Nonspecific Binding of Biotherapeutics and the Extracellular Matrix: Implications for Drug Delivery

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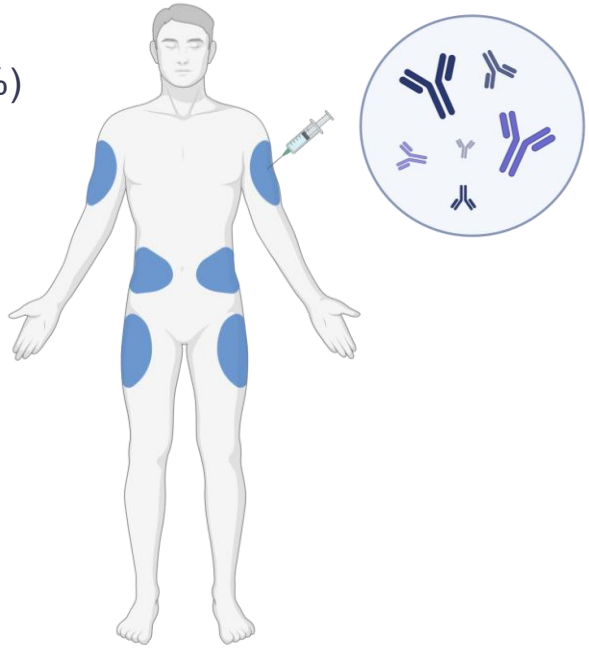
NEXT-GENERATION DELIVERY INNOVATIONS

Subcutaneous (SC) Delivery is Increasing in Prevalence for the Administration of Monoclonal Antibody Therapeutics (mAbs)

- mAbs often face challenges with oral delivery routes and are administered intravenously (~70%) or subcutaneously (~30%)
- SC injection is increasingly preferred by patients due to its many advantages

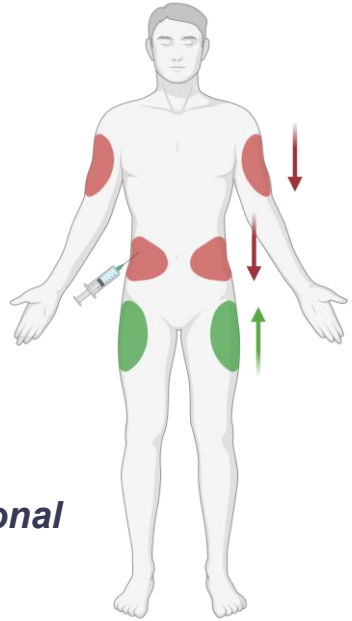
Intravenous (IV) Injections	Subcutaneous (SC) Injection
× Requires hospitalization	✓ Can be self-administered at home
× Low patient mobility during infusion	✓ High patient mobility – no IV pumps
× Increased risk for infection at the injection site	✓ Low risk for infection near the injection site

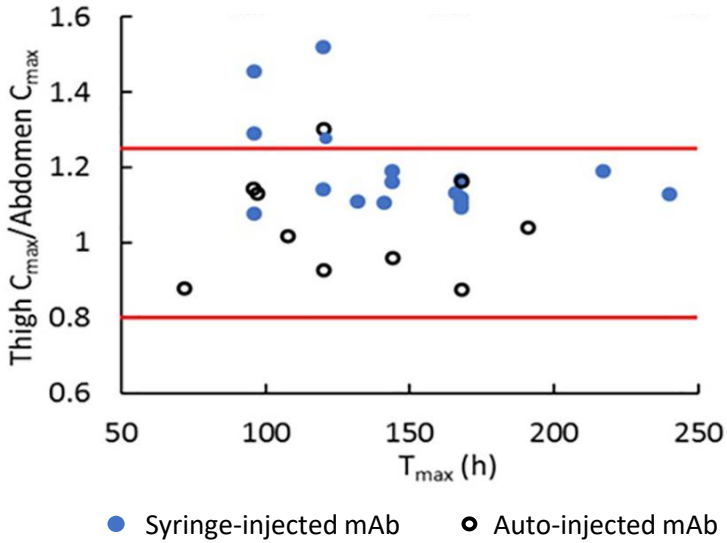


Challenge with SC Delivery: Regional Differences in Bioavailability and Drug Uptake

- mAb biodistribution after SC injection varies depending on injection location
- Thigh injections yielded faster mAb absorption compared to arm/abdomen injections

What could be causing these regional differences in bioavailability?





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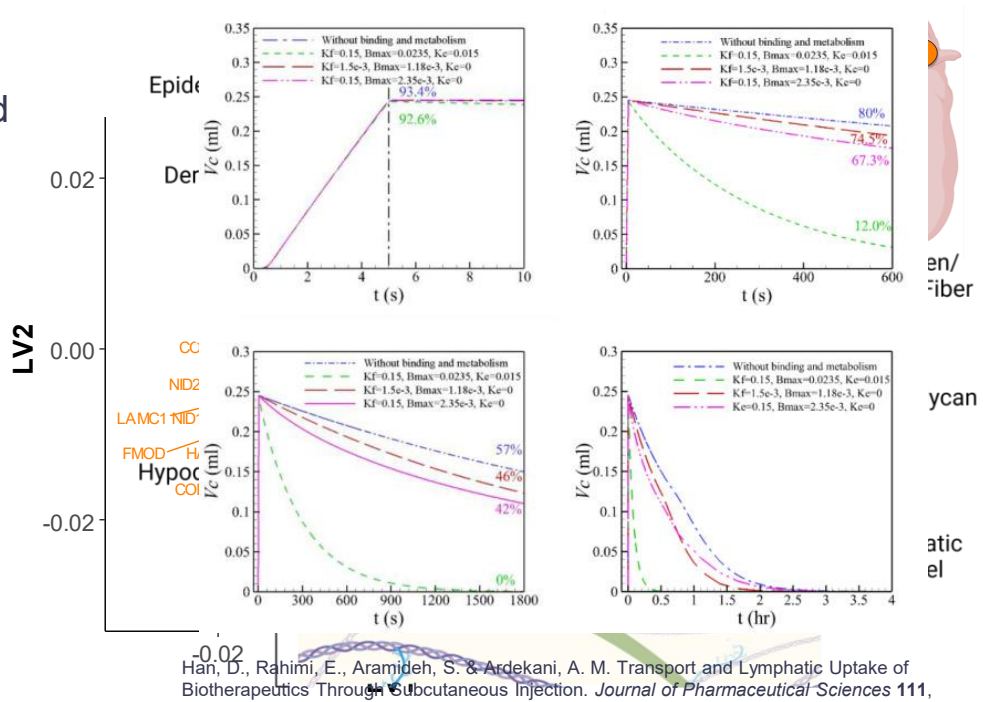
Binding to the Extracellular Matrix (ECM) May Explain Regional Differences in Bioavailability

- ECM composition has been demonstrated to be heterogeneous in porcine model
- ECM binding may slow or limit the movement of drug through the interstitial space, resulting in less efficient uptake and delayed systemic exposure
- Mathematical model of SC biodistribution showed stronger binding significantly limited amount of bioavailable drug

Which ECM molecules are involved in mAb-ECM binding interactions?



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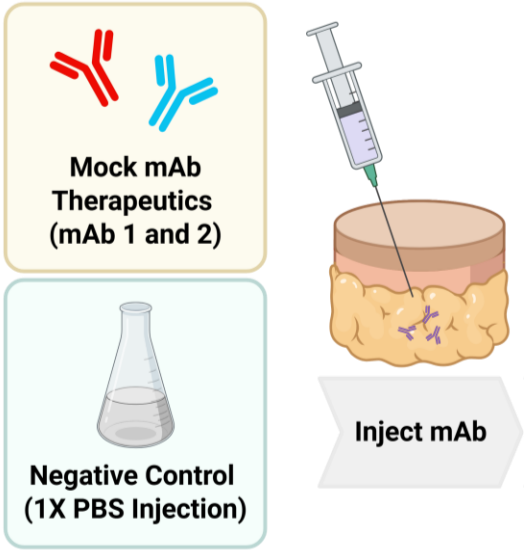
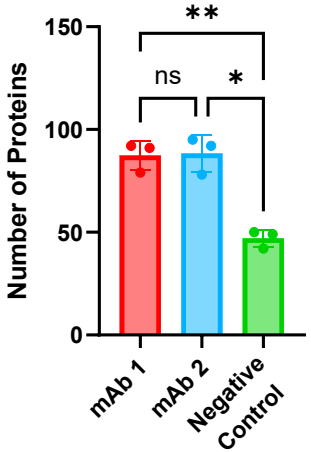
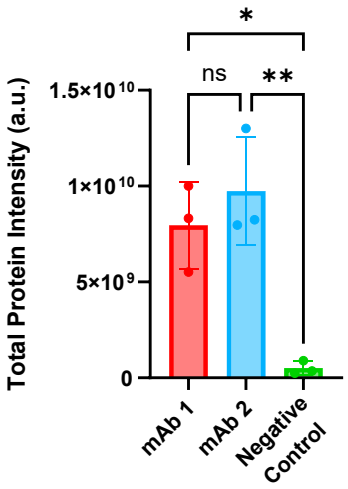
Immunoprecipitation Coupled with Mass Spectrometry Allows for the Identification of Bound ECM Molecules Post-SC Injection

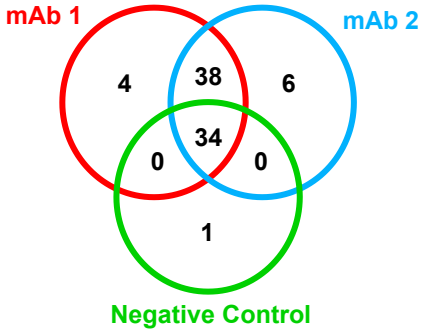


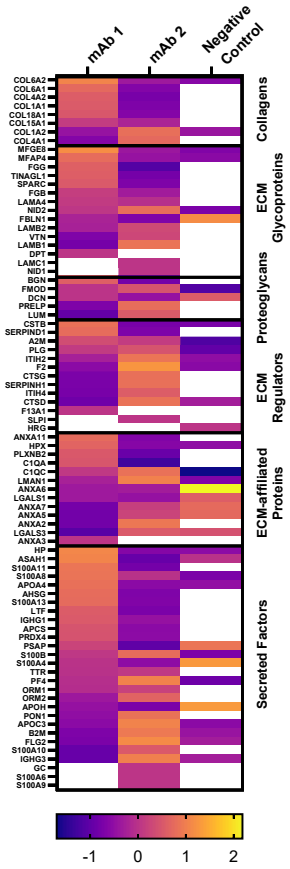
In-Solution Digestion Identified a Core Set of ECM Barriers

- 38 ECM proteins were uniquely bound to both mAbs, indicating a core set of ECM molecular barriers









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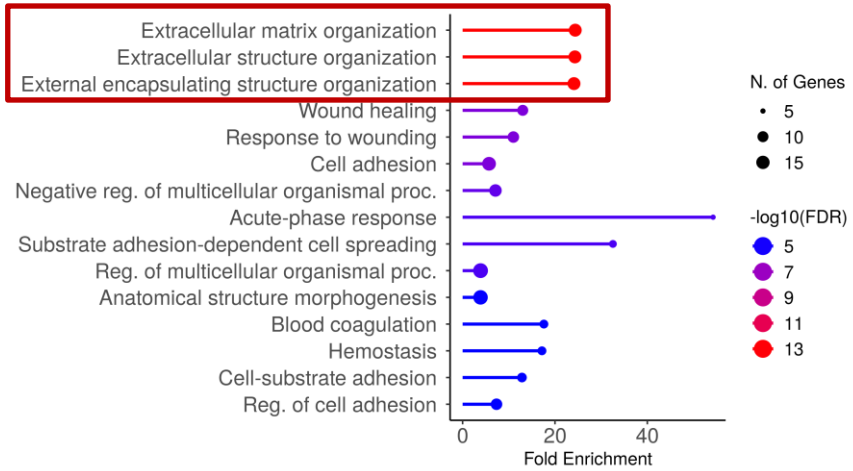
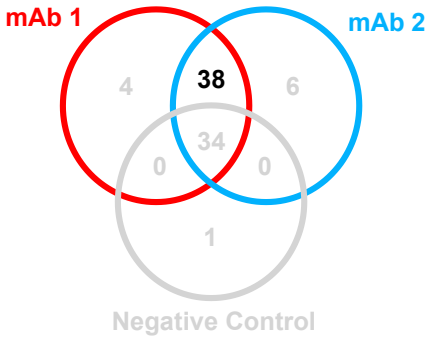
- GO analysis reveals these are largely structural components of the ECM



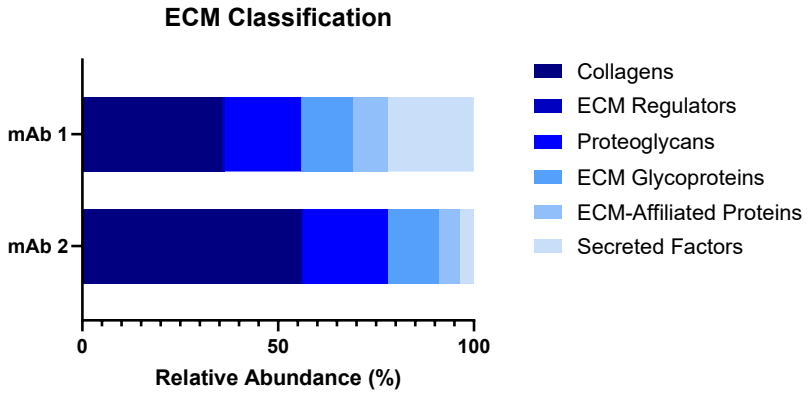


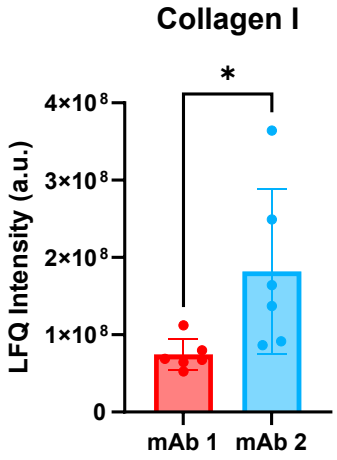
Proteins Uniquely Bound to mAb 1 and mAb 2	
COL15A1	SERPINH1
COL18A1	ANXA11
COL1A1	ANXA2
COL4A1	C1QA
COL4A2	PLXNB2
COL6A1	AHSG
FBLN1	APCS
FGB	IGHG1
FGG	LTF
LAMA4	ORM1
LAMB1	ORM2
LAMB2	PON1
SPARC	PRDX4
TINAGL1	S100A10
VTN	S100A11
BGN	S100A13
LUM	TTR
PRELP	
CTSG	
ITIH4	
SERPIND1	

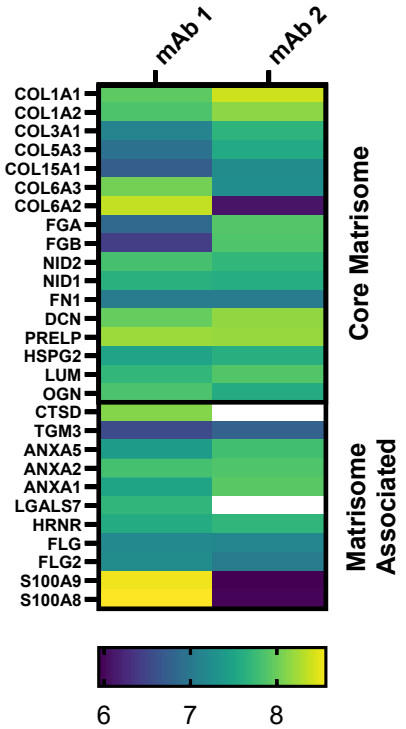
In-Gel Digestion Revealed Collagens are the Most Tightly Bound ECM Proteins

- Collagen type-I was found significantly more adhered to mAb 2 compared to mAb 1

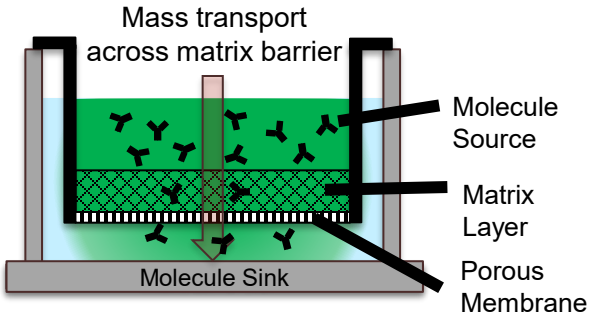
What effect do these binding interactions have on SC transport?



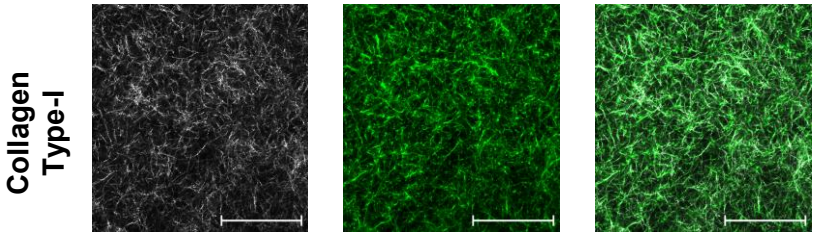


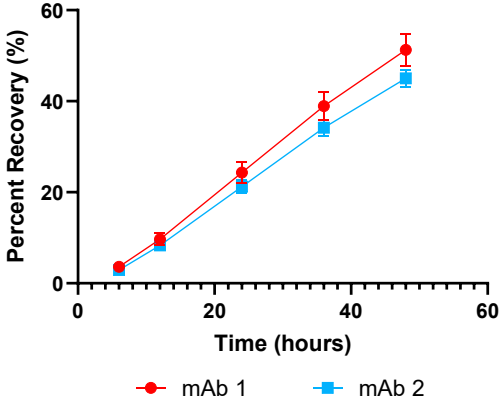


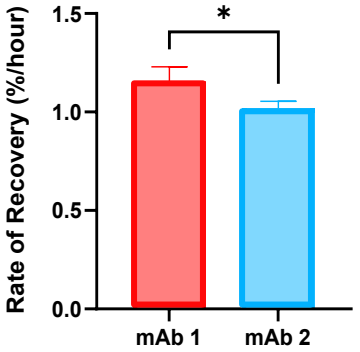
Transwell Model Highlights Collagen Type-I Significantly Limits Mass Transport in the SC Interstitial Space



- mAb recovery through a collagen type-I gel was measured over a period of 48 hours
- mAb 2 demonstrated significantly lower rate of recovery compared to mAb 1 – corroborating LC-MS/MS findings

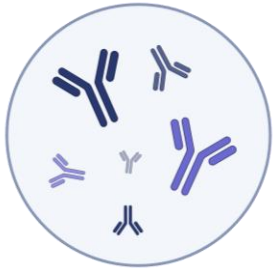


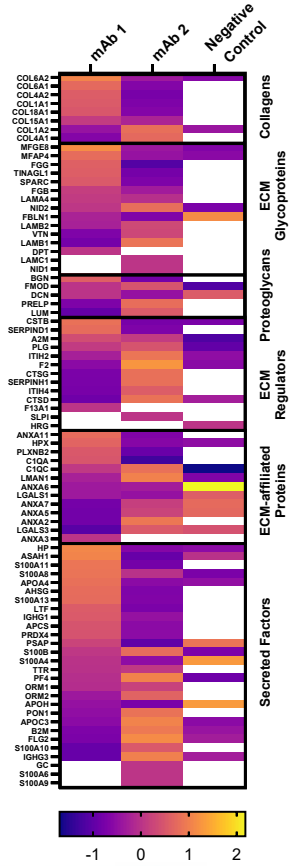




Conclusions

- mAb-ECM binding interactions may slow interstitial diffusion and reduce lymphatic uptake
- Proteomic analysis identified a set of 38 ECM proteins that may act as molecular barriers to SC transport
- Collagen type-I exhibited strong binding to both mAbs, with a slight preference for mAb 2, leading to reduced mobility in *in vitro* transport assay
- Mapping these binding interactions can inform biotherapeutic design, formulation, and injection site decision strategies





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Thank You!

Any Questions?

Path of a SC Injected mAb – A Simplified Story

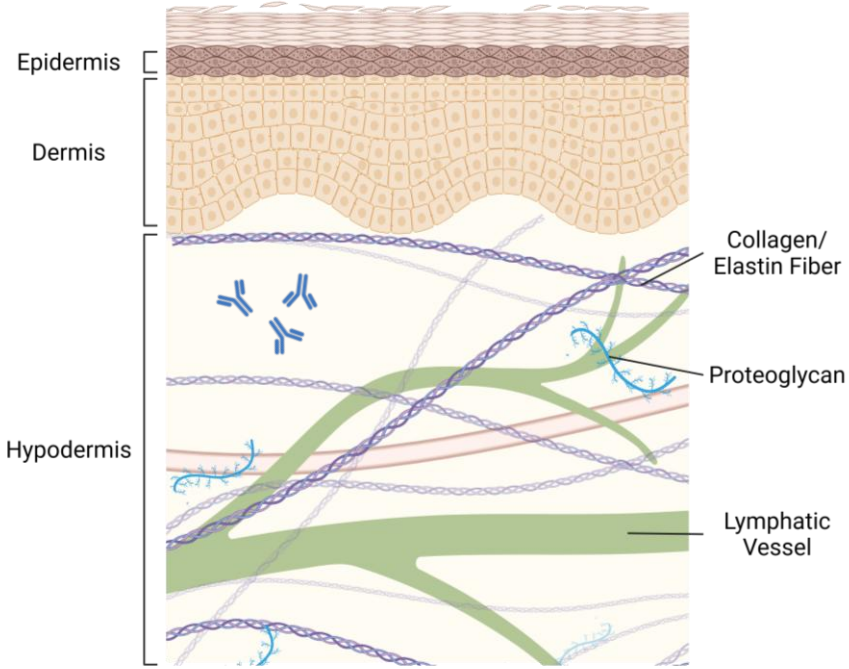
1) mAb is injected into the interstitial space in the hypodermis

2) Diffusion through the extracellular matrix (ECM) within the interstitial space

3) Entry into initial lymphatic vessels via primary lymphatic valves

4) Transport through lymphatic vessels

5) Drainage into systemic circulation



In-Solution Digestion Identified a Core Set of ECM Barriers

- Both mAbs exhibited preferential binding to proteins rich in glutamic acid and deficient in serine
- Suggests that binding could be arising from electrostatic interactions