Human Collagen's Role within Artificial Extracellular Matrices used for in vivo Analysis of Injectable Formulations

Probing how material characteristics are affected by the integration of collagen and how this integration can achieve a more biomimetic response from biologic injections within the Scissor *in vitro* platform

BACKGROUND

No preclinical animal model has been identified that can predict pharmacokinetic properties of biopharmaceutical formulations intended for subcutaneous (SC) injection in man.1 To fill this gap, in vitro tools have been developed to investigate distinct events that can affect the uptake properties of a material from the SC injection site to reach systemic circulation, an overall outcome that defines its bioavailability. Typically, these methods of analysis employ a membrane, hydrogel, and/or cellular components to mimic the in vivo environment, chemically discriminating between the diffusion of a formulation's components and/or between formulations.2 We have developed an in vitro method to monitor the release of injectable pharmaceutical following injection into a dialysis membrane encased glycosaminoglycan-based hydrogel.1,4 Herein, we look to probe the influence collagen (type I, II, III @ 0, 0.05, and 0.1 mg/mL) has on the material and chemical characteristics that dictate the in vitro bioavailability responses of previously described artificial extracellular matrices (ECMs). Here, we examine the influence collagen (type I, II, III) has on viscoelastic properties of the hydrogel format and compare collagen's impact on drug release properties in this in vitro model of the SC injection site.

METHOD

The rheological characteristics of the novel and previously described artificial extracellular matrices1,3 were analyzed using an Anton-Paar® MCR102e rheometer. Samples were deposited between 25mm parallel plates. frequency scans were conducted from 100-0.01 s-1 at 1% strain and time-dependent responses were taken at 1 s-1 at 1% strain for 2 hours in a hydrating environment. All samples were analyzed in an environment where 2-dimensional diffusion off of the sample stage was allowed.

Multiple formulations including caffeine, rapid & basal insulins, and denosumab were analyzed using the SCISSOR® system (Pion Inc.), seen in figure 1.1,3,4 Concentrations of injectates 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.

RESULTS

Optical Density

Spectroscopic analysis showed that ECM with 0.1 mg/mL collagen type I had an optical density of 0.014 ± 0.016 absorbance units (AU), whereas ECM with 0.1 mg/mL collagen type II was 0.006 ± 0.010 AU, compared with a baseline of -0.002 ± 0.002 AU for ECM with no collagen.

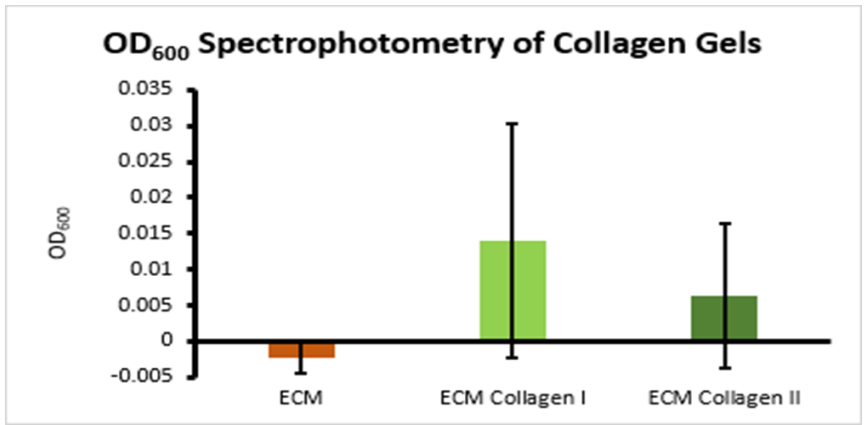

Figure 1. Optical density of the ECM (orange), ECM-Col1 (light green) and ECM-Col2 (dark green)

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Material Characteristics

Rheometric analysis of fresh ECMs showed no significant difference between the ECM with 0 mg/ml collagen and 0.1 mg/mL ECM-ColF (ECM with collagen from human fibroblast, mix of type I and III) complex viscosity during a frequency scan @ 1% strain, while 0.1 mg/mL ECM-ColF showed a more viscous ($p>0.05$), and more stable behavior. After ≥100 hour assays ECM showed a ~99% loss in complex viscosity @ ~1 Hz, from 0.74 ± 0.01 to 0.004 ± 0.001 Pa·s. The addition of 0.05 and 0.1 mg/mL collagen resulted in a viscosity loss of 0.72 ± 0.01 to 0.013 ± 0.004 Pa·s (data not shown), and 0.76 ± 0.01 to 0.035 ± 0.04 Pa·s, respectively.

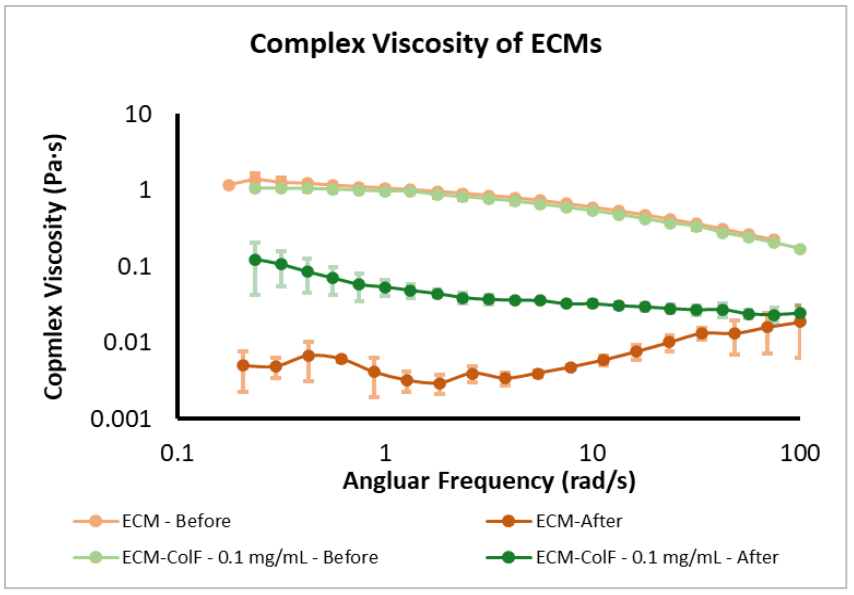

Figure 2. Frequency scans @ 1% strain of ECM with no collagen before (light orange) and after (dark orange), and ECM-ColF samples before (light green) and after (dark green) SCISSOR assay lasting ≥ 100 hours. N=3

Insulin Injections

Rapid and basal insulin (50-200uL) were injected and monitored over 4 days. 0.05 mg/ml ECM-ColF allowed rapid insulin to reach a maximum of 73 ± 2% release within 12 hours, while basal insulin plateaued at 67 ± 9% release over the duration of the experiment. The ECM with no collagen had complete release of rapid insulin within 20 hours. The presence of collagen appears to result in a more biomimetic degradation of both formulations, as aggregation and/or degradation occurred after release. Basal insulin also exhibited a more stable complex after release from the ECM-ColF.

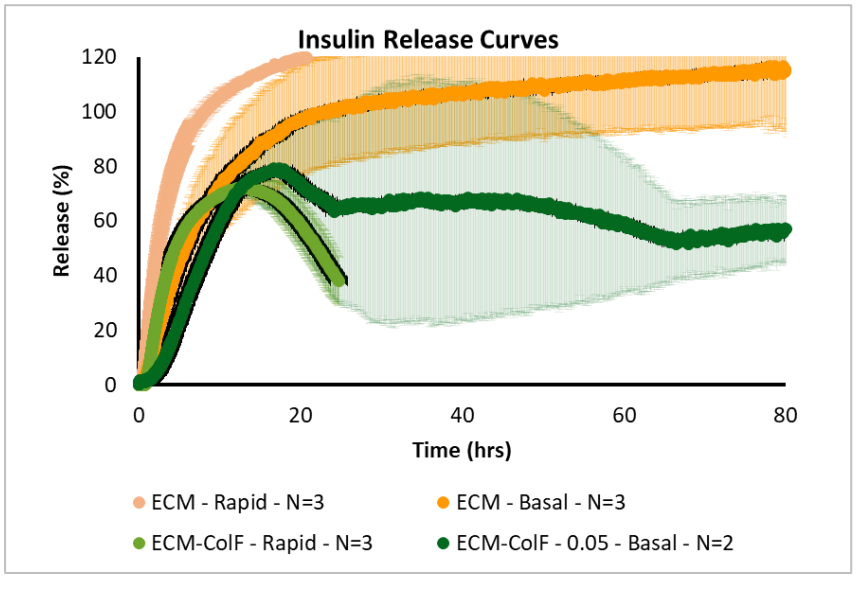

Figure 3. Release profiles resulting from the injection of 200µL of rapid (light) and basal (dark) insulin into the ECM with no collagen (green) and ECM-ColF with 0.05 mg/mL ColF (orange)

Caffeine Injections

Caffeine release profiles were collected using all variations of artificial ECMs (N=3). The caffeine release from all models indicated complete release at 24 hours. The single trial which displays early release (orange) still approaches the maximum in the same timeframe. The addition of 0.1 mg/mL collagen increases the lag time and delays complete release by ~20%.

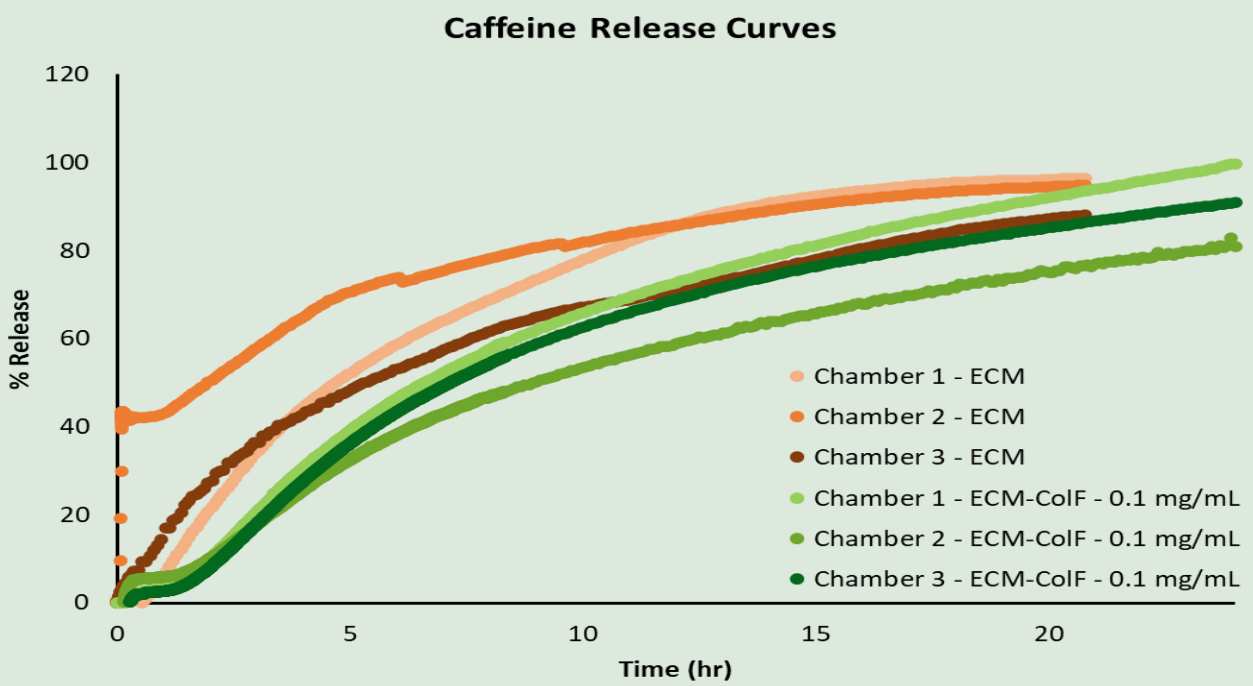

Figure 4. Release profiles of 200 µL of 10 mg/mL caffeine in aqueous solution from the ECM with no collagen (orange) and ECM-ColF (green) from each SCISSOR chamber

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Dextran Injections

Release of 4 kDa and 70 kDa dextran solutions (200 µL, N=3) were monitored over 1 week. The ECM release of the 4 and 70 kDa dextrans plateaued within 30 hours for both injections. The ECM-ColF cartridge appeared to plateau at 81 ± 5% and 75 ± 5% for 4 and 70 kDA, respectively, at ~70 hours.

Injections of the 4 kDa dextran molecule show typical bolus formation with both matrices. The ECM-ColF appears to complex the bolus in-place for a longer duration, resulting in a more homogenous diffusion throughout the cartridge before release.

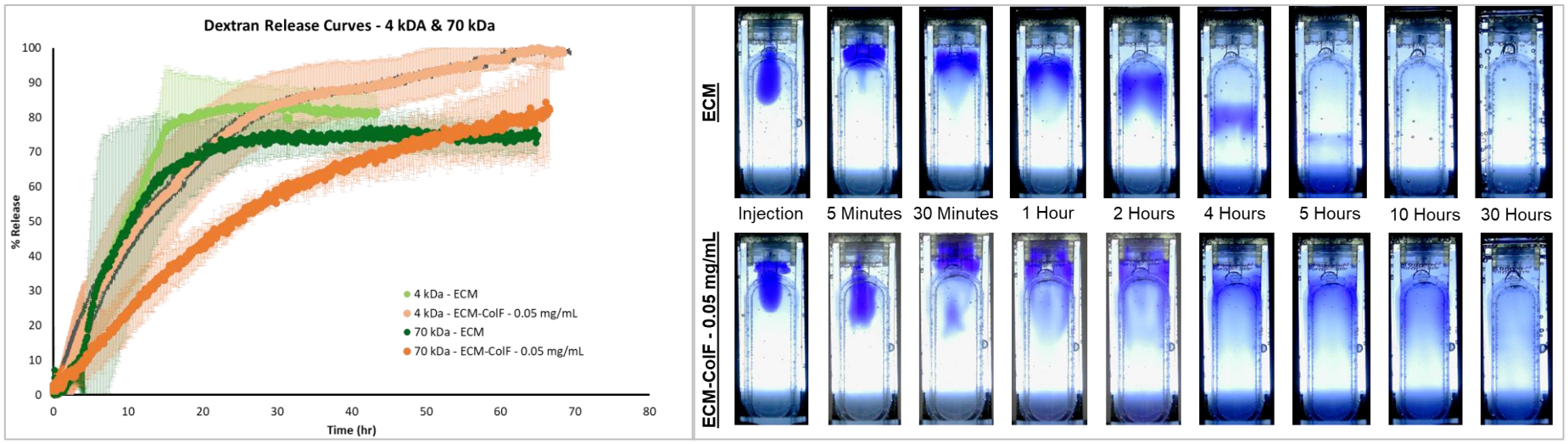

Figure 65 (Left) Release profiles of 200 µL injections of 5 mg/mL 4kDA (light) and 70 kDa (dark) Dextran molecules tagged with DICM-FC and Texas Red®, respectively, from the ECM with no collagen (green) and 0.05 mg/mL ECM-ColF (orange). (Right) Images of the ECM with no collagen and ECM-ColF cartridges after injection of the 4kDa Dextran molecule.

Denosumab Injections

Release of denosumab formulations (200 µL) were monitored over 1 week. The ECM allowed 100% release within 72 ± 6 hrs, whereas the 0.05 mg/mL ECM-ColF resulted in a plateau at 62 ± 3%, while the ECM-ColF with 0.1 mg/mL collagen plateaued at 58 ± 5%. Both ECM-ColF cartridges resulted in statistically similar maximum release ($p<0.05$).

The ECM-ColF cartridge over 1 week of assay showed how the denosumab LAI formulation complexes within the artificial ECM, unlike the pristine ECM.

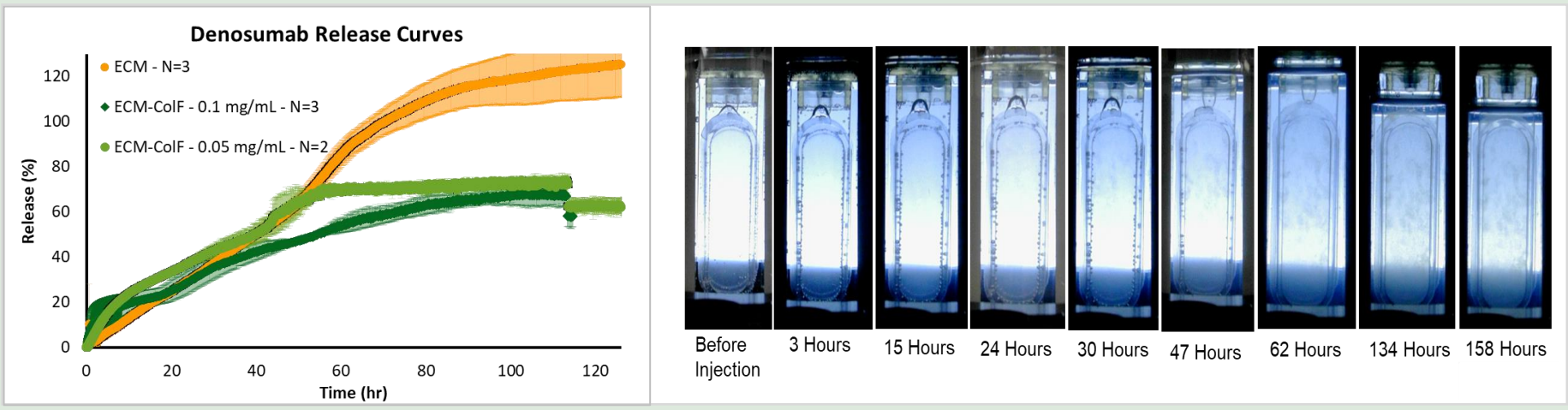

Figure 6. (Left) Release profiles of 200 µL injections of a commercial formulation of denosumab from the ECM with no collagen (orange), 0.05 mg/mL ECM-ColF (green). (Right) Images of the ECM with no collagen and ECM-ColF cartridges after injection of the denosumab formulation.

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CONCLUSIONS

Artificial ECMs containing collagen types I, II, and III and concentrations (0, 0.05, 0.1 mg/mL) were evaluated using five model injectables within Pion's subcutaneous injection site simulator (SCISSOR®).

The increased retention of viscosity-over-time indicates that collagen complexes with the artificial ECM components in an attractive manner, resulting in increased stability without an initial increase in viscosity.

Caffeine injections into each model showed analogous behavior over short time scales. However, the collagen-containing ECMs exhibited a more complex release profile, with multiple points of inflection and increased lag time.

Commercially available formulations of insulin analogs and denosumab were injected to elucidate peptide and monoclonal antibody release behavior with and without collagen's inclusion within the artificial ECM further showcasing how the addition of collagen effects post-injection events within SCISSOR.

In conclusion, collagen not only plays a role in supporting the architecture of these artificial ECMs, but it can directly affect the release profiles of all classes of injectables without significant deviation to the initial material characteristics.

This content was authored by Conor Gomes and Balint Sinko of Pion inc., Billerica, MA in collaboration with Kate Gridley and Randy Mrsny at the Department of Pharmacy and Pharmacology, University of Bath, Bath, UK.

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