APPLICATION NOTE

Evaluating the Impact of Hyaluronidase on Subcutaneous mAb Formulations Using the SCISSOR N3 Platform

Subcutaneous (SC) delivery of monoclonal antibodies (mAbs) is a desirable route of administration as drug developers seek alternatives to intravenous (IV) infusion. However, SC administration presents several challenges, including limited injection volumes, high local tissue viscosity, and variability in absorption kinetics.

Pion's SCISSOR N3

To overcome these constraints, hyaluronidase can be included in SC mAb formulations. Hyaluronidase degrades hyaluronic acid (HA), a major structural component of the extracellular matrix (ECM) within subcutaneous tissue. By temporarily breaking down hyaluronan, the enzyme acts as a spreading factor, reducing tissue resistance and viscosity at the injection site. This facilitates dispersion of larger injected boluses and enhances the movement of mAbs through the ECM.

In addition to enhanced dispersion, incorporation of hyaluronidase provides several important advantages in SC mAb delivery:

- **Increased Volume Capacity**: Larger injection volumes can be administered, which would otherwise be restricted by the ECM.
- **Improved Pharmacokinetics**: Reduced depot retention time at the injection site enables faster absorption and increased bioavailability, often approaching that achieved via IV administration.
- **Improved Patient Experience**: Faster administration and reduced injection site discomfort can lead to improved tolerability and convenience.

Despite the growing clinical adoption of hyaluronidase-enabled SC formulations, there remains a limited number of predictive *in vitro* tools capable of evaluating how these formulations behave at the injection site prior to *in vivo* studies. Understanding how hyaluronidase alters release kinetics, interactions, and physical stability in a bio-relevant environment can accelerate formulation

screening and de-risk development programs before they reach patient studies.

In this study we assess this use case with the SCISSOR N3™ (Subcutaneous Injection Site Simulator) platform and Rainbow™ spectrometer which are specialized for ranking and derisking formulations in a biorelevant, *in vitro* SC environment.

In this study, the SCISSOR N3 platform was used to evaluate the release behavior of a mAb formulation under bio-relevant SC conditions. The platform is a two-compartment system designed to simulate the SC injection site and subsequent uptake into systemic circulation.

- **Injection Site Simulator (Donor Compartment)**: The formulation is injected into a cartridge containing an artificial ECM, containing hyaluronic acid, designed to mimic the physical and biochemical environment of the SC space.
- **Sink Chamber (Acceptor Compartment)**: Following injection, the active pharmaceutical ingredient (API) diffuses from the Cartridge ECM into a larger outer chamber, representing transport toward blood and lymphatic uptake.

Quantification of API release into the outer SCISSOR chamber was performed using Rainbow, a UV–Vis spectrophotometer operating as a real-time, fiber-optic concentration monitoring device. The Rainbow enables continuous, non-destructive monitoring of API concentration throughout the experiment, allowing high temporal resolution release profiling.

Study Design and Formulations Evaluated

A blinded mAb formulation (mAbM) was evaluated under three conditions:

1. Standard formulation (no hyaluronidase), fast injection

2. Formulation containing hyaluronidase, slow injection

3. Standard formulation (no hyaluronidase), slow injection

All formulations were tested at a concentration of 150 mg/mL with a 1 mL injection volume into the cartridge, suspended in a chamber filled with 300mL bicarbonate buffer. The SCISSOR assay was conducted over 6 hours to best capture the full release profile of this immediate-release mAb under SC-like conditions.

Injection speed was varied to assess whether delivery rate had an impact on release. Rainbow measurements were acquired continuously to quantify API release into the outer chamber.

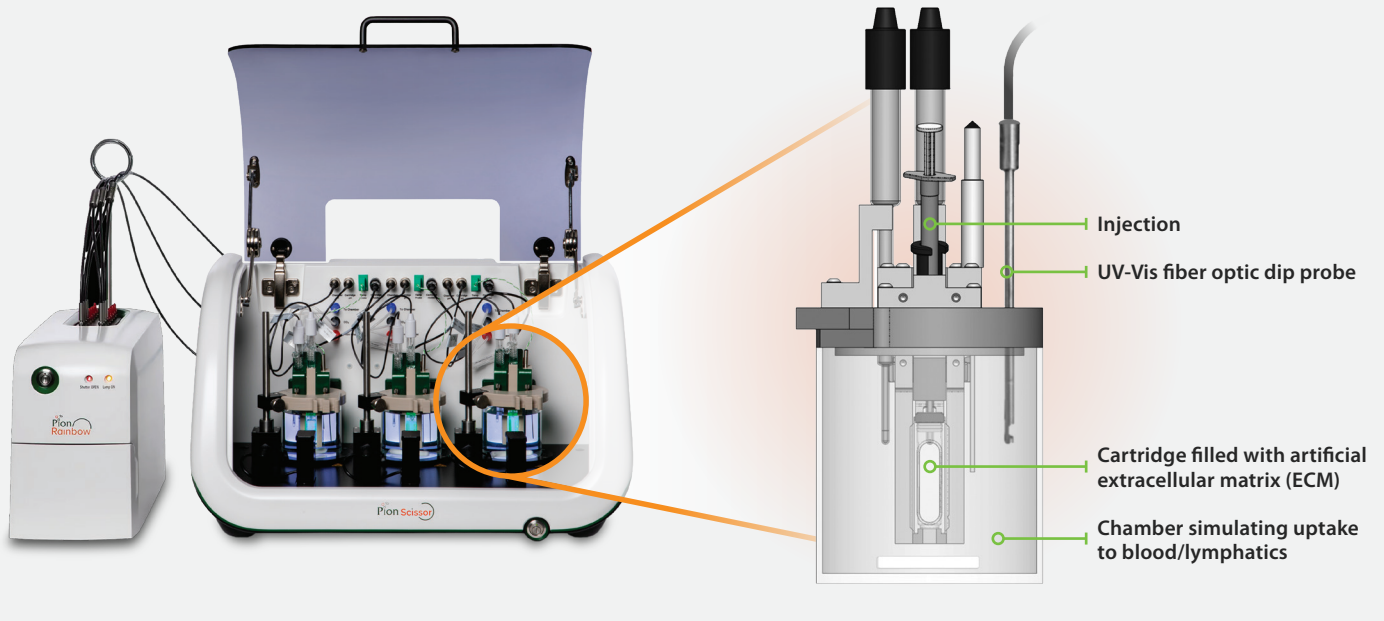

Figure 1. SCISSOR N3 system (center) with Rainbow spectrophotometer (left). Right: schematic of the SCISSOR N3 chamber showing the components and the internal placement of the Rainbow fiber-optic probe.

Formulation Behavior Upon Injection

Each formulation was supplied as a ready-to-inject solution and introduced directly into the SCISSOR cartridge. Upon injection, a sub-visible bolus was formed and observed to migrate through the cartridge to the lower region before releasing into the outer chamber. Importantly, no evidence of aggregation was observed in images collected immediately after injection or following 6 hours assay duration (Figure 2) indicating that the samples - both with and without hyaluronidase - were stable upon injection into the simulated SC environment.

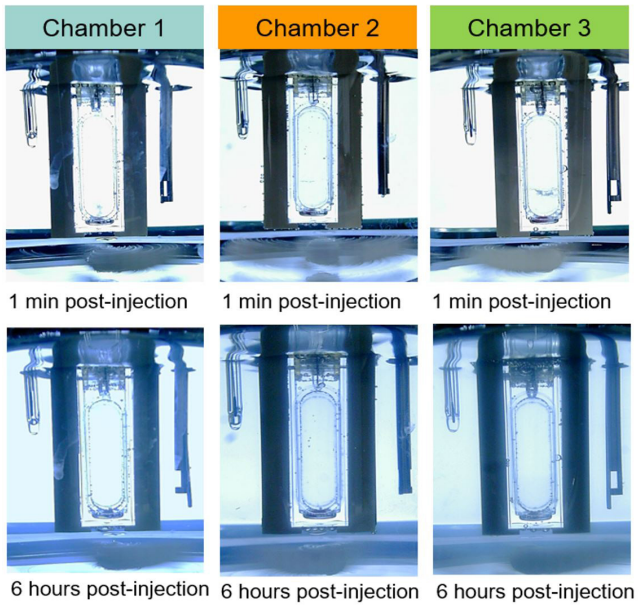

Figure 2. Representative images collected by SCISSOR during analysis of chamber 1 (mAb M, fast injection), chamber 2 (mAb M + hyaluronidase, slow injection) and chamber 3 (mAb M, slow injection).

API Release Kinetics and Rank Ordering

Release profiles generated by the Rainbow spectrophotometer revealed a clear distinction between formulations with and without hyaluronidase added as an excipient (Figure 3).

- Formulations **without hyaluronidase** exhibited slower and incomplete release from the ECM, consistent with diffusion-limited transport through an intact HA matrix.
- The **hyaluronidase-containing formulation** demonstrated rapid and near-complete release, indicating efficient degradation of the HA-based ECM and accelerated API transit into the outer chamber.

Complete release of the API from the SCISSOR cartridge was observed within minutes for the hyaluronidase-containing formulation, whereas formulations without hyaluronidase showed markedly slower kinetics over the same timeframe.

Importantly, injection speed (2 seconds vs. 10 seconds) had no measurable impact on release behavior, suggesting that formulation composition, rather than

	Injection speed	Formulation
Chamber 1	2 seconds	mAb M
Chamber 2	10 seconds	mAb M + Hyaluronidase
Chamber 3	10 seconds	mAb M

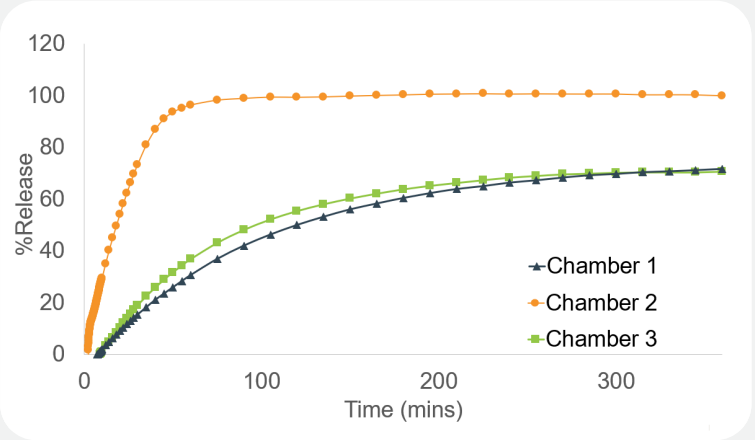

Figure 3. %Release curves generated by Rainbow showing rank order obtained with mAb M + Hyaluronidase releasing significantly faster than the two mAb samples with no Hyaluronidase.

delivery rate, is the dominant factor influencing SC release kinetics in this experiment.

Conclusions and Future Directions

These results demonstrate that the SCISSOR™ platform can:

- Differentiate formulations with and without hyaluronidase
- Correctly rank-order SC release kinetics
- Provide important information about injection site behavior
- Support excipient and formulation screening in a bio-relevant, *in vitro* system

For developers of SC mAbs, the SCISSOR N3 platform offers a practical approach to evaluating how formulation choices impact release behavior prior to animal or clinical studies.

While this study focused on formulation composition and injection speed, future work should evaluate the combined effects of injection volume and hyaluronidase concentration, as increased volume is a primary driver for incorporating hyaluronidase into SC products. Additionally, further investigation is needed to link the release performance observed in SCISSOR N3 for hyaluronidase-containing injections with human *in vivo* outcomes to assess translatability.

Why Use Pion

The SCISSOR technology, originally developed and validated primarily using mAbs, and now backed by 10+ years of experience in *in vitro* subcutaneous testing, is designed to mimic the human subcutaneous environment—enabling researchers to compare drug release kinetics and potential immunogenicity risk in a controlled setting. By providing insights into how a drug behaves post-injection, SCISSOR aids in optimizing formulations and predicting clinical outcomes without relying on animal testing.