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Artificial Extracellular Matrices as Tools for in vivo Injectable Formulation Analysis

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PURPOSE

Striking differences have been observed in measured bioavailability between pre-clinical models and human clinical outcomes following subcutaneous delivery, particularly in the case of biopharmaceutical drugs.[1] To this end, the subcutaneous injection site simulator (SCISSOR) was designed and validated to better mimic the human subcutaneous extracellular matrix (ECM) environment with a series of monoclonal antibodies.[2] To expand on this body of work we present data for a range of pharmaceutical motifs (small molecules, peptides, proteins, and monoclonal antibodies) examined in the SCISSOR with artificial ECMs.

METHOD(S)

Material Characterization of Artificial Extracellular Matrices

The rheological characteristics of the SCISSOR artificial extracellular matrix (ECM) and extended-release artificial extracellular matrix (ECM-XR) were analyzed before and after assay using an Anton-Paar® MCR102e rheometer. ECM and ECM-XRs were deposited between 25mm parallel plates and frequency scans were conducted from 100-0.01 s^{-1} at 1% strain.

SCISSOR assay of therapeutics

Multiple formulations including caffeine, insulin, denosumab, and superpositive (+) and supernegative (-) green fluorescent protein (GFP) were analyzed using the SCISSOR® system. Concentrations of API in the receiving chambers were monitored in real-time using *in situ* fiber optic dip probes connected to the Rainbow® UV-Vis spectrometer (Pion Inc.) or offline analysis was carried out with an Agilent A1100 HPLC after sampling.

Figure 1. The Pion SCISSOR® (right) and Rainbow® R6 (left) systems were used to monitor *in situ* release of a formulation.

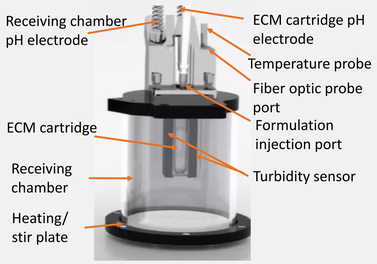

Figure 2. The Pion SCISSOR® chamber assembly labelled with tools and functionalities.

OBJECTIVE: SCISSOR can accurately predict relative *in vivo* pharmacokinetic behavior of subcutaneously injected pharmaceuticals over 1 week with use of the ECM-XR

RESULT(S)

ECM & ECM-XR Analysis

After >100 hour assays the ECM showed a ~99% loss in complex viscosity, from 1.99 ± 0.7 to 0.004 ± 0.001 Pa·s, while the ECM-XR conserved viscosity with a drop from 2.0 ± 0.1 to 0.6 ± 0.4 Pa·s. The conserved viscosity will allow for longer analysis within SCISSOR.

Table 1. ECM and ECM-XR rheological parameters before and after >100 hour assays, 1% strain @ 0.9 s^{-1}. N=4 or 5 for the pre- and post-assay measurements, respectively.

Prototype	Complex Viscosity (Pa·s)	Storage Modulus (Pa)	Loss Modulus (Pa)
ECM	1.8 ± 0.3	0.45 ± 0.8	1.6 ± 0.3
ECM-XR	3.3 ± 0.3	2.9 ± 0.3	0.4 ± 0.1

≥100 hours

Prototype	Complex Viscosity (Pa·s)	Storage Modulus (Pa)	Loss Modulus (Pa)
ECM	0.005 ± 0.002	0.002 ± 0.002	0.004 ± 0.002
ECM-XR	0.6 ± 0.1	0.5 ± 0.1	0.16 ± 0.08

→ **ECM-XR conserved storage modulus through assay**

Caffeine

Caffeine release profiles were collected using the ECM and ECM-XR (50uL injection, N=3). The caffeine release from both models indicated complete release at 24 hours, with 1 trial exhibiting early release.

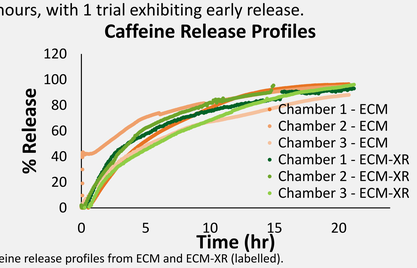

Figure 3. Caffeine release profiles from ECM and ECM-XR (labelled).

Superpositive (+) Green Fluorescent Protein

Superpositive GFP was injected into the ECM and ECM-XR to evaluate non-specific release. +GFP had 10% release over 15 hours in the ECM, while the ECM-XR released 0%. **The ECM-XR demonstrated correct electrostatic complexing of the +GFP, inhibiting release.**

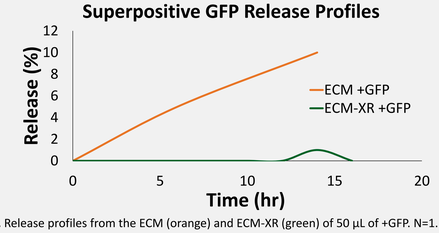

Figure 4. Release profiles from the ECM (orange) and ECM-XR (green) of 50 µL of +GFP. N=1.

Supernegative (-) Green Fluorescent Protein

Supernegative GFP was injected into the ECM and ECM-XR to investigate the non-specific release of oppositely charged GFP. -GFP had 100% release over 20 hours within the ECM, while the ECM-XR plateaued at 25% release at 5 hours. The **ECM-XR's material properties resulted in incomplete release.**

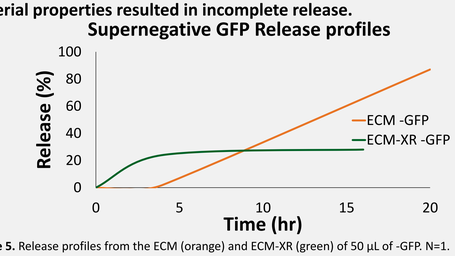

Figure 5. Release profiles from the ECM (orange) and ECM-XR (green) of 50 µL of -GFP. N=1.

→ **The ECM-XR allowed for prolonged interactability post-injection in both experiments (+/- GFP).**

Denosumab

Release of denosumab injections (50 uL, N=3) were monitored over 1 week. ECM injections released to 100% within 72 ± 6 hrs, where the ECM-XR release plateaued at 73 ± 10% at 160 hours.

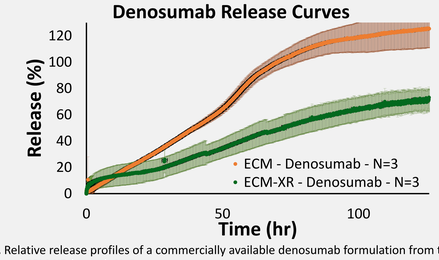

Figure 6. Relative release profiles of a commercially available denosumab formulation from the ECM (orange) and ECM-XR (green). N=3.

Insulin

Rapid and basal insulin (50 uL, N=2) were injected into the ECM and ECM-XR and monitored over 4 days. In both the ECM and ECM-XR, rapid insulin reached 100% release in < 1 day. Basal insulin plateaued at <20% release over the duration of the experiment.

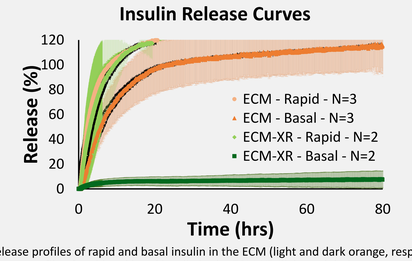

Figure 7. Release profiles of rapid and basal insulin in the ECM (light and dark orange, respectively) along with the release profile of rapid and basal insulin in the ECM-XR (light and dark green, respectively).

SCISSOR GFP Injections

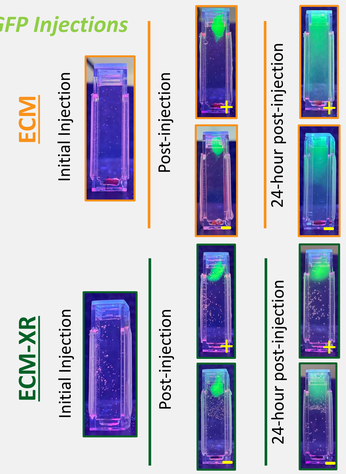

Figure 8. Representative images of -/+ GFP (labelled) diffusing through the ECM (orange) and ECM-XR (green) at pre-, 1 hour, and 24 hours post-injection.

CONCLUSION(S)

Two artificial ECMs (ECM & ECM-XR) were evaluated using 5 model injectables within Pion's subcutaneous injection site simulator (SCISSOR). The release profiles were compared to show how each model released over smaller time scales, while the **ECM-XR could sustain release over 1 week**.

Caffeine injections into each model showed similar behavior over short time scales for both the ECM and ECM-XR, **demonstrating the ECM-XR's analogous behavior in shorter release studies**.

Injections of +/- GFP showed that the ECM-XR could appropriately complex +GFP for days, as opposed to the ECM release over the same time frame, **demonstrating the expanded stability of the ECM-XR**.

Lastly, commercially available formulations of insulin analogs and denosumab were injected to **elucidate peptide and monoclonal antibody release behavior within the ECM-XR**.

In conclusion, although both the ECM and ECM-XR correctly model the environment of the human subcutaneous space, the ECM-XR demonstrated fitness for prolonged release profiles – up to 1 week.

1.Kinnunen, H. M., Sharma, V., Contreras-Rojas, L. R., Yu, Y., Alleman, C., Sreedhara, A., . . . Mrsny, R. J. (2015). A novel in vitro method to model the fate of subcutaneously administered biopharmaceuticals and associated formulation components. *Journal of Controlled Release*, 94-102.

2.Bown, H. K., Bonn, C., Yohe, S., Yadav, D. B., Patapoff, T. W., Daugherty, A., & Mrsny, R. J. (2018). In vitro model for predicting bioavailability of subcutaneously injected monoclonal antibodies. *J Control Release*, 13-20.