

# 3D Printed Microneedles for Breast Cancer Biomarker Discovery in Dermal Interstitial Fluid

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# Two Goals

1. Create a microneedle system for reliable painless skin ISF sampling
2. Discover breast cancer protein biomarkers in skin ISF



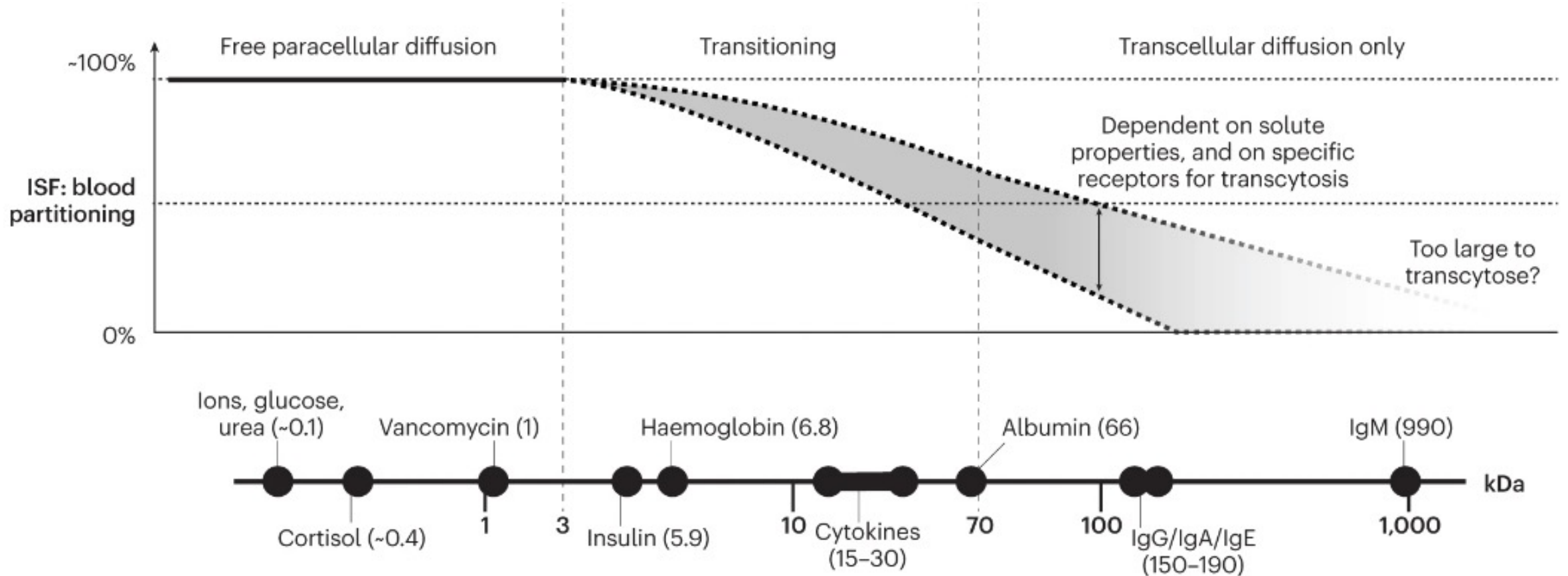
# ISF is a Promising Diagnostic Compartment

**Table 2 | Comparison of analytes representing several classes found in blood plasma, ISF, saliva and sweat**

	<b>Na<sup>+</sup></b>	<b>K<sup>+</sup></b>	<b>Lactate</b>	<b>Glucose</b>	<b>Cortisol</b>	<b>Drugs</b>	<b>Cytokines</b>	<b>Antibodies</b>
<b>Molecular weight (Da)</b>	23	39	90	180	362	Mostly hundreds of daltons	More than five to tens of kilodaltons	Hundreds of kilodaltons
<b>Lipophilicity</b>	Very low (charged)	Very low (charged)	Very low (charged)	Low (hydroxyls)	High	Often high	Very low	Very low
<b>Blood plasma</b>	135-145 mM	3.5-5 mM	0.5-10 mM (resting to nonresting)	4.1-6.9 mM (venous, resting)	Hundreds of nanomolar total; tens of nanomolar unbound fraction	Mostly equivalent to unbound in plasma	Picomolar to nanomolar	Varies; total ~0.4-16 mg ml <sup>-1</sup>
<b>ISF<sup>a</sup></b>	Similar to plasma	Similar to plasma	Similar to plasma	Similar to plasma	Unbound similar to plasma (p)	Many equivalent to unbound in plasma (p)	80% of plasma (a,p)	15-25% of plasma



# ISF is a Promising Diagnostic Compartment

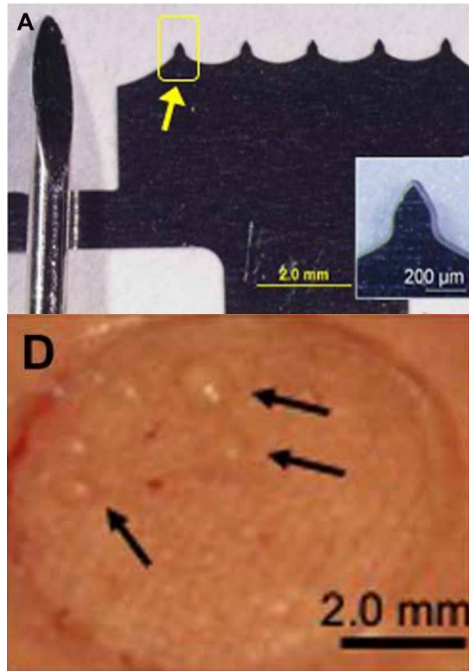


# ISF Continuous Glucose Monitoring



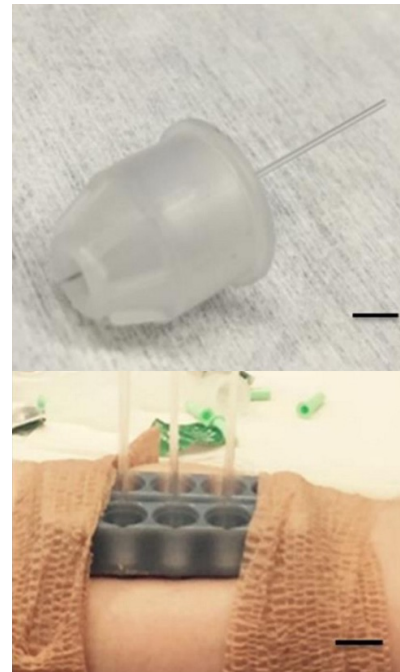
# Literature Benchmarks

Microneedling  
+ Vacuum



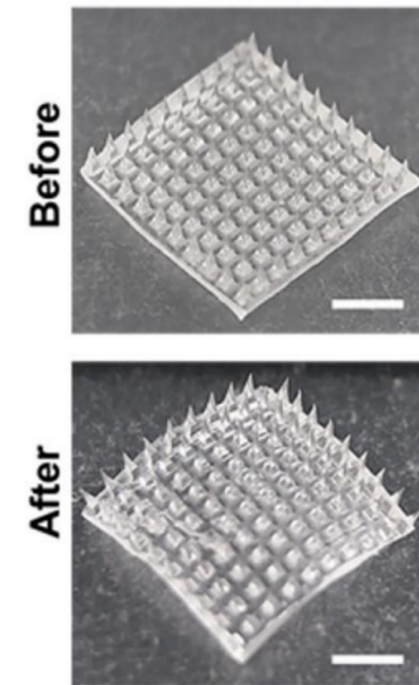
2-5  $\mu\text{L}$  in 20 min

32G Needles  
+ Positive pressure



>10  $\mu\text{L}$  in <3 hr (64%)

Hydrogel  
Microneedles



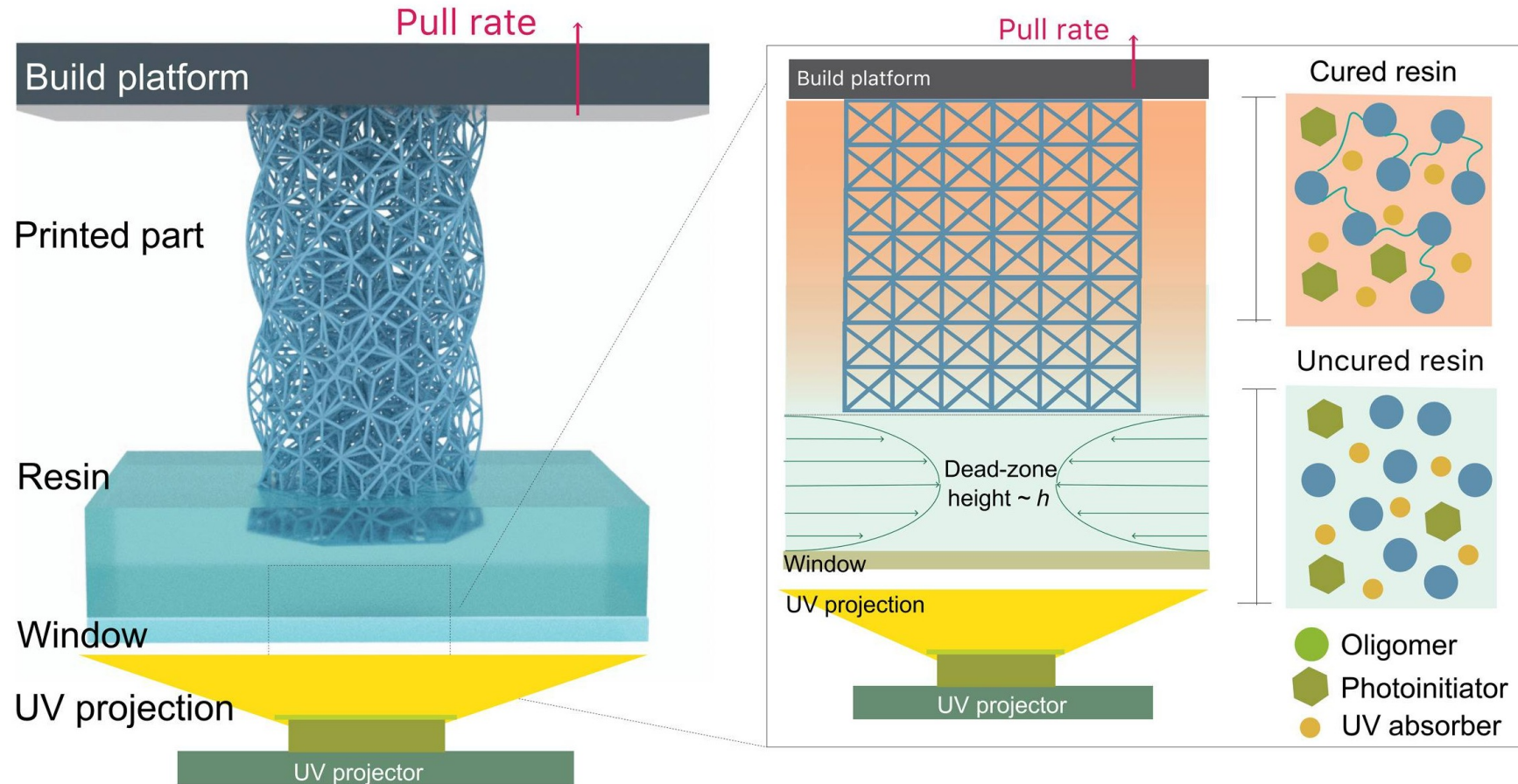
4-8  $\mu\text{L}$  in 3 min

Can we leverage 3D printing to  
improve ISF collection?

# Design Criteria

- Collect 50 uL in 20 minutes (max est. 60  $\mu\text{L}/\text{cm}^2$ )
- Consistent penetration
- Mechanically robust
- Easy ISF retrieval from device
- Easy to apply
- Can sterilize

# Continuous Liquid Interface Production (CLIP)









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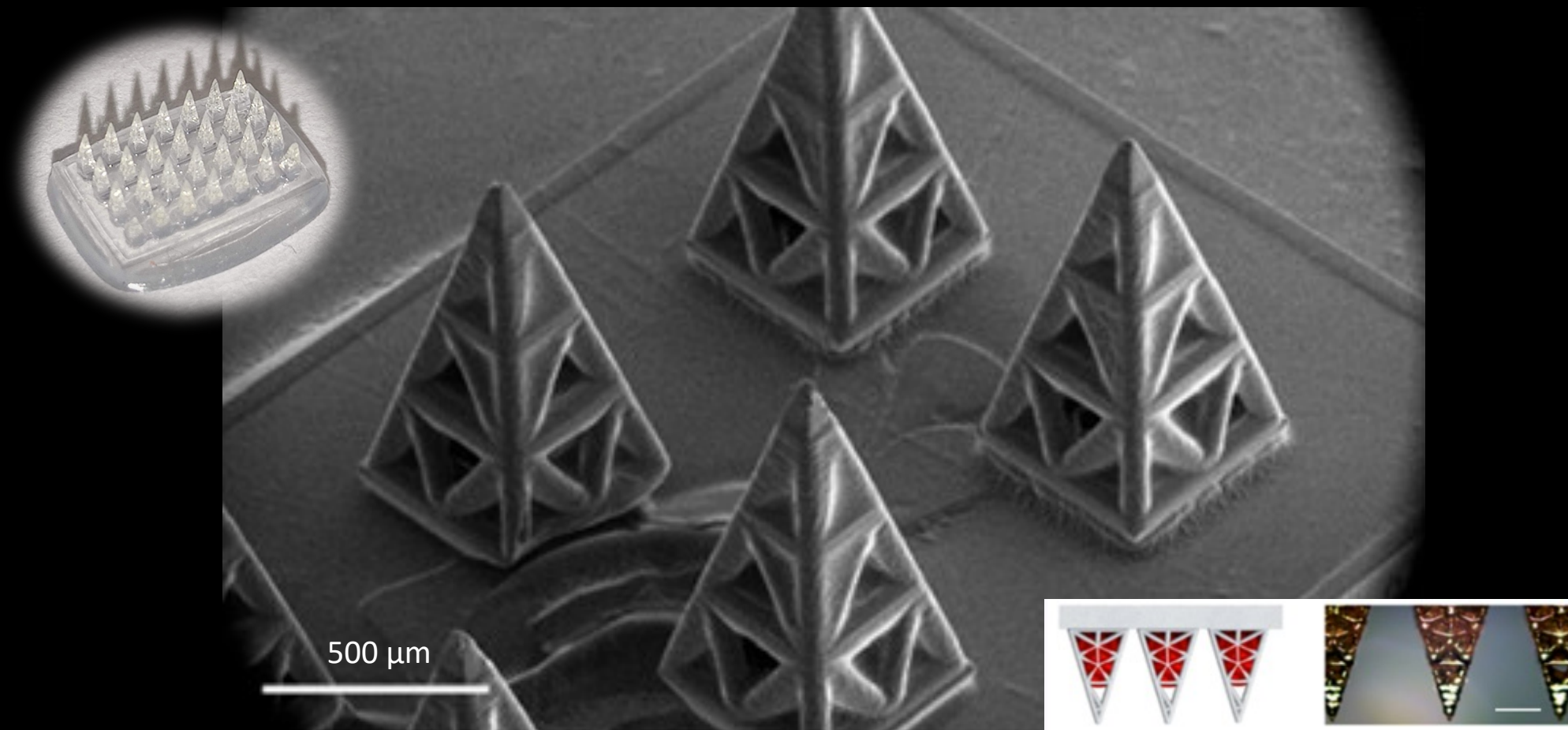
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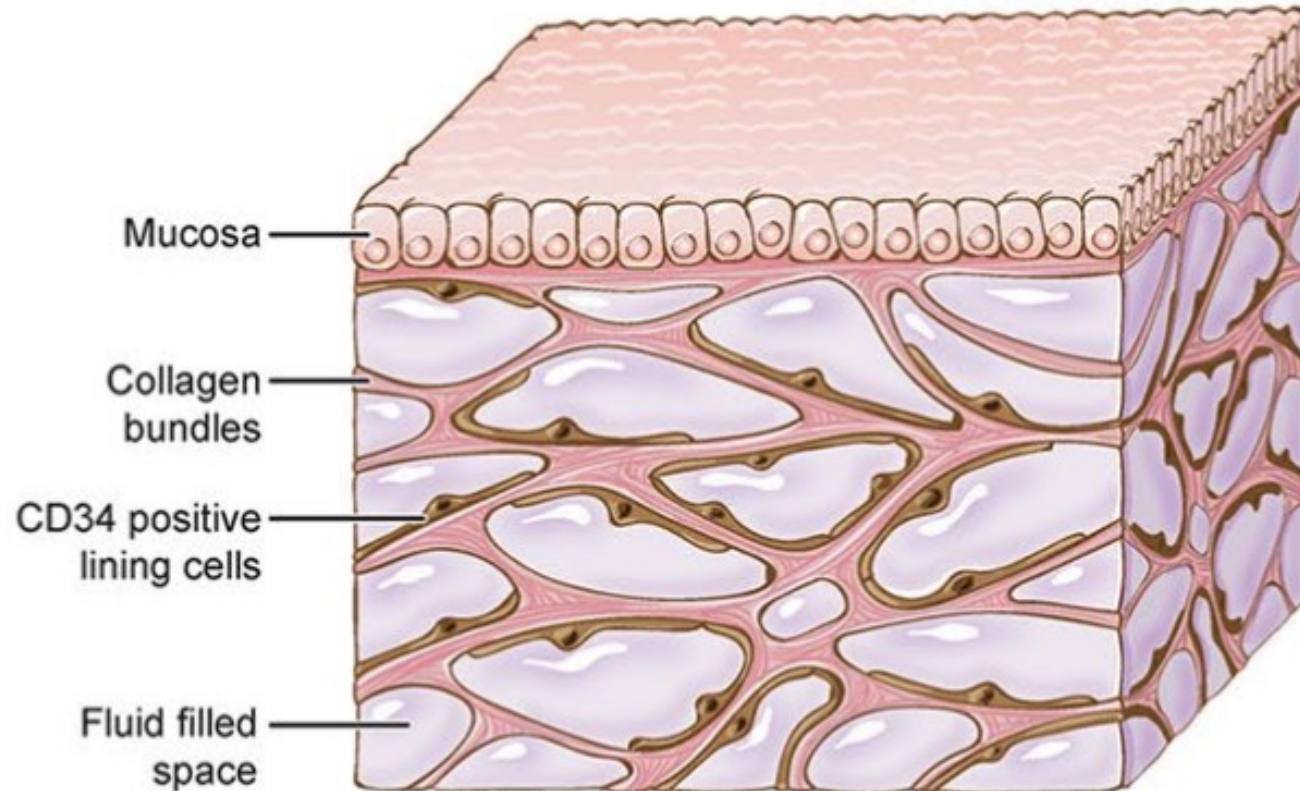




# Lattice Microneedle Array Patch (LMAP)



# Fluid in skin is not free fluid

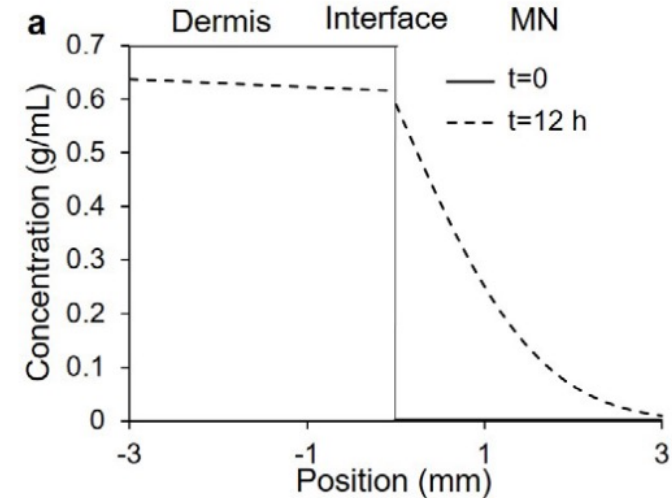
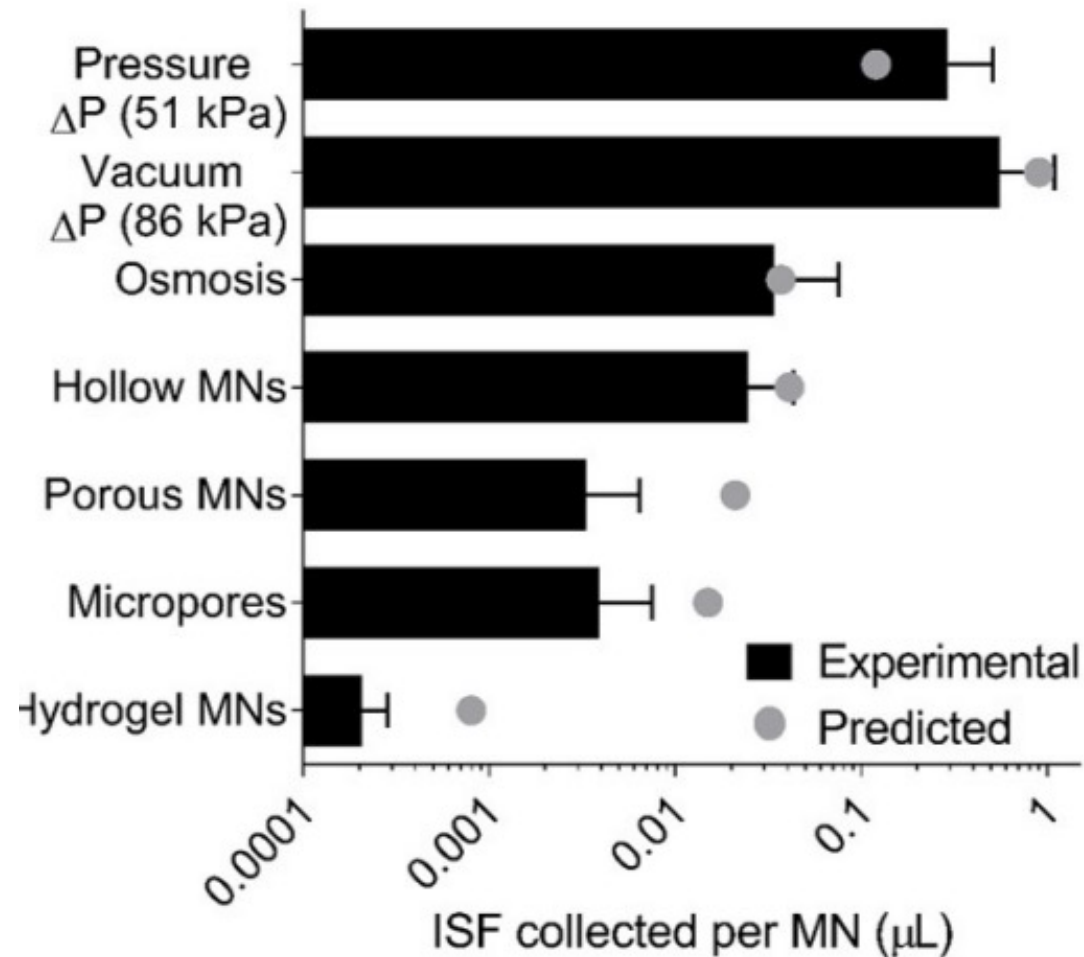


*J Gregory ©2016 Mount Sinai Health System*



- Proteoglycans
- Glycoproteins
- Collagen
- Hyaluronic acid

# Diffusion alone is probably not enough

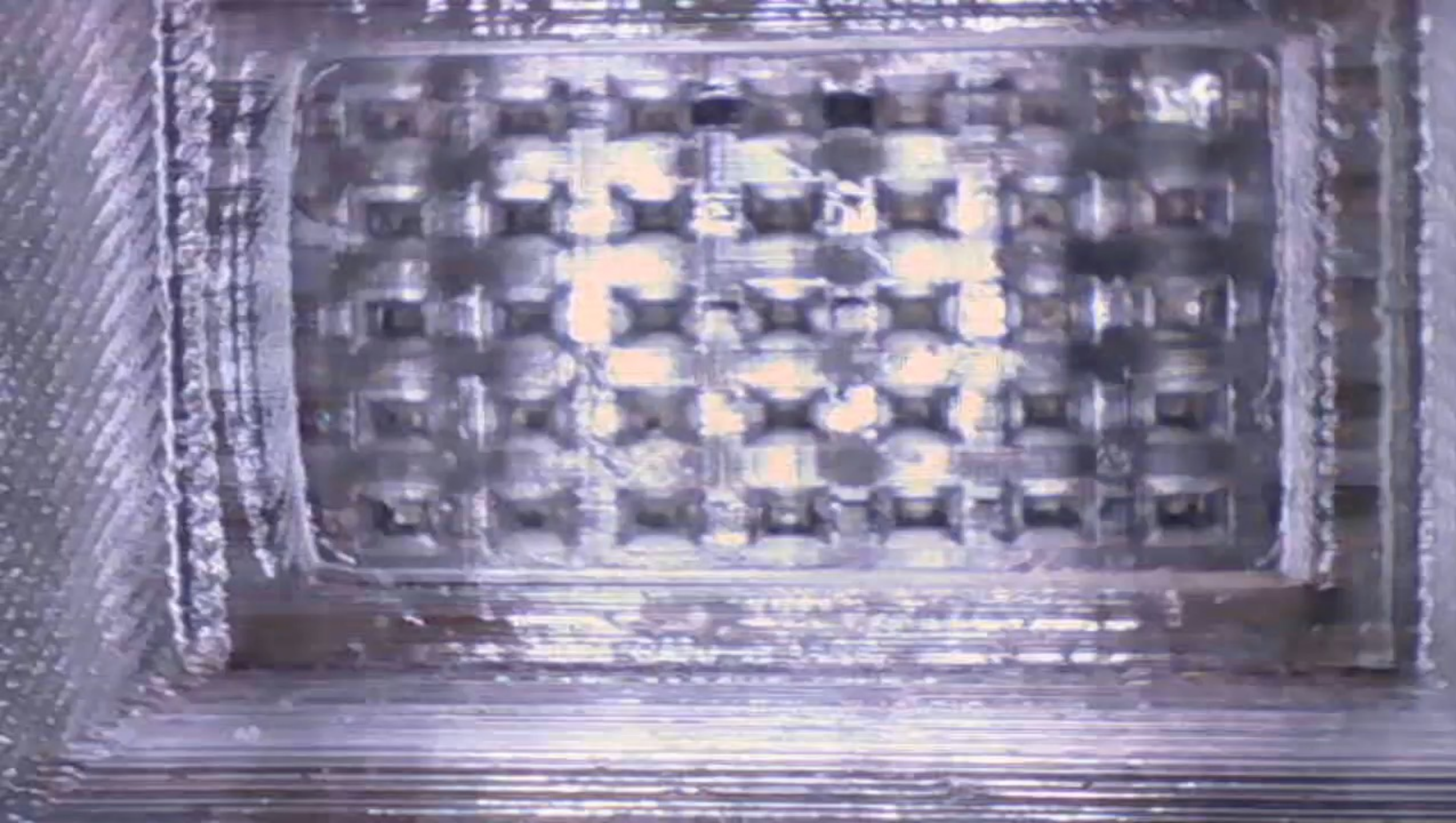


Self Diffusion Coefficient

Dermal ISF:  $8.7 \times 10^{-10} \text{ m}^2 / \text{s}$

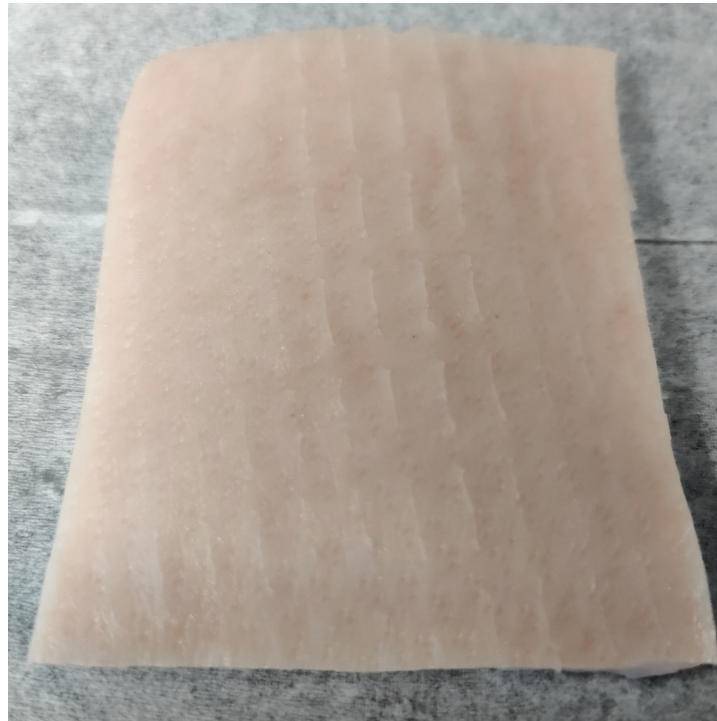
Water  $40^\circ\text{C}$ :  $3.2 \times 10^{-9} \text{ m}^2 / \text{s}$





# Ex vivo Pig Back Skin Model

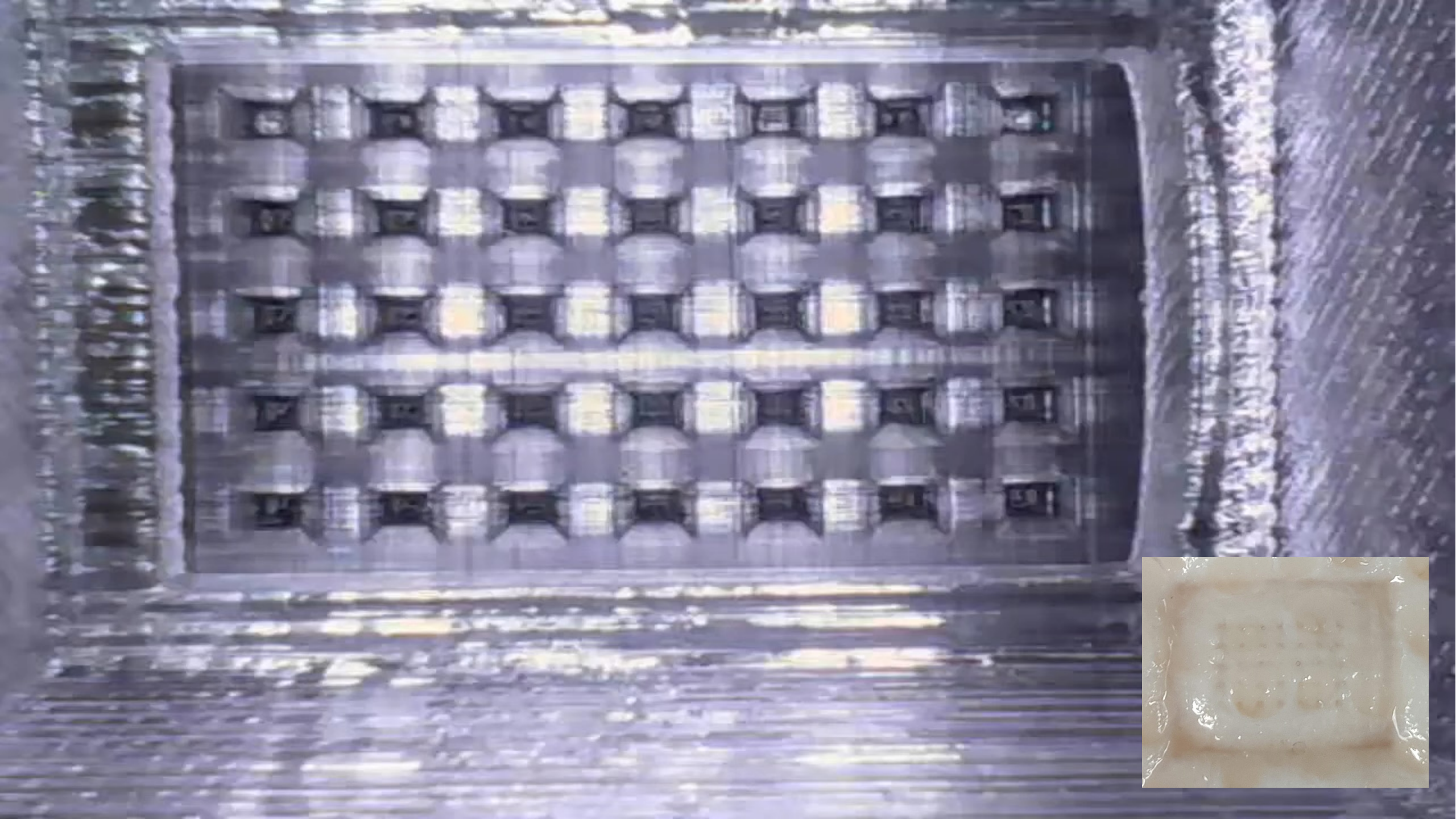
Epidermis side



Dermis side





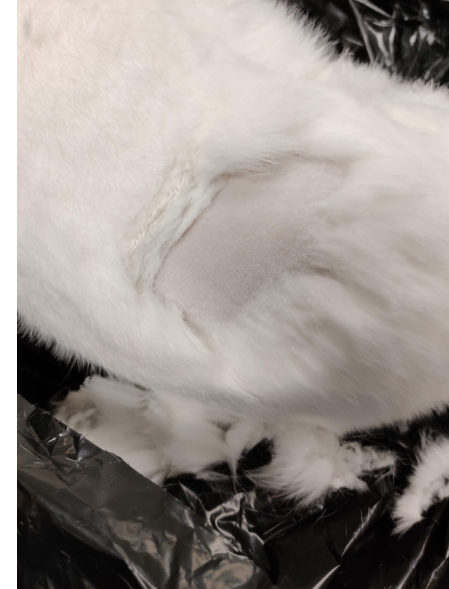
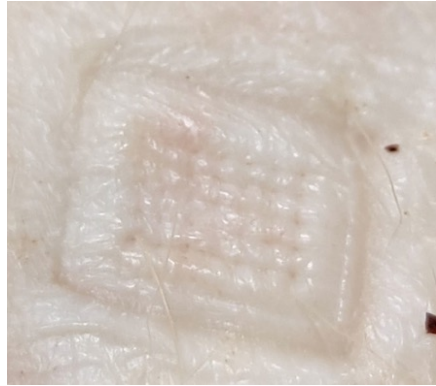


# ISF extraction only works on the dermis side

	Frozen Pig Back Skin	Frozen Pig Ear Skin	Fresh Pig Ear Skin	Frozen Human Skin
Epidermis				
	0.6 mg ISF	6.1 mg ISF	0.7 mg ISF	2.8 mg ISF
	Vacuum	Vacuum	Vacuum + pressure	Pressure
	3 sites x 14 min total	1 site x 15 min	1 site x 5 min	1 site x 5 min
	1.2 mm MAP	1.2 mm MAP	2.0 mm MAP	2.0 mm MAP
Dermis				
	29.2 mg ISF	32.2 mg ISF	18.2 mg ISF	25.1 mg ISF
	Pressure	Pressure	Pressure	Pressure
	1 sites x 5 min	1 sites x 5 min	1 site x 5 min	1 site x 5 min
	1.2 mm MAP	1.2 mm MAP	2.0 mm MAP	2.0 mm MAP

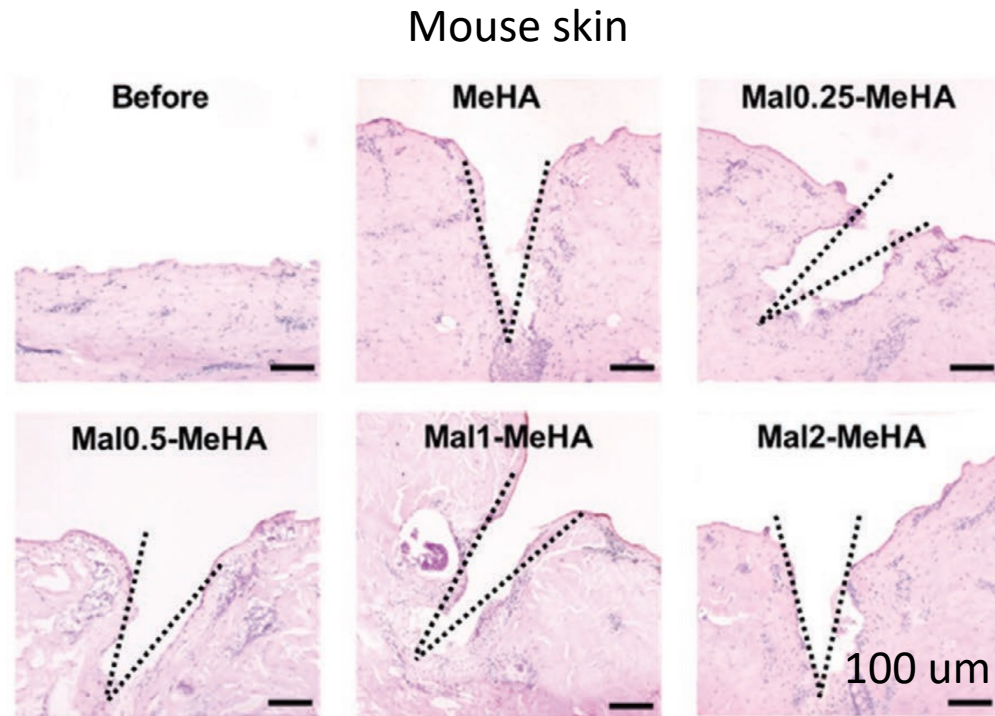


# Failures in Postmortem Pigs and Rabbit

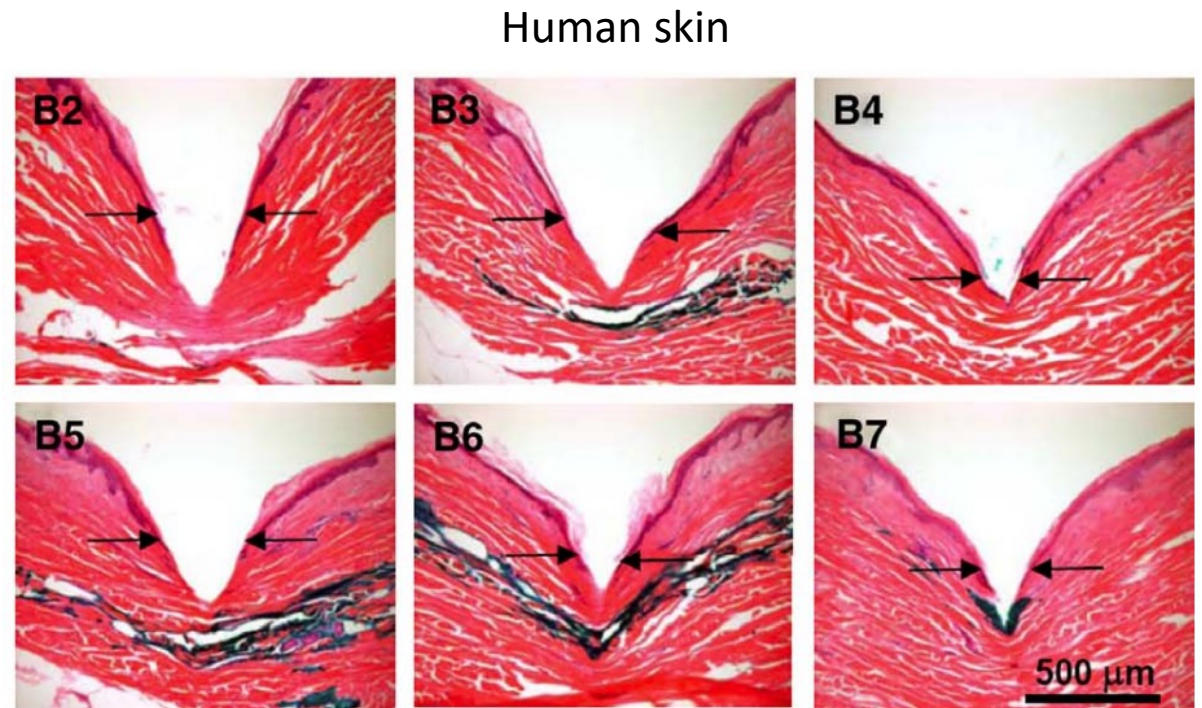




# Hypothesis: shallow penetration due to indentation



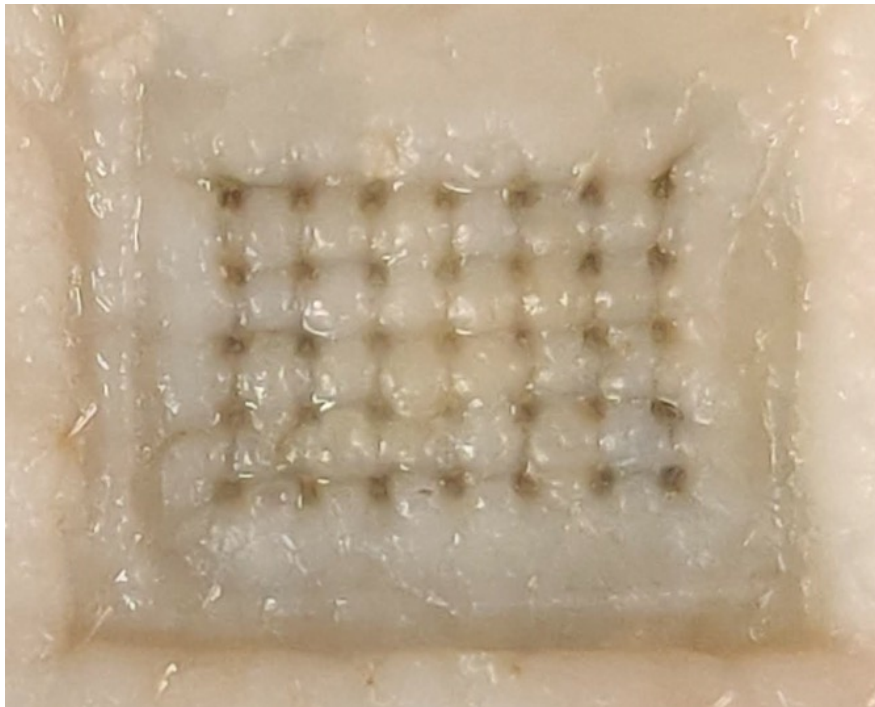
Zheng, Adv Health Mat 9 (2020) 1901683



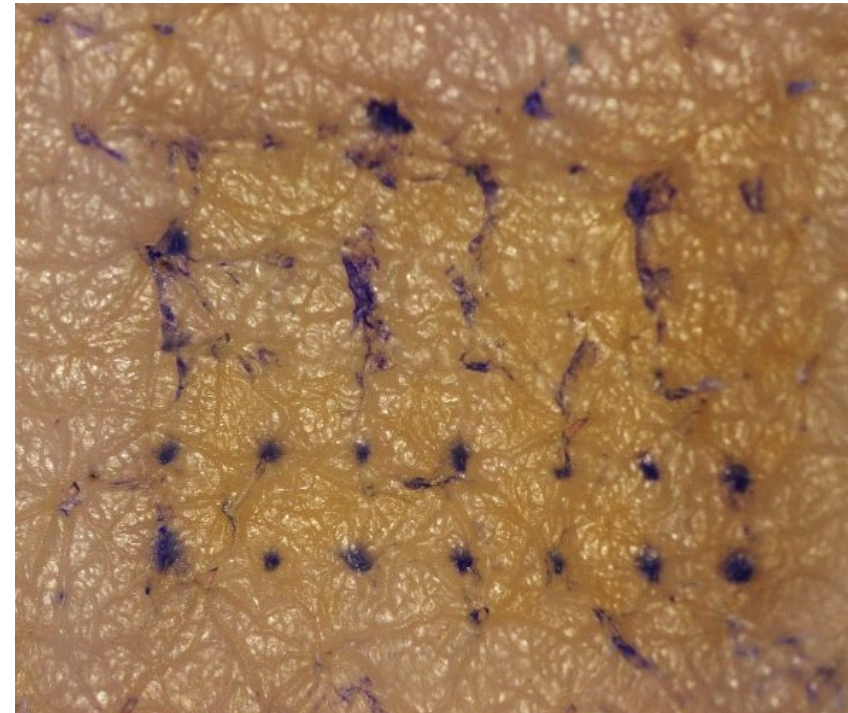
Prausnitz, J Contr Release 112 (2006) 357

# Inconsistent penetration of stratum corneum

No staining



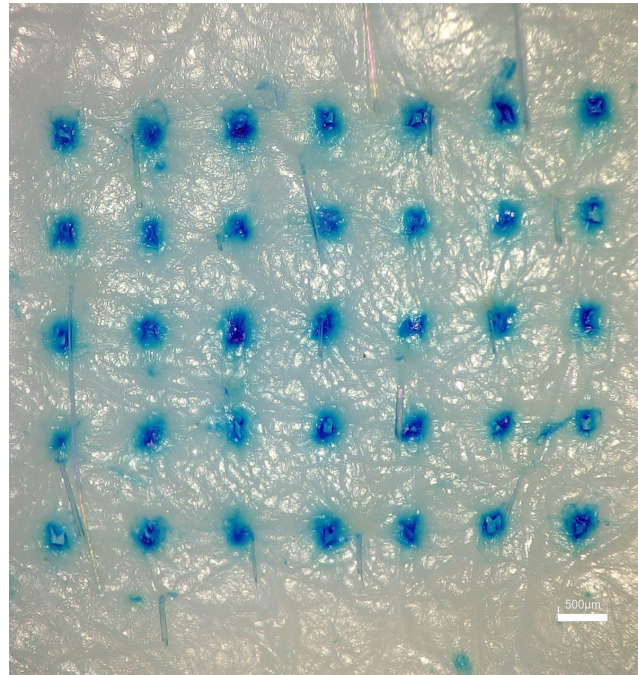
After Gentian Violet



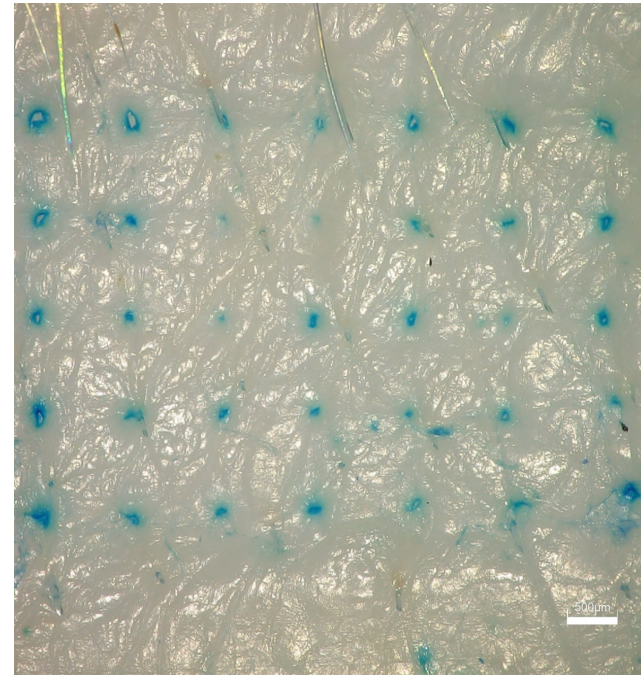


# Spring-loaded applicator for more consistent penetration of stratum corneum

Applicator

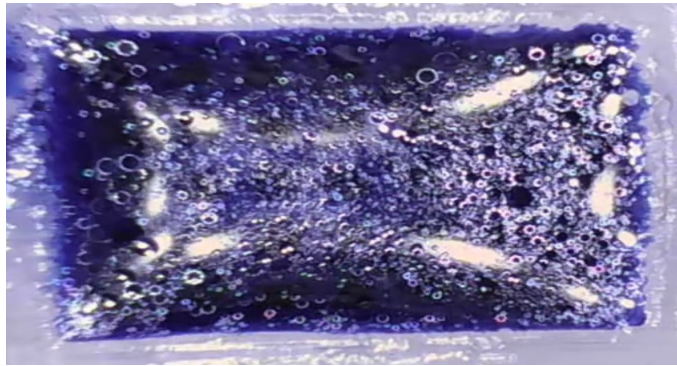


No applicator

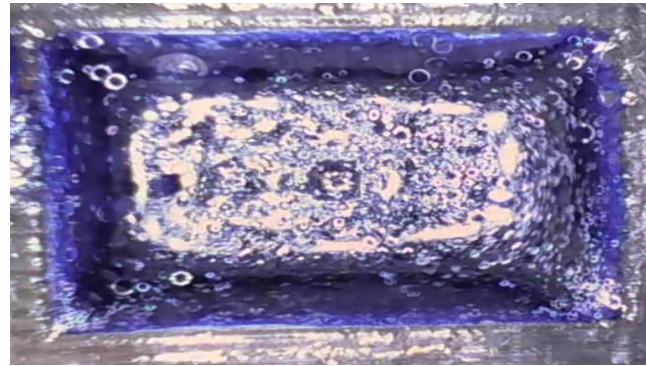


# ISF leaks back upon microneedle removal

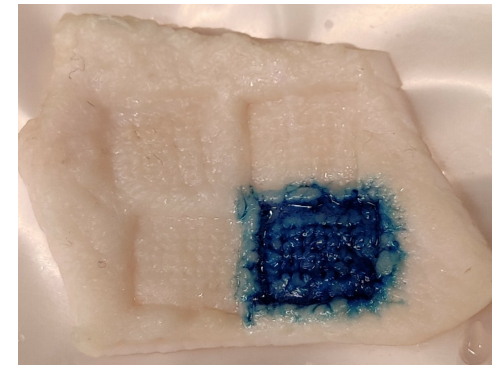
Full reservoir



After skin insertion x 10 s  
and removal



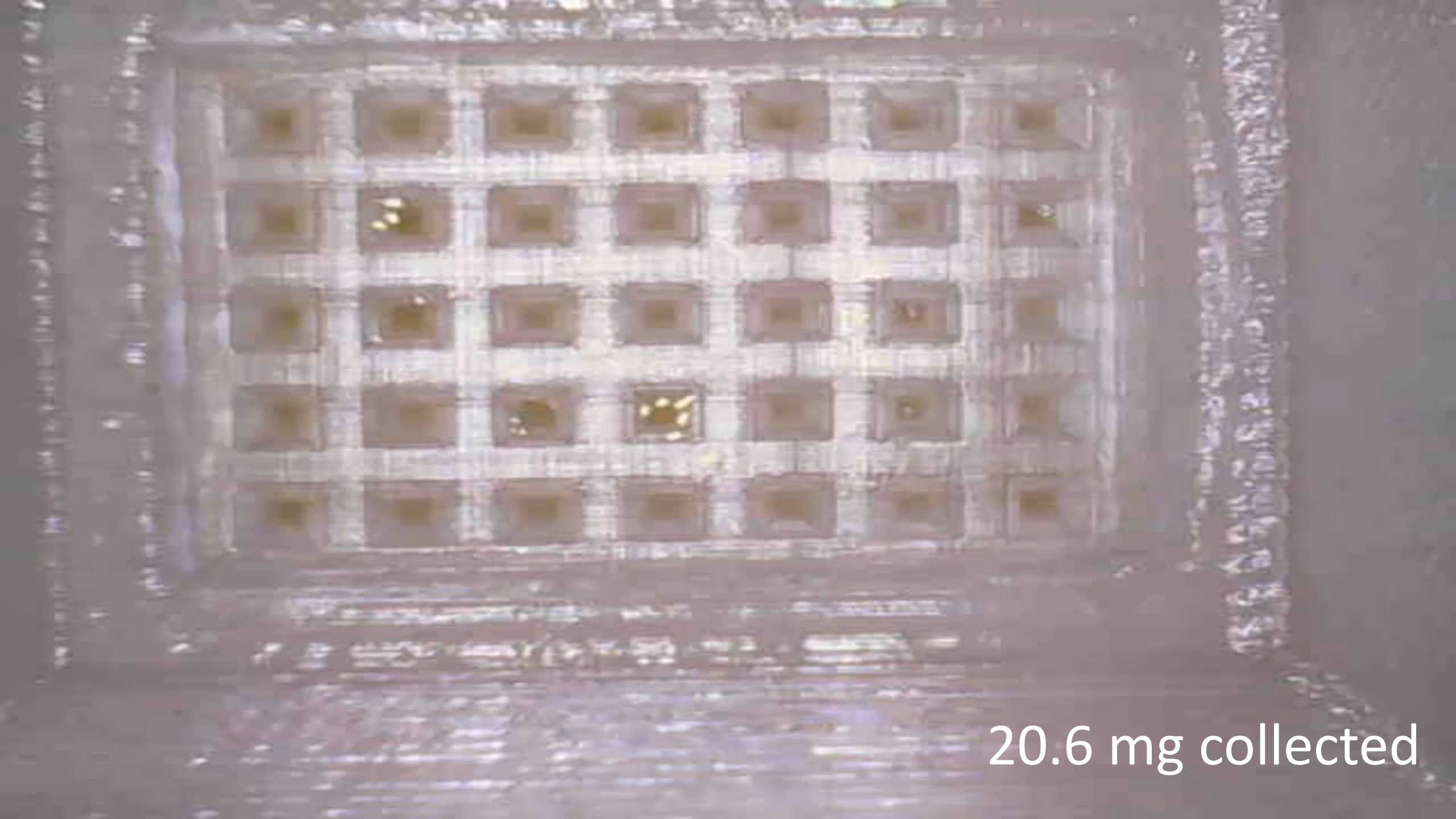
Dye leaks onto  
skin



# Putting it all together

- **Applicator** for consistent penetration
- Attach handheld vacuum for **visual monitoring**
- Apply **pressure** x 5 min
- Turn on **vacuum momentarily** while withdrawing microneedle





20.6 mg collected

# Conclusions

- Collect 50 uL in 20 minutes: **20 mg in 5 min in pig skin**
- Consistent penetration: **Applicator**

# Next Steps

- Ex vivo human skin
- Post-mortem animal studies
- Device iteration

# Acknowledgement

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