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

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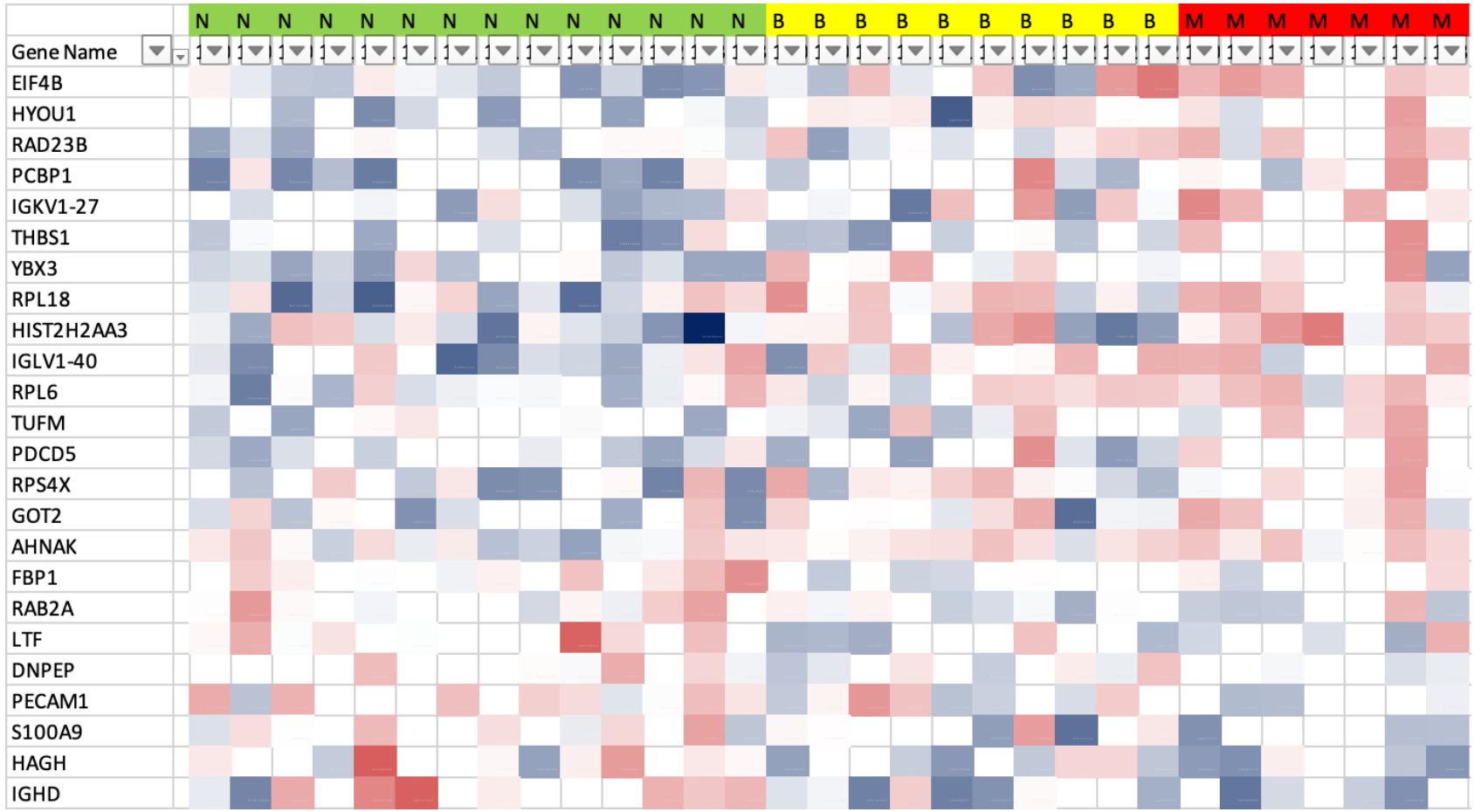
1/11/2023

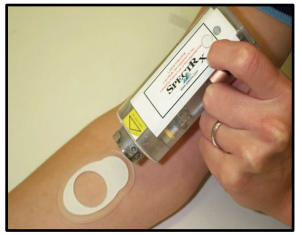
Two Goals

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

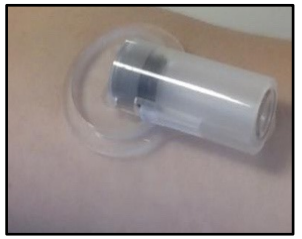
A Pilot Study of Breast Cancer ISF Biomarker



Laser micropores



1 hr vacuum



5 µL ISF

Bermudez, Early Cancer Detection Conference Poster, 2020

ISF is a Promising Diagnostic Compartment

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

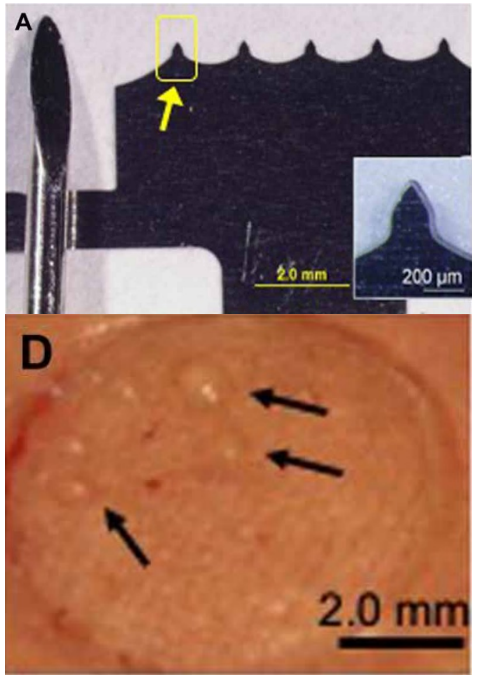
	Na^+	K^+	Lactate	Glucose	Cortisol	Drugs	Cytokines	Antibodies
Molecular weight (Da)	23	39	90	180	362	Mostly hundreds of daltons	More than five to tens of kilodaltons	Hundreds of kilodaltons
Lipophilicity	Very low (charged)	Very low (charged)	Very low (charged)	Low (hydroxyls)	High	Often high	Very low	Very low
Blood plasma	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^{-1}
ISF[a]	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

Heikenfeld, Nat Biotechnol 37, 407–419, 2019

ISF Continuous Glucose Monitoring

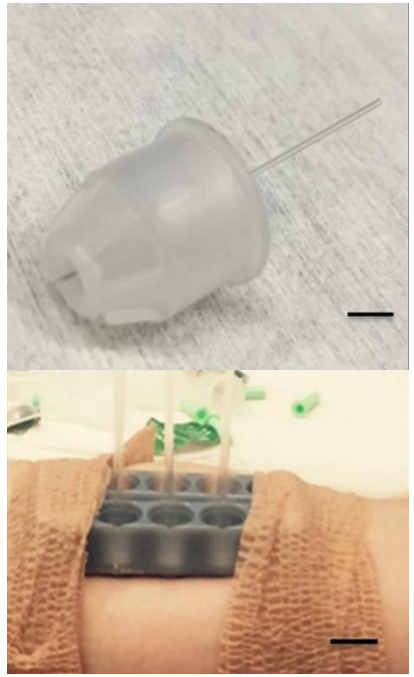
ISF Sampling Strategies

Microneedling + Vacuum



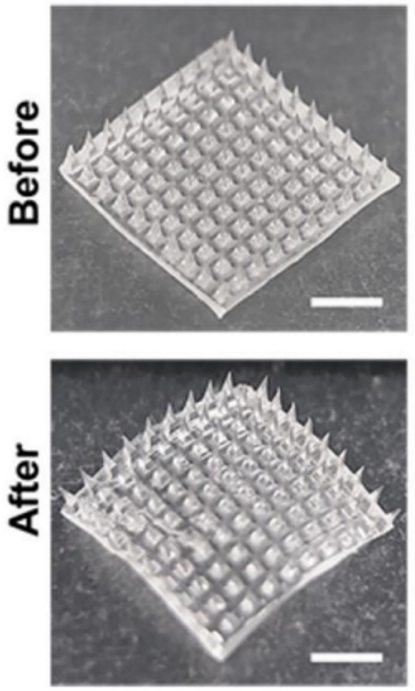
2-5 µL in 20 min

32G Needles + Positive pressure



10 µL in 30 min (64%)

Hydrogel Microneedles



4-8 µL in 3 min

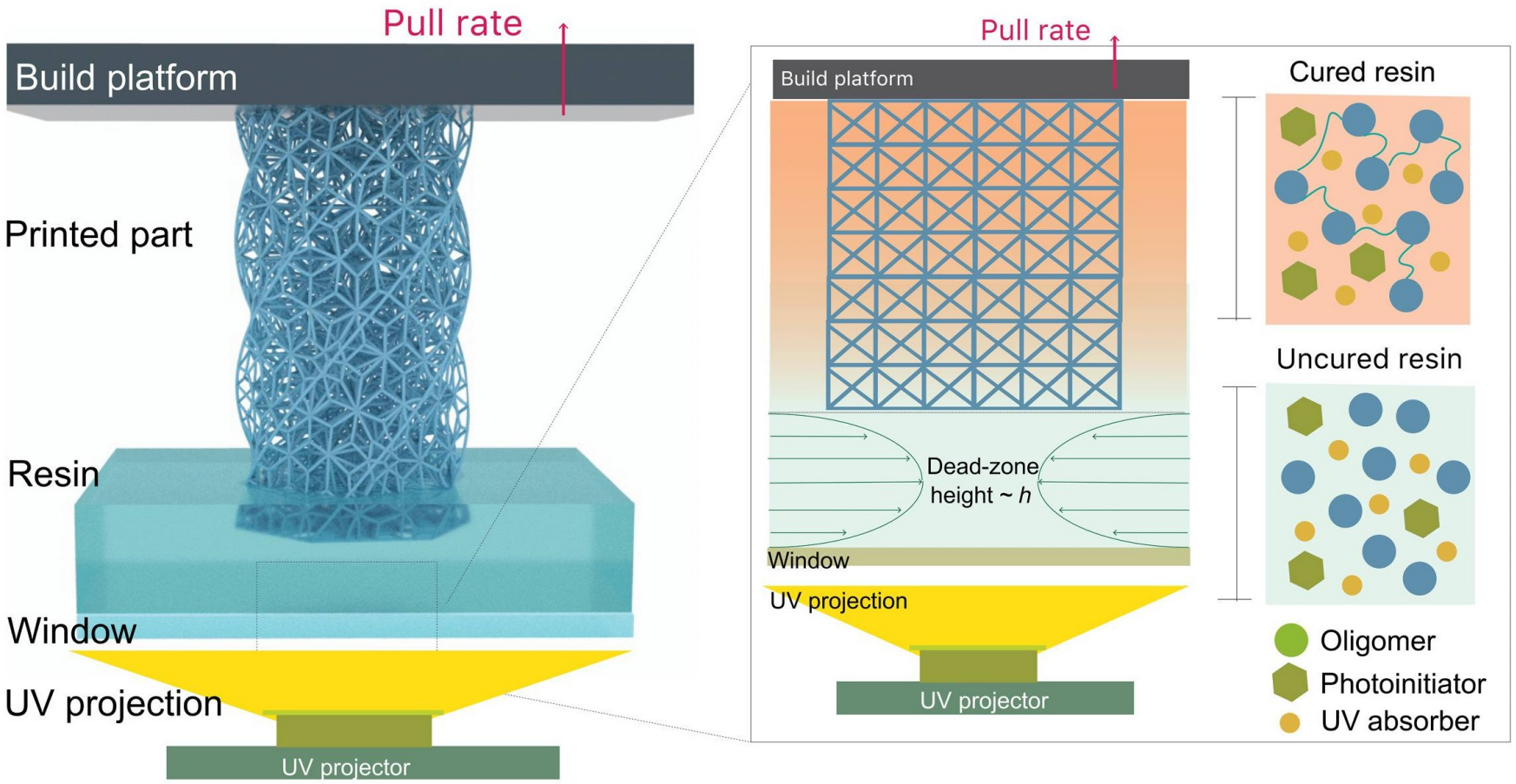
Samant, Sci Transl Med 12, eaaw0285, 2020; Miller, Nat Comm Biol 1, 173, 2018; Zheng, Adv Health Mater 9, 1901683, 2020

Can we leverage 3D printing to improve ISF collection?

Design Criteria

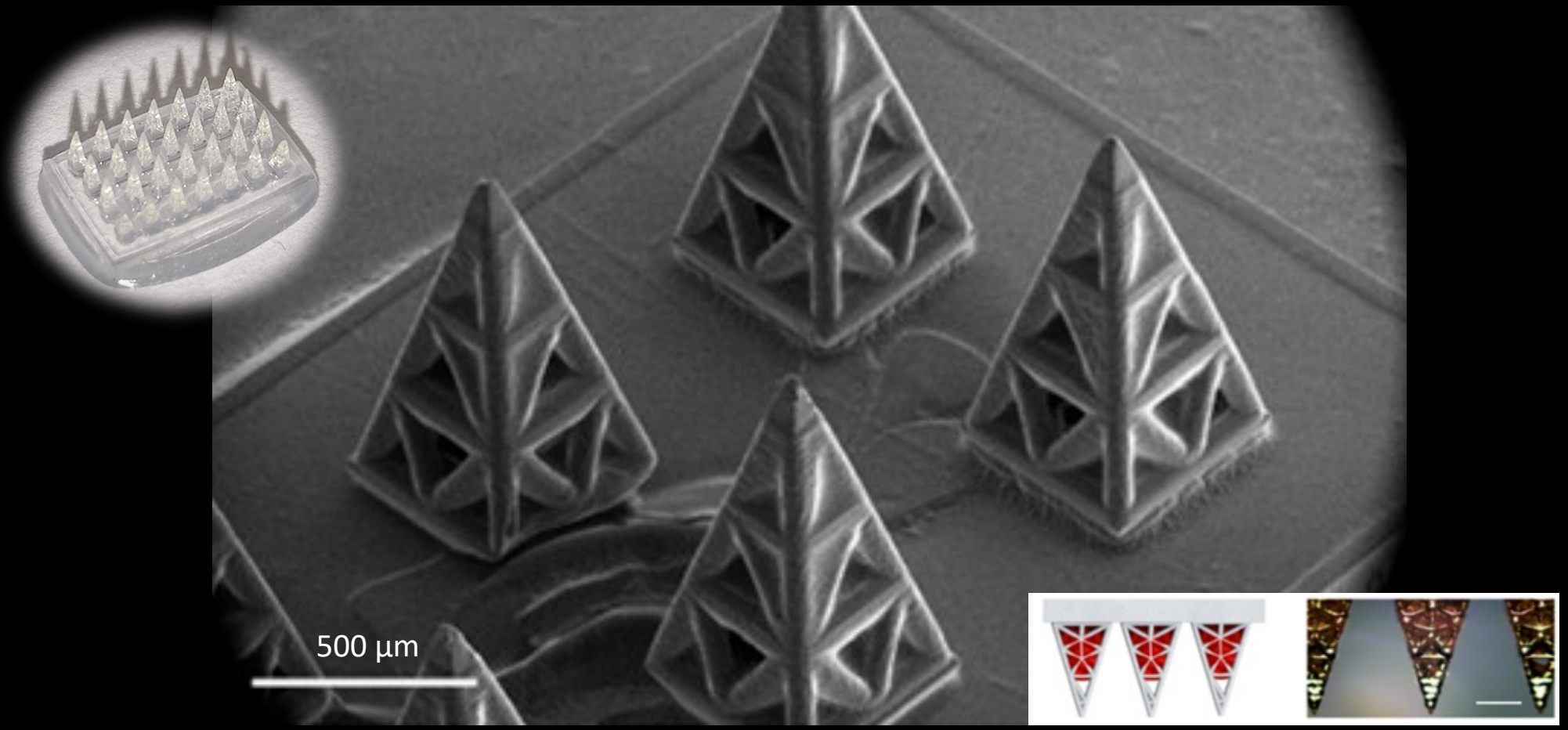
- Collect 50 uL in 20 minutes (max est. 60 µL/cm^2)
- Consistent penetration
- Mechanically robust
- Easy ISF retrieval from device
- Easy to apply
- Can sterilize

Continuous Liquid Interface Production (CLIP)



Hsiao, Sci Adv 8, eabq2846, 2022

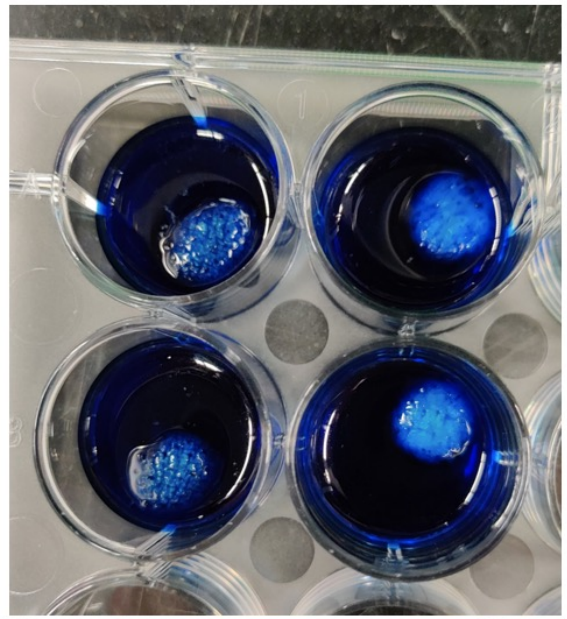
Lattice Microneedle Array Patch (LMAP)

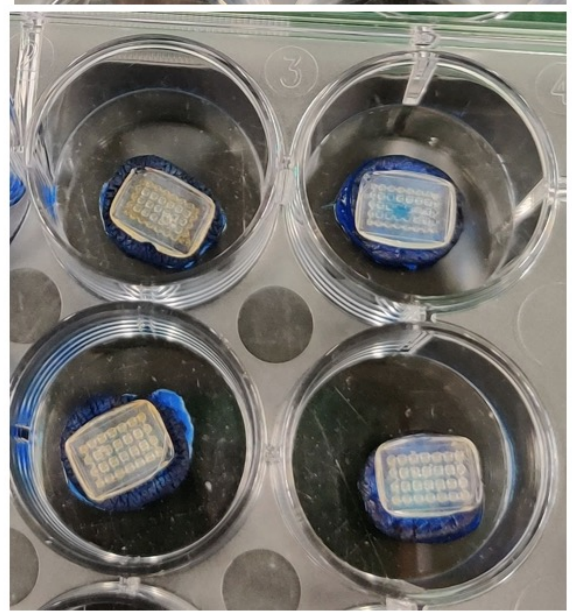


Rajesh, *JACS Au* 2, 2426–2445, 2022

Does LMAP collect ISF in skin?

Human skin Pig skin





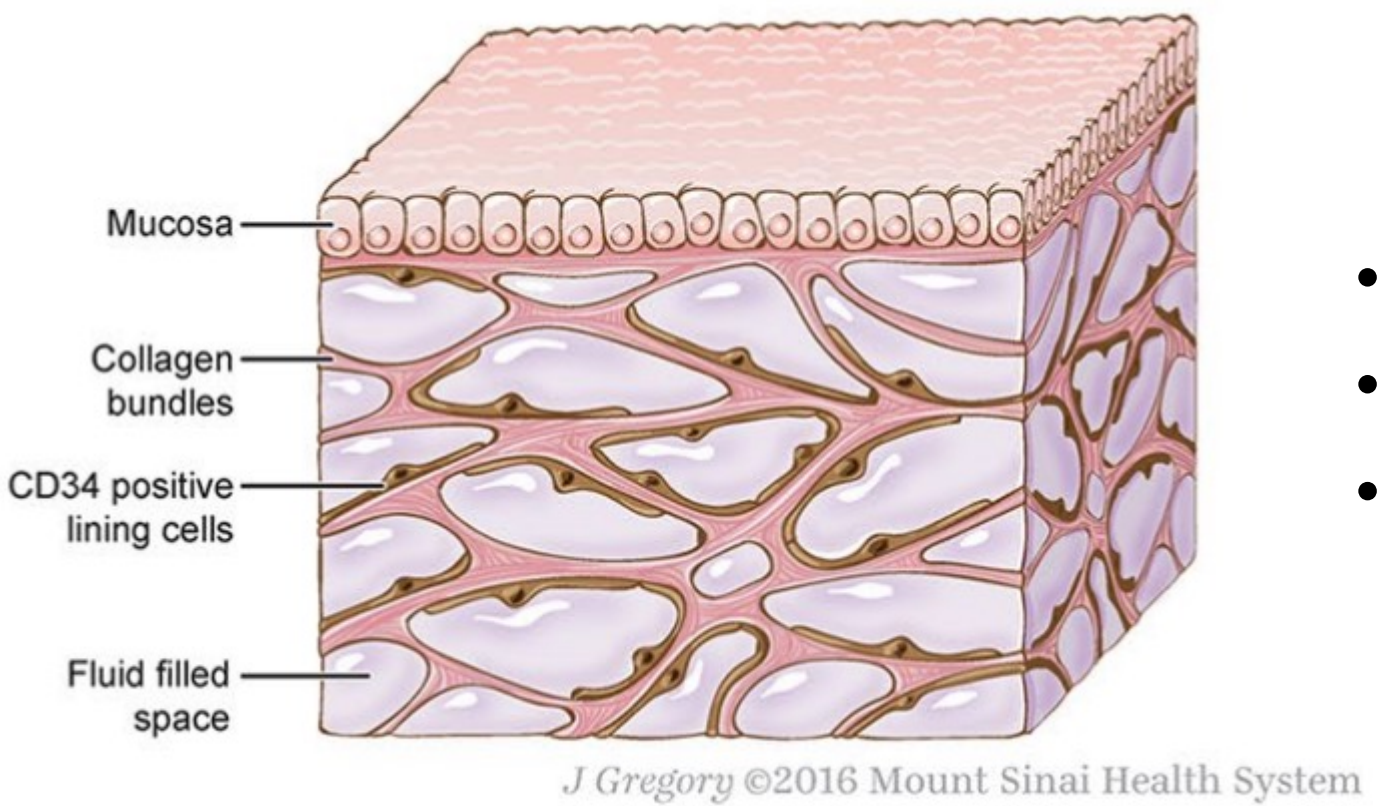
40 MN Array

28 MN Array

Collected ISF in uL
Top # = per patch
Bottom # = per MN

1.9 0.048	0.35 0.009
1.3 0.046	0.61 0.022

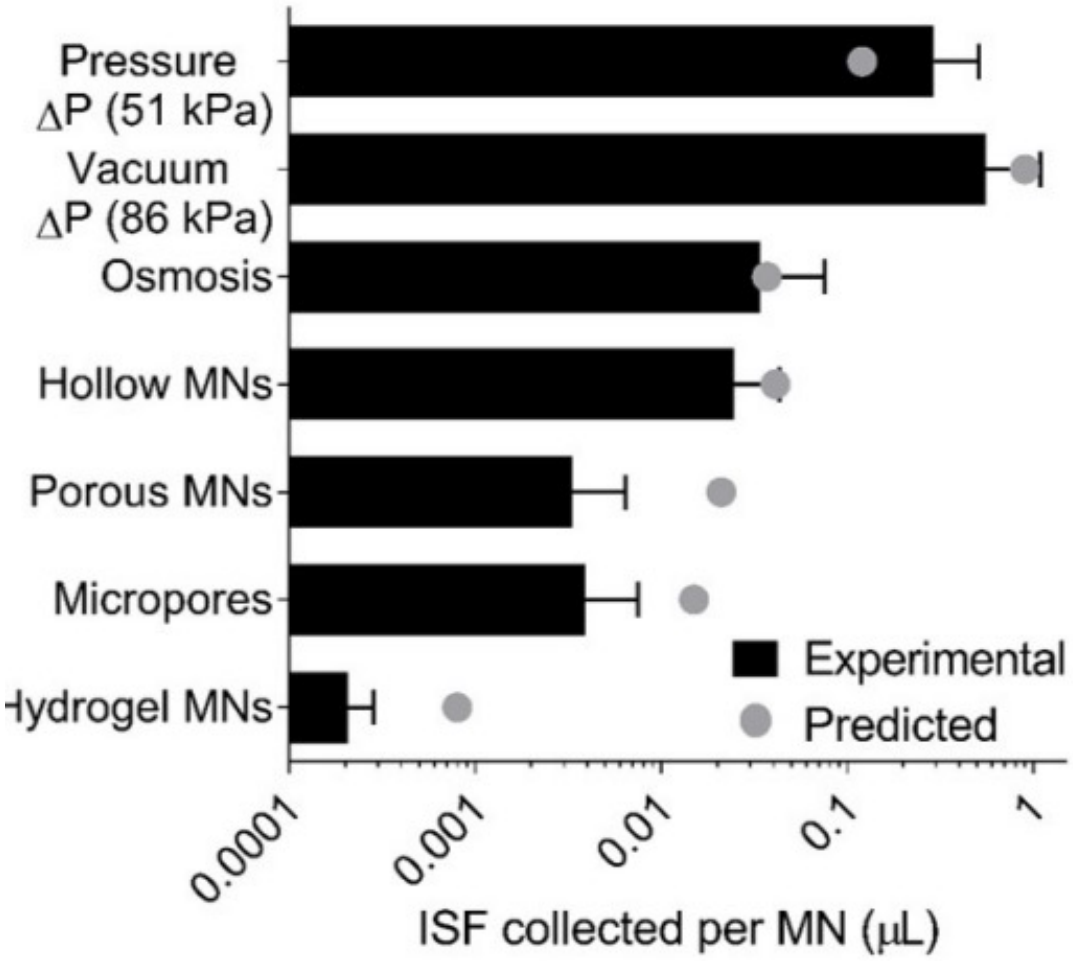
Fluid in skin is not free fluid

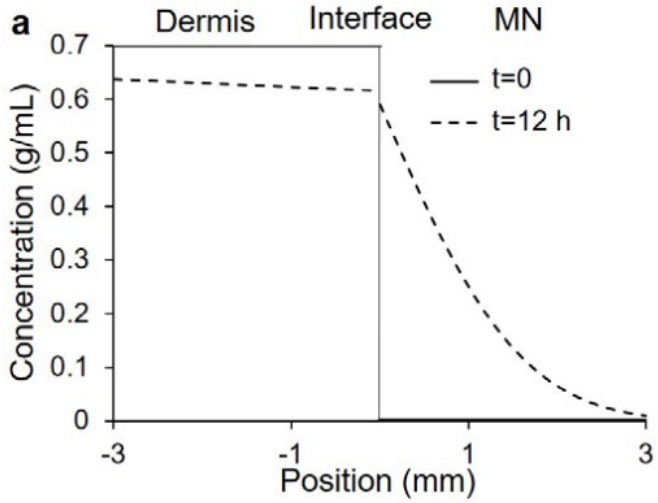


- Proteoglycans
- Glycoproteins
- Hyaluronic acid

Benias, Sci Rep 8, 4947, 2018

Diffusion alone is probably not enough





Self Diffusion Coefficient

Dermal ISF: 8.7×10^{-10} m^2 /s

Water 40°C: 3.2×10^{-9} m^2/s

Prausnitz, PNAS, 115(18), 4583-4588, 2018

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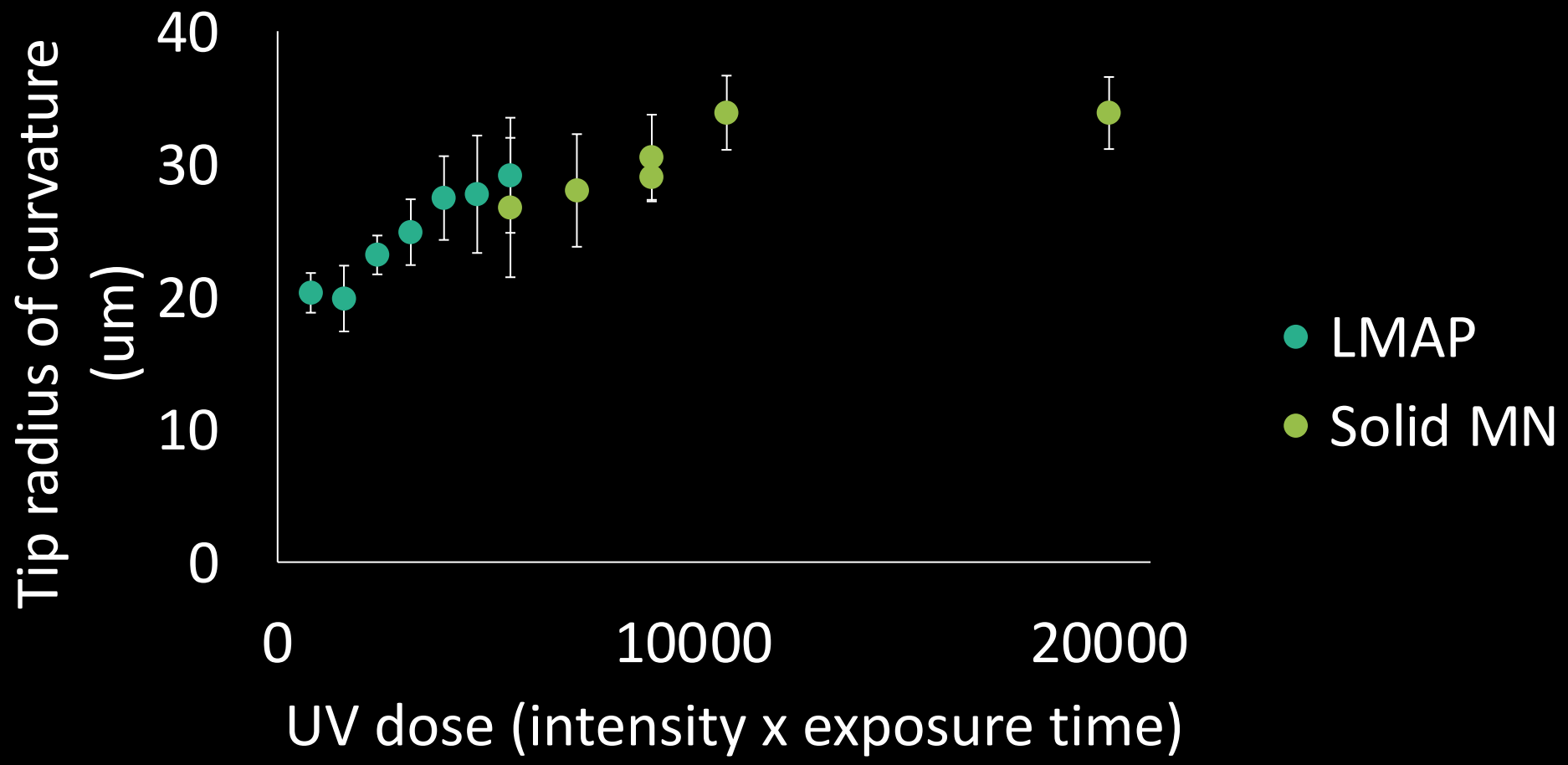
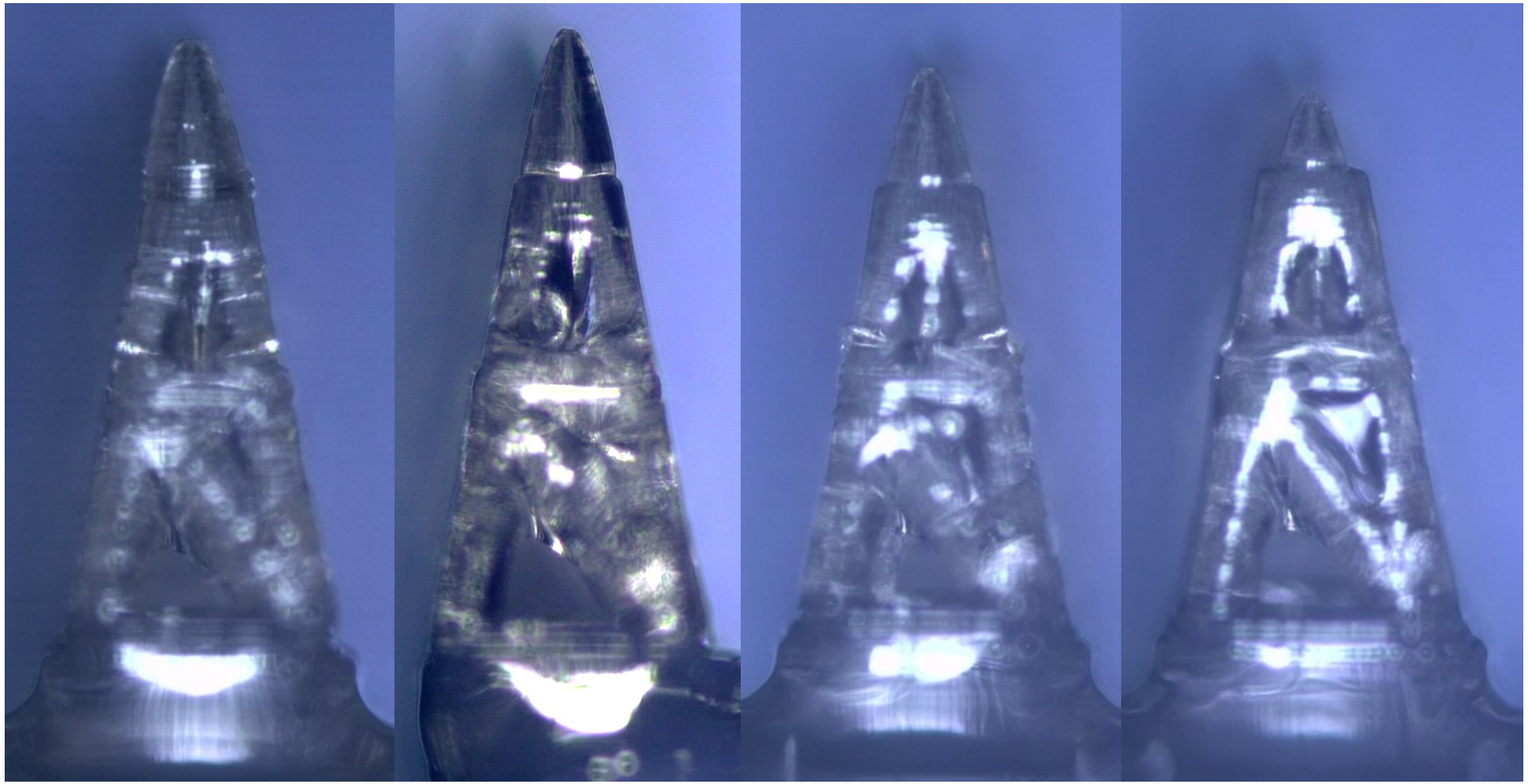
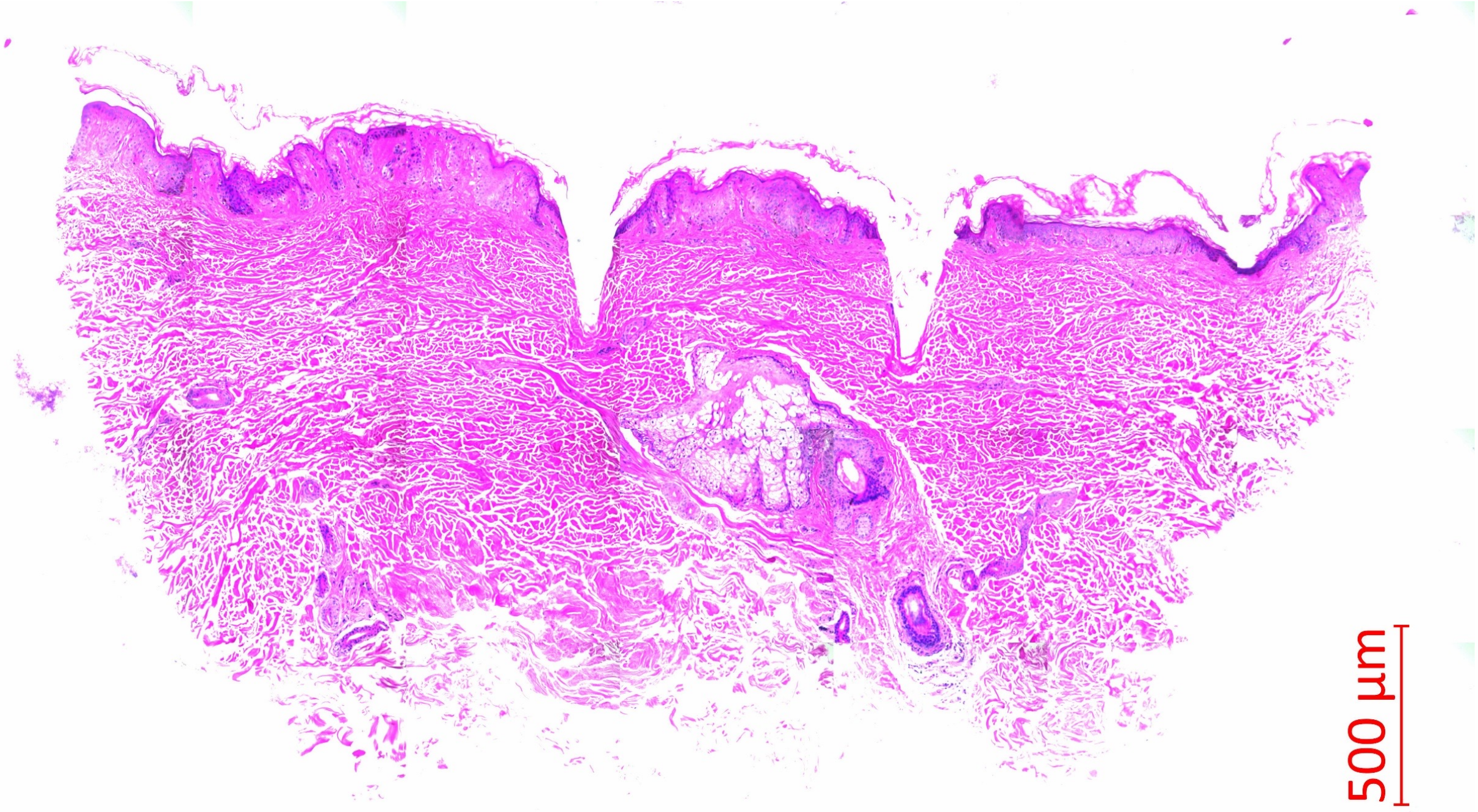
Optimize UV dose for tip sharpness



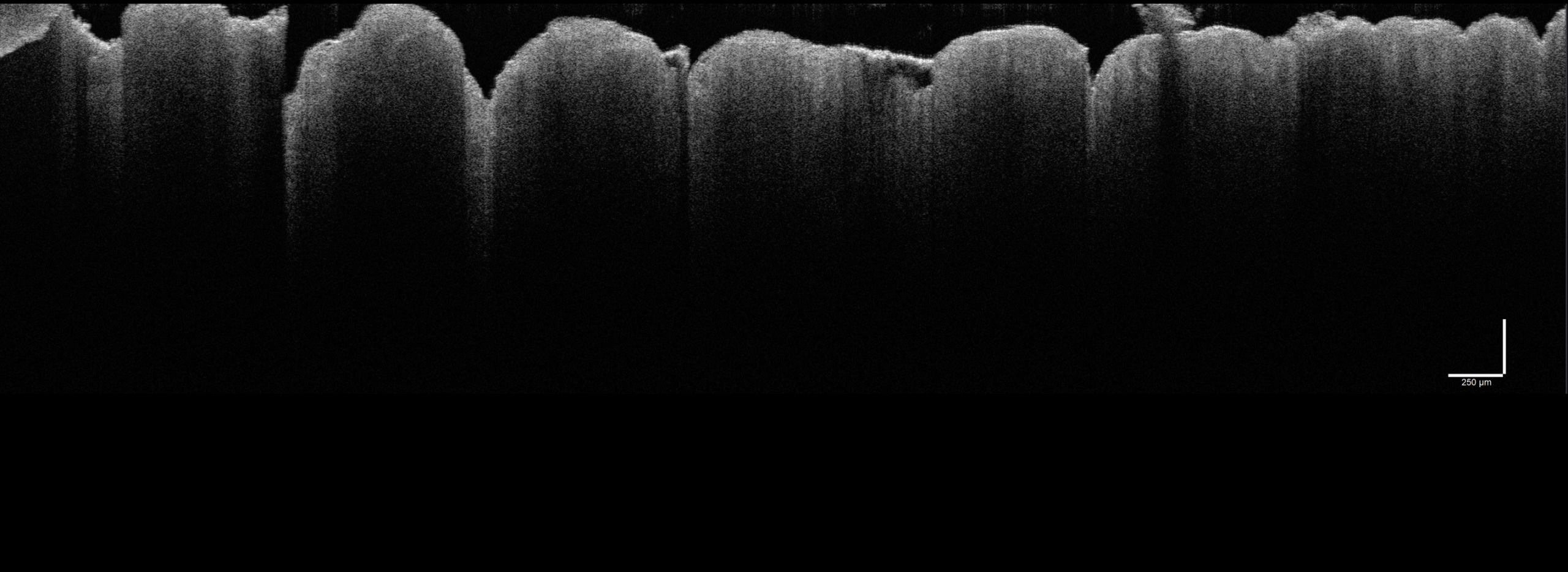
UV Dose (a.u.)	5600	2400	1600	800
Tip Radius (um)	29 ± 4	23 ± 1	20 ± 2	20 ± 1



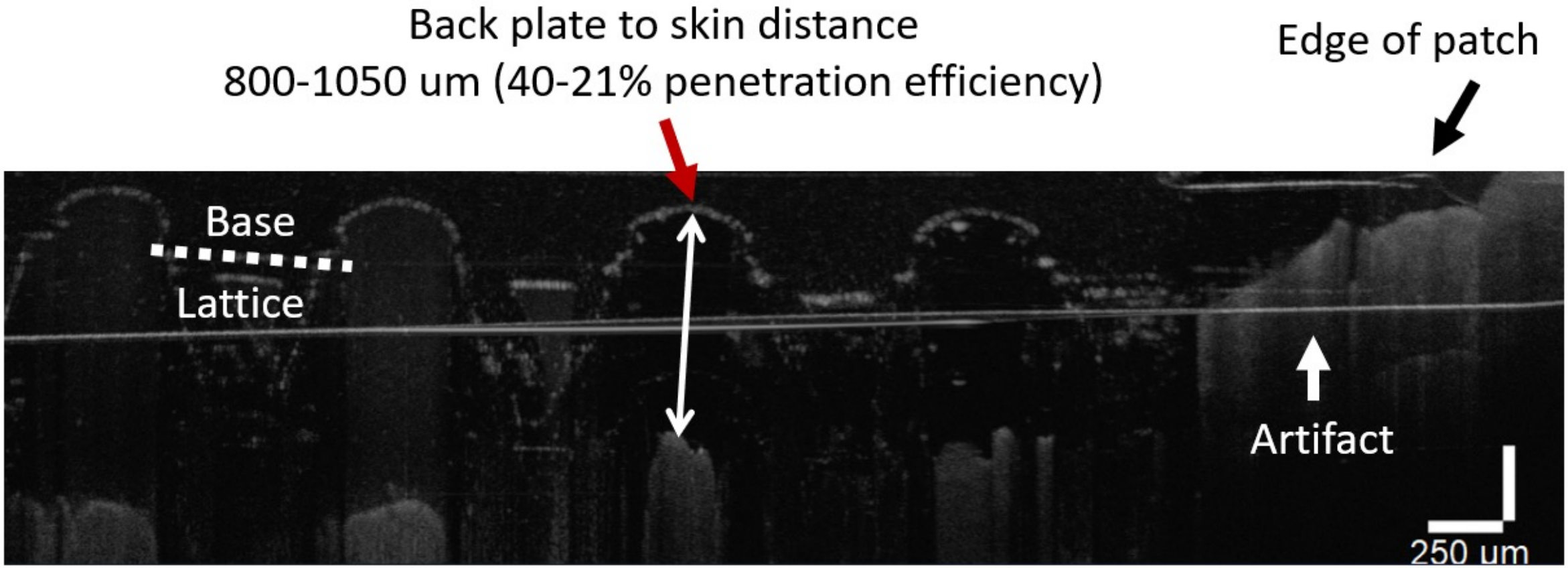


Collaboration with Davis Lab

OCT of human skin after LMAP application



Use OCT to assess penetration



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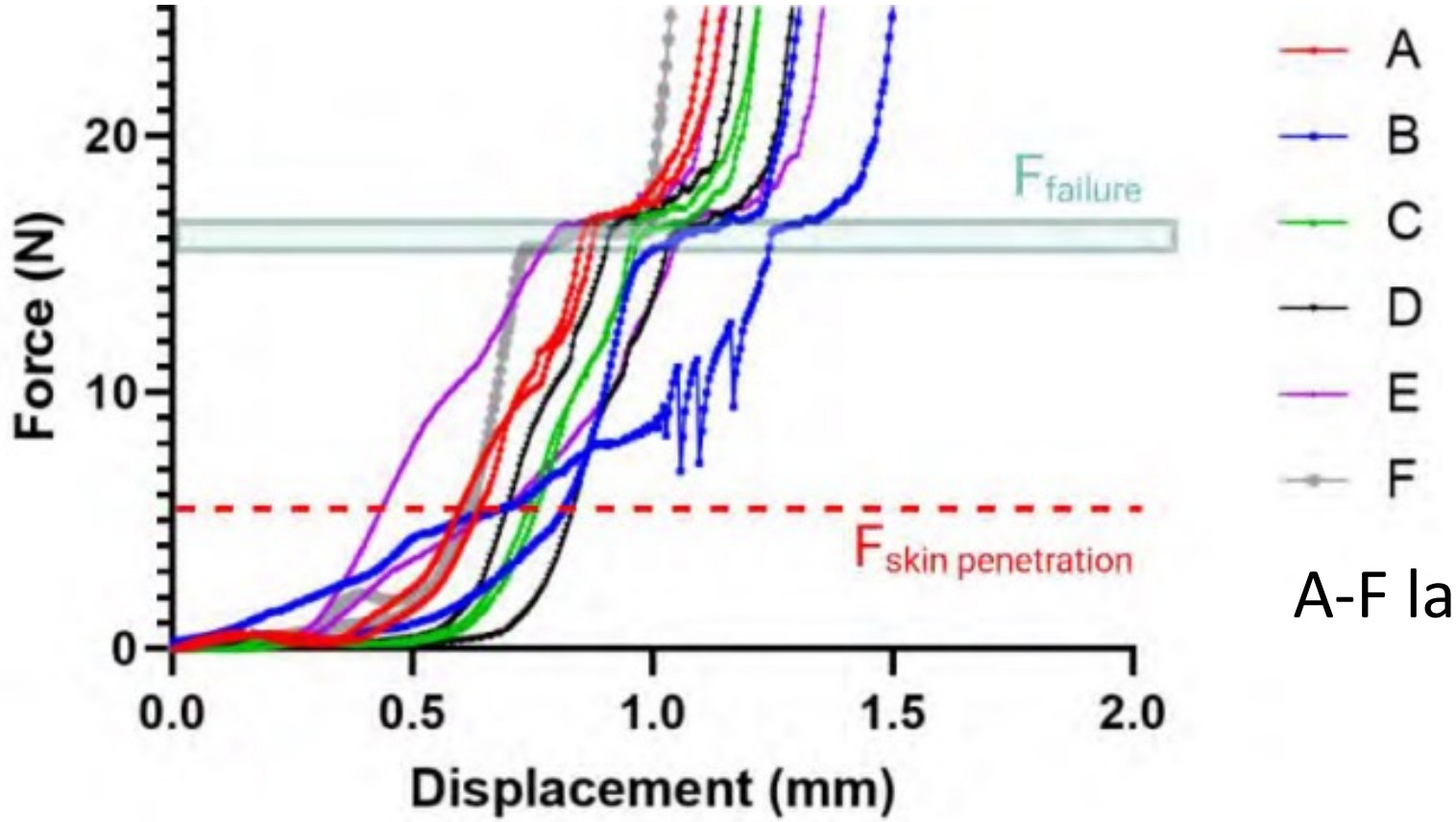
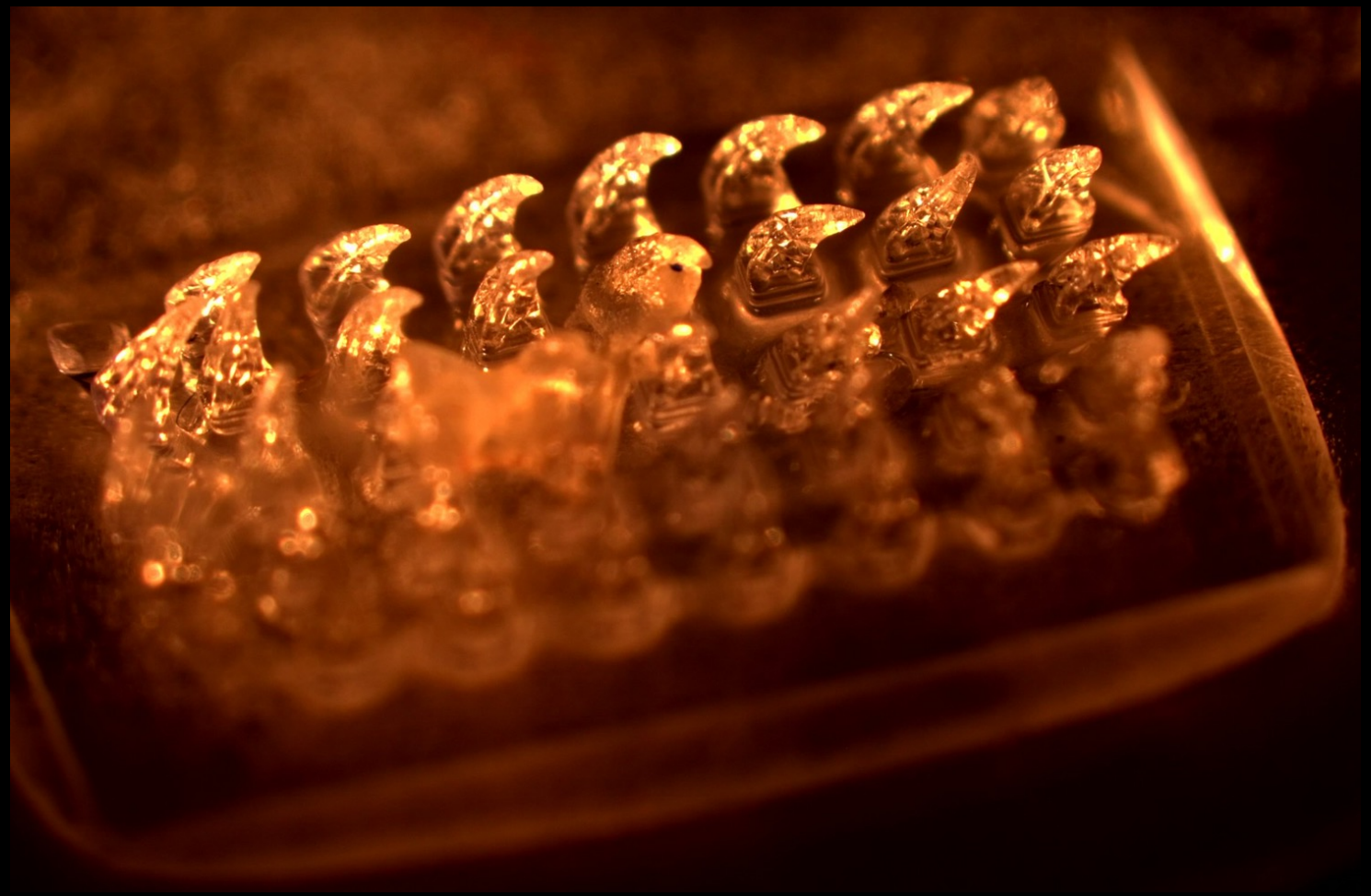
A Safety Factor of 3 in Axial Loading



A-F label different designs

Courtesy of Netra Rajesh

Microneedles bend without fracture



Learnings

- Collect 50 uL in 20 minutes: ΔP likely necessary
- Consistent penetration: 20-30 µm sharpness ok, 50% of design height penetrates, consistency TBD
- Mechanically robust: axial loading ✓, lateral force evaluation needed
- Easy ISF retrieval from device
- Easy to apply
- Can sterilize

Acknowledgement

Mentors: Joseph DeSimone, Sharon Pitteri, Steven Poplack

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Stanford Cancer Imaging Training Program