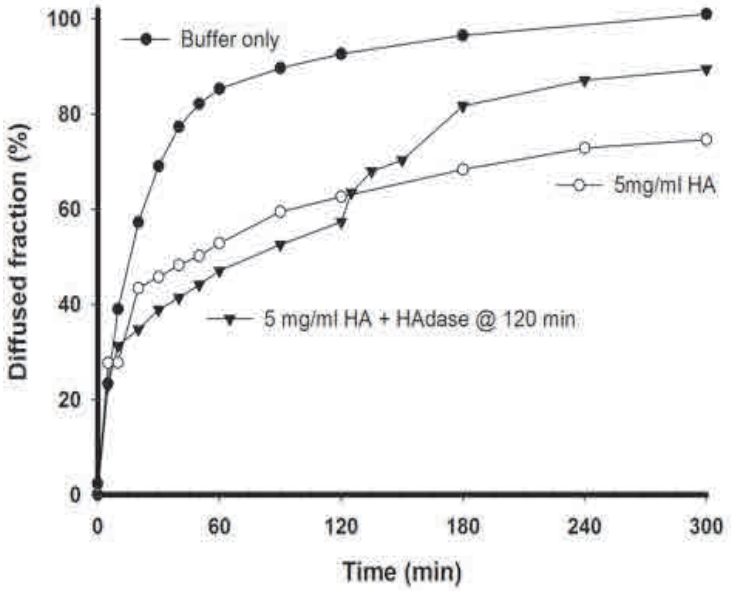
Comparison of mAb-HA Interactions



Events in the Injection Chamber

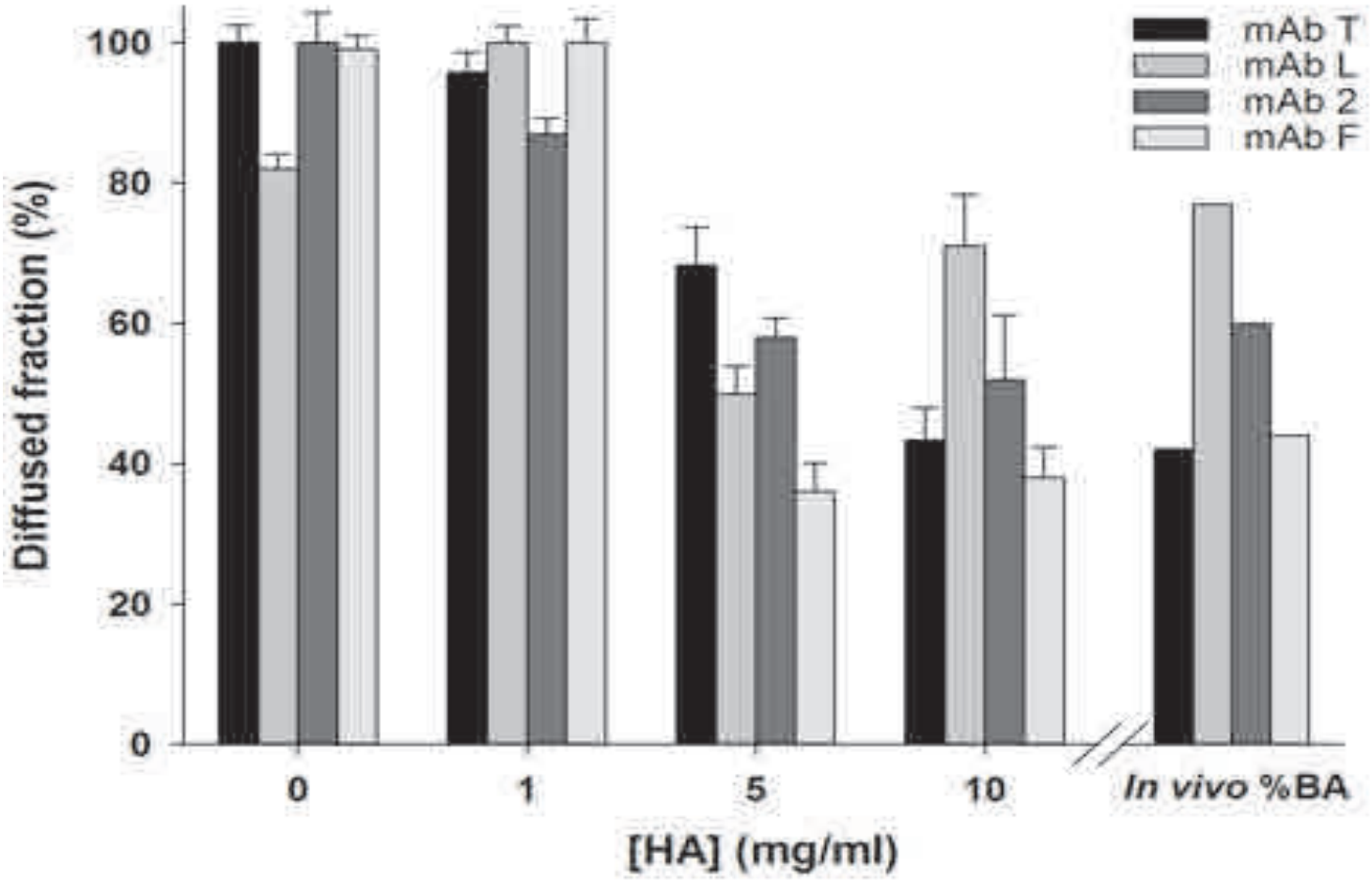


Composite graph of real-time changes in pH and percent light transmission (%T) for the mAb T formulation with 5 mg/mL HA in the injection cassette



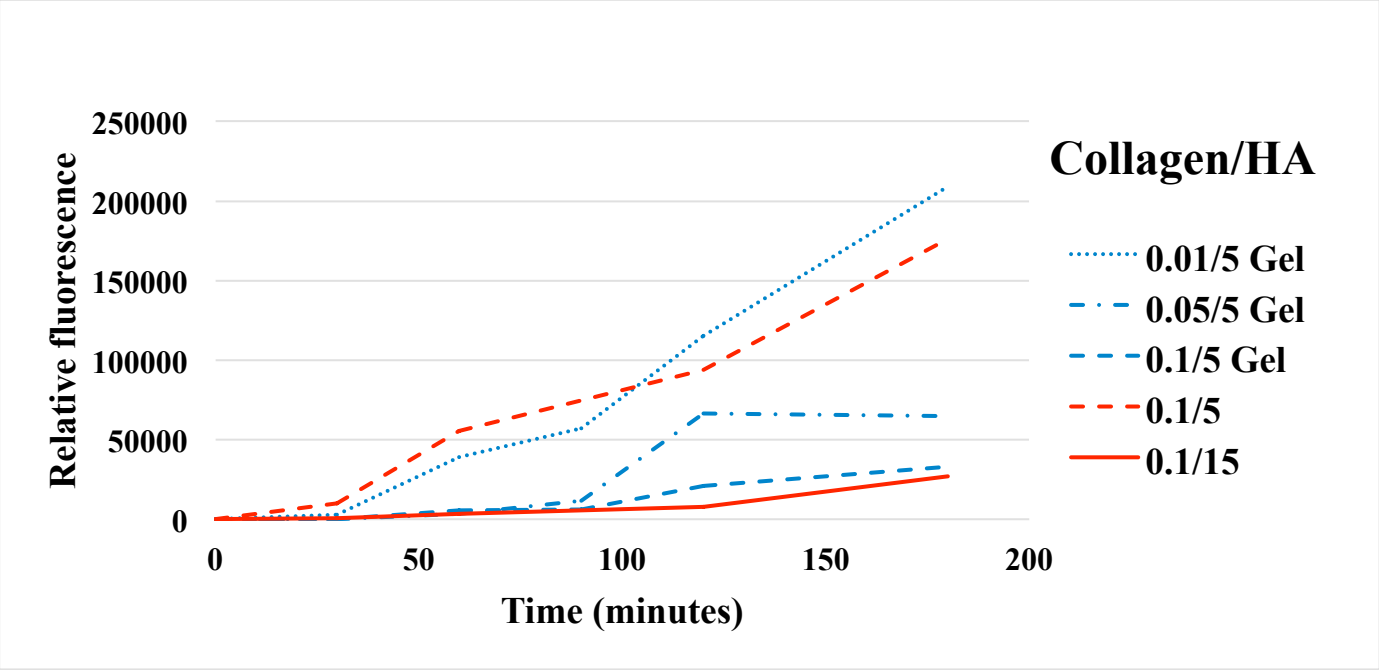
Addition of hyaluronidase at 2 h into the cassette containing 5 mg/mL HA increased the released fraction of mAb T

An Interesting Correlation

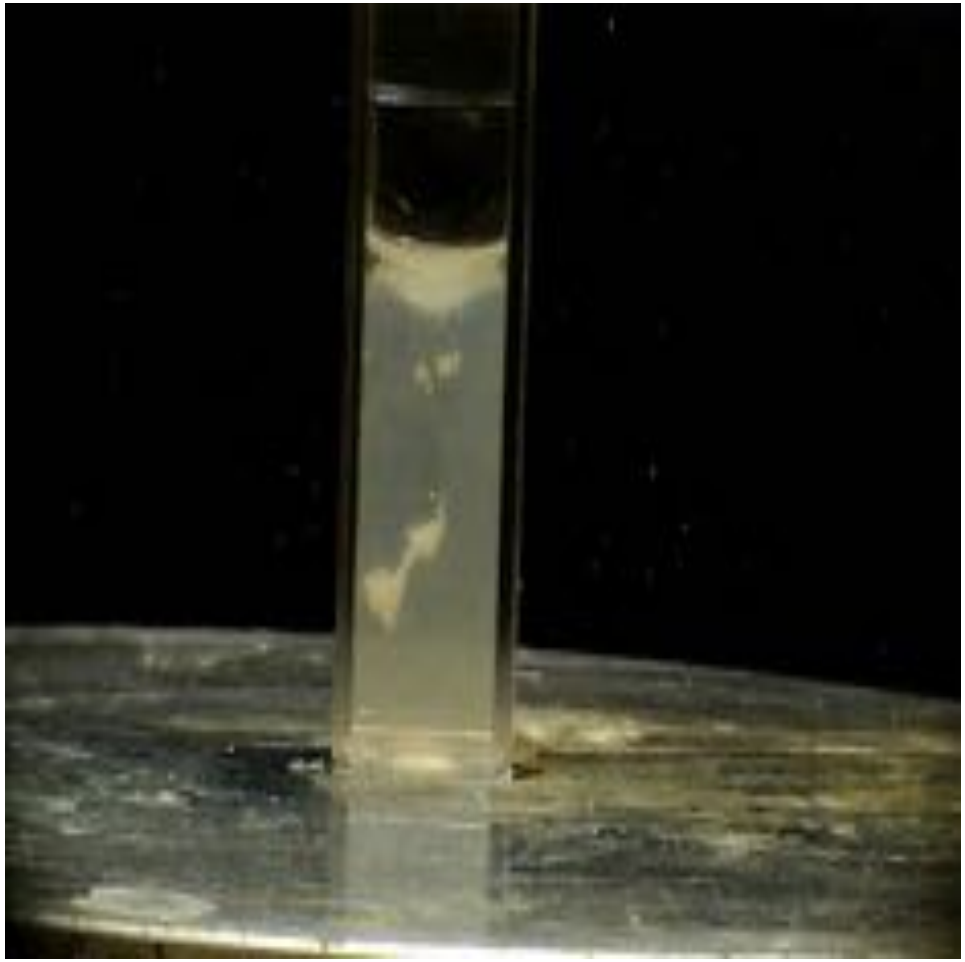


A Gelled System for Sustained Release

- ↑collagen and HA concentration ↓ dextran diffusion
- Solute diffusion was slower in gelled formats
- Dextran release from 0.1% collagen/ 5 mg/mL HA gel ≈ 0.1% collagen 15 mg/mL HA solution



Sustained release formulation injected into gelled matrix



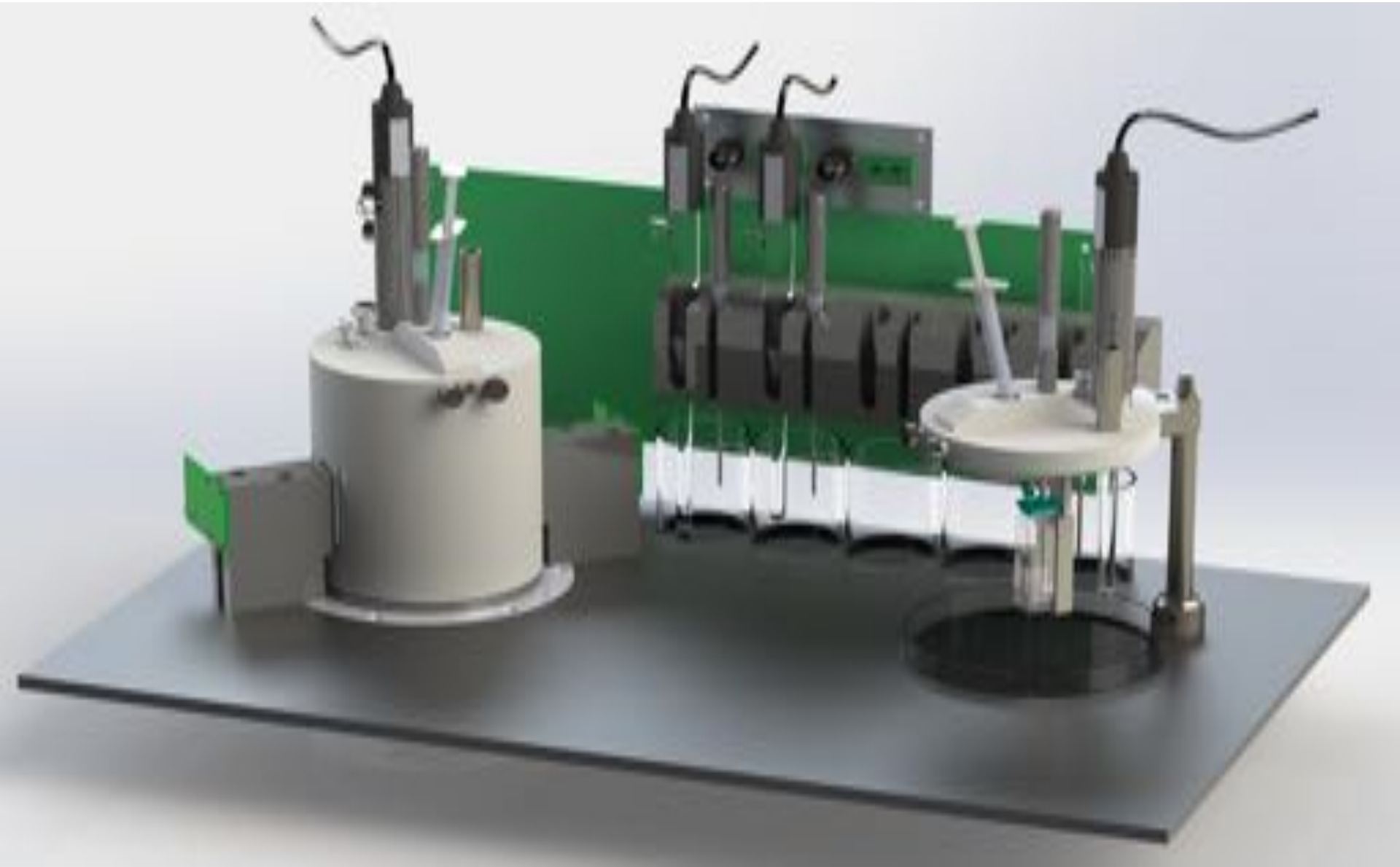
0.1% Collagen, 5 mg/mL HA gel format

Summary of Our Approach

- No animal model has been identified that will correlate with human *in vivo* outcomes – we have set up an *in vitro* model that simulates dynamic events occurring at the SC site after the injection of a biopharmaceutical.
- The model monitors ECM interactions, pH changes, protein turbidity, excipient concentrations and spectroscopic properties.
- The system is run over several hours at body temperature and under conditions that examine specific parameters for examination related to excipient-based actions on protein stability.
- Future studies will focus on correlation of this data with human *in vivo* outcomes.
- Our model does not examine cell-mediated or immune responses in the short (several hours) timeframe

What is Happening Now

Inside the breadbox



Those who made it happen

University of Bath
Hanne Kinnunen
Jenni Solbe
Alison Evans
Matt Young
Alistair Taverner

Sirius Analytial
John Comer
Karl Box
Rebecca Biloune
Brett Hughes

Genentech
Sreedhara Alavattam
Ann Daugherty
Stefan Fischer
Tom Patapoff
Vikas Sharma