Bioprinting on 3D nanostructures with the Alveole PRIMO

ENGR 241 Fall 2019
Final Presentation

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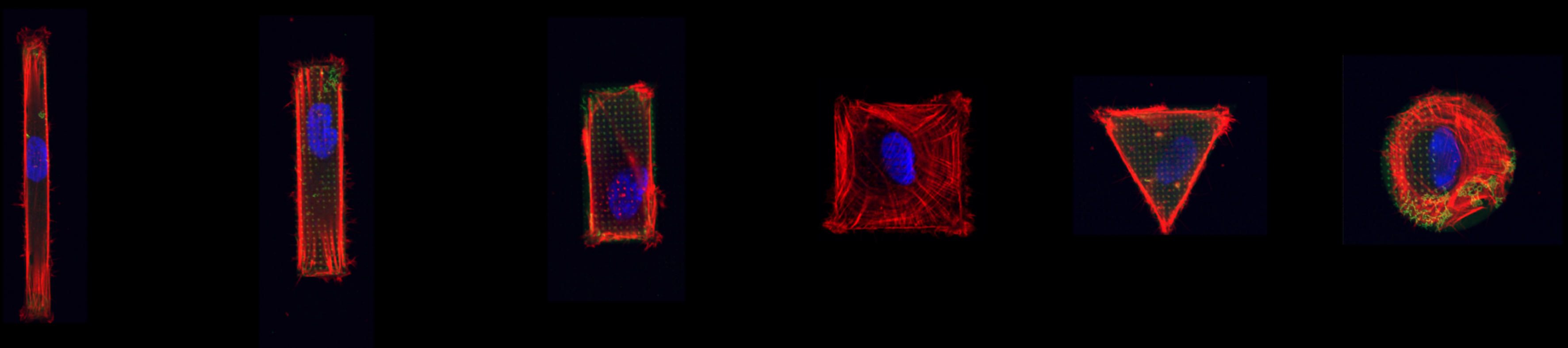


Motivation & Goals

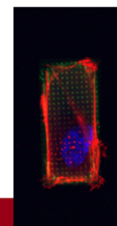
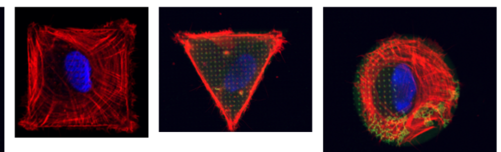
- **To fabricate large amounts of vertical nanostructures of <u>various shapes</u> using <u>photolithography & dry etching.</u>** ***Part A***

  This procedure will shrink the nanostructures by dry & wet etching to achieve a resolution down to 200 nm without the need of E-beam lithography. It's less costly and much more efficient.

  Xiao Li et al. ***Nature Protocols***, vol. 14, p1772–1802 (2019)

- **To pattern biomolecules on 3D nanostructures using <u>PRIMO</u> to allow stable cell adhesion in given regions.** ***Part B***

  1. Because the surface of nanostructures are not flat, the biomolecules adsorbed on the surface might not be evenly distributed. We will finely tune the Primo Pattern to create a platform for the uniform and stable coating of biomolecules.
  2. Control cell adhesion on a defined area, a more systematic way to modulate cell tension, which allows us to study tension-based mechanobiological pathways (YAP, mTOR, Hippo, etc).

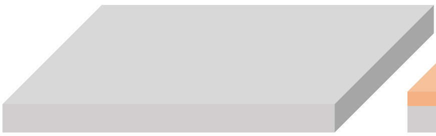



Xiao Li et al. ***Manuscript to be submitted***

Materials & Methods

Part A - Nanostructured Chip Fabrication

1

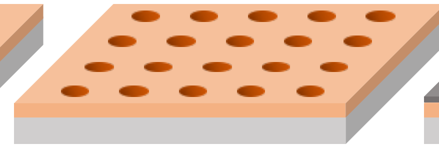


- Quartz Wafer Cleaning using SRD
- HMDS coating using YES Oven

2

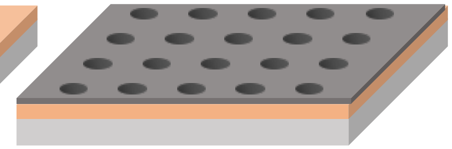
- 3612 photoresist coating using svgcoat

3



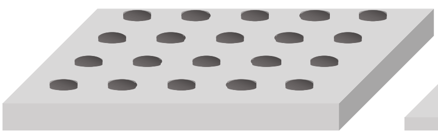
- **Nanopillar patterning using Heidelberg II**
- Developer coating using svgdev

4



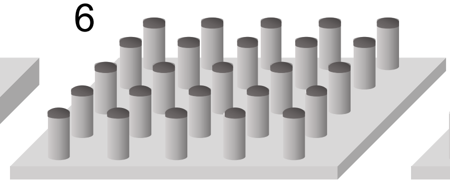
- Cr deposition using AJA Evaporator

5



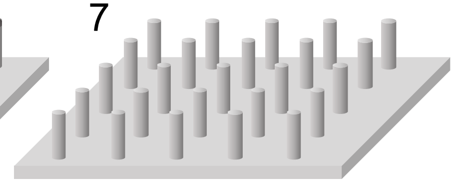
- lift-off with acetone and IPA

6



- **Dry etching using PT-Ox to create nanostructures of various shapes**

7



- Wet etching using 20:1 BOE to shrink vertical nanostructures isotropically

8

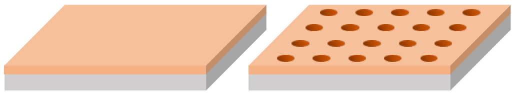
- Wafer cutting into small pieces (chips)

Chips decorated with ~
1-μm-tall,
2.5-μm-pitch,
200-nm-diameter vertical
nanostructures

Results (Part A) – Nanostructures Patterning using Heidelberg2

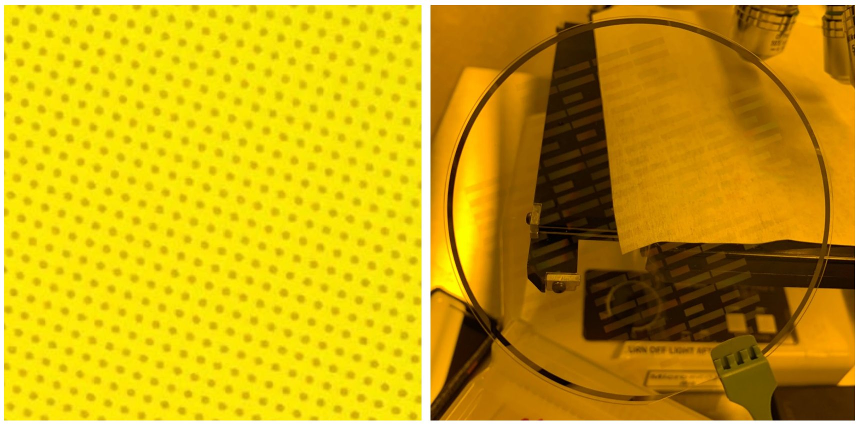
Photolithography Patterning

2 3



- 3612 photoresist coating using svgcoat

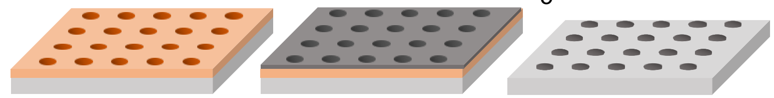
- **Nanopillar patterning using Heidelberg II**
- Developer coating using svgdev



After Resist Developing; Before Cr deposition
(Featured Pillar diameter ~700 nm)

Cr Deposition and Lift-Off

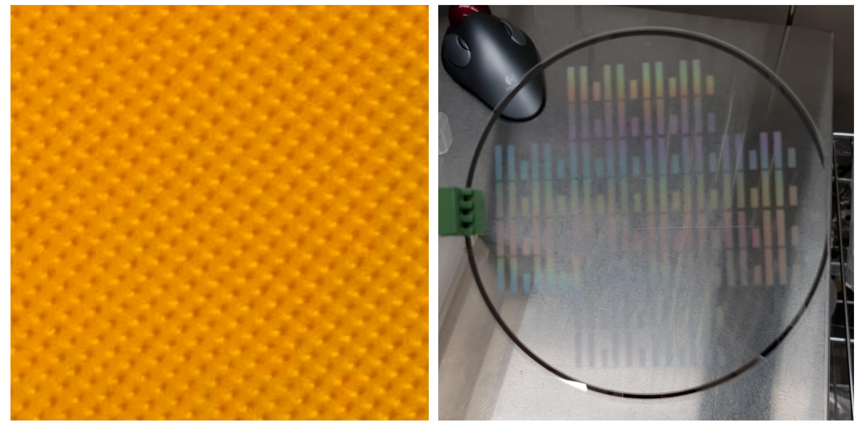
3 4 5



- **Nanopillar patterning using Heidelberg II**
- Developer coating using svgdev

- Cr deposition using AJA Evaporator

- lift-off with acetone and IPA

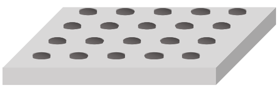


After Cr deposition and Lift-off
(Featured Pillar diameter ~700 nm)

Results (Part A) –Dry Etching using PT-Ox

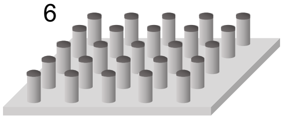
Data of Dry Etch Experiments

5



- lift-off with acetone and IPA

6

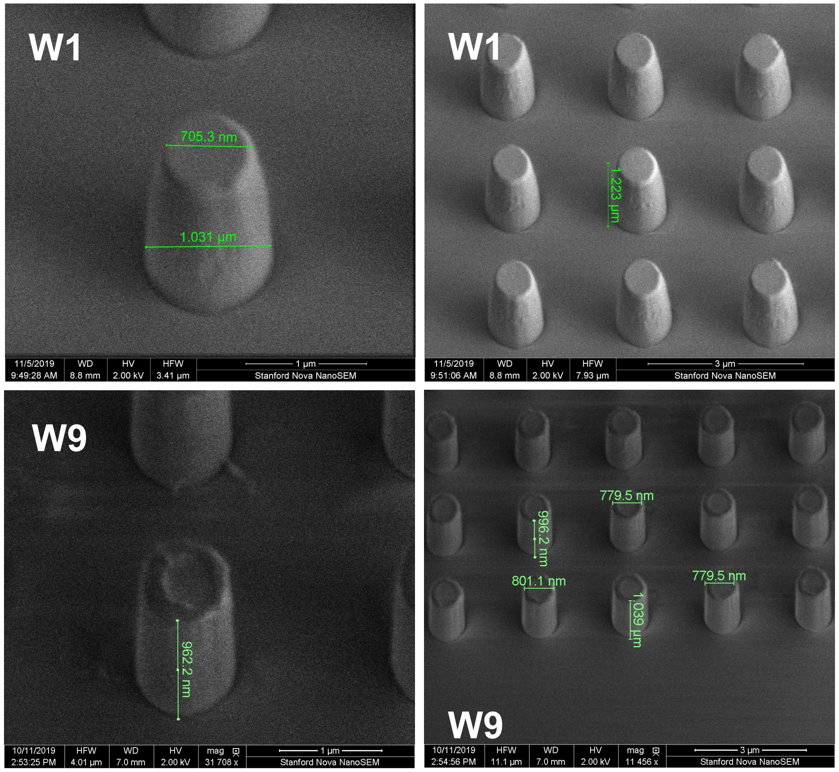


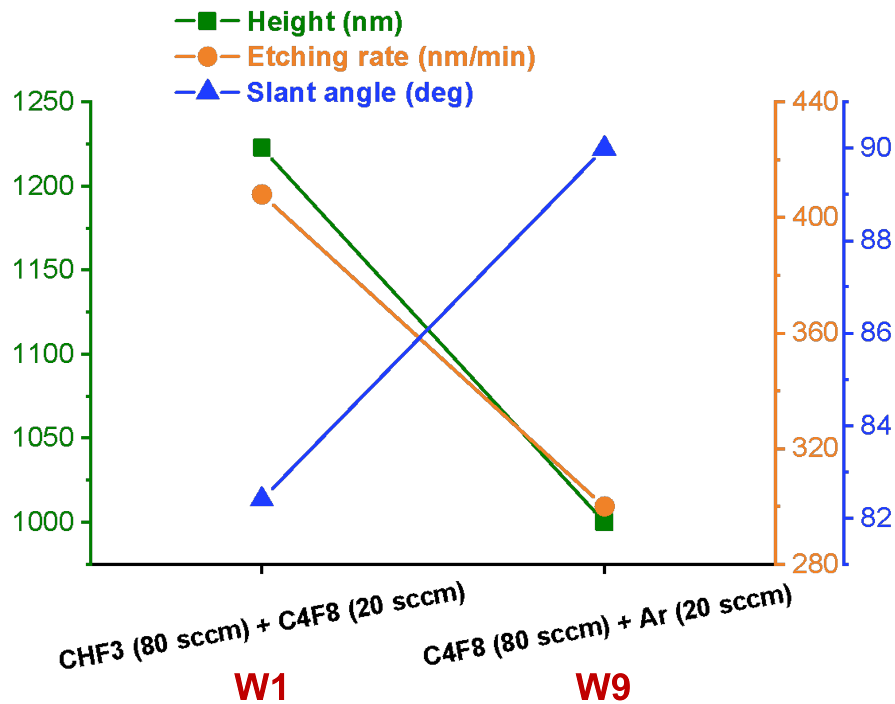
- **Dry etching using PT-Ox to create nanostructures of various shapes**

No.	Pattern	CHF_3 Flow Rate (sccm)	C_4F_8 Flow Rate (sccm)	H_2 Flow Rate (sccm)	Ar Flow Rate (sccm)	Bias power (W)	Etching Time (min)	Height (nm)	Diameter (nm)	Etching Rate (nm/min)	Slant Angle (deg)	Optimized Parameters
W1	+++−+	80	20	40	0	200	3	~1223	~705 (top) ~1031 (bottom)	~408	~82.4	*CHF_3 flow rates:* 80 sccm *ICP Power:* 1500 W *Pressure:* 7 mT
W2	+++−+	80	20	40	0	200	3+3	~2150	~613 (top) ~1263 (bottom)	~309	~81.4	
W3	+++−+	80	20	40	0	200	3+3+3	~2984	~511 (top) ~1186 (2/3-height) ~1288 (bottom)	~278	~66.6	
W4	+−+−+	80	0	40	0	200	3	~909	~724 (top) ~967 (bottom)	~303	~82.4	
W5	+−+−−	80	0	40	0	100	6	~1200	~815 (top) ~1230 (bottom)	~200	~80.2	
W6	+−++−	80	0	40	10	50	10	~566	~790 (top) ~1205 (bottom)	~56.6	~69.9	
W7	+−−−+	80	0	10	0	200	3	~1009	~722 (top) ~1103 (bottom)	~336	~79.3	
W8	+−−−−	80	0	10	0	100	6	~1293	~775	~216	~90.0	
W9	−++++	0	80	40	20	200	3.3	~1000	~780	~300	~90.0	*No CHF_3*

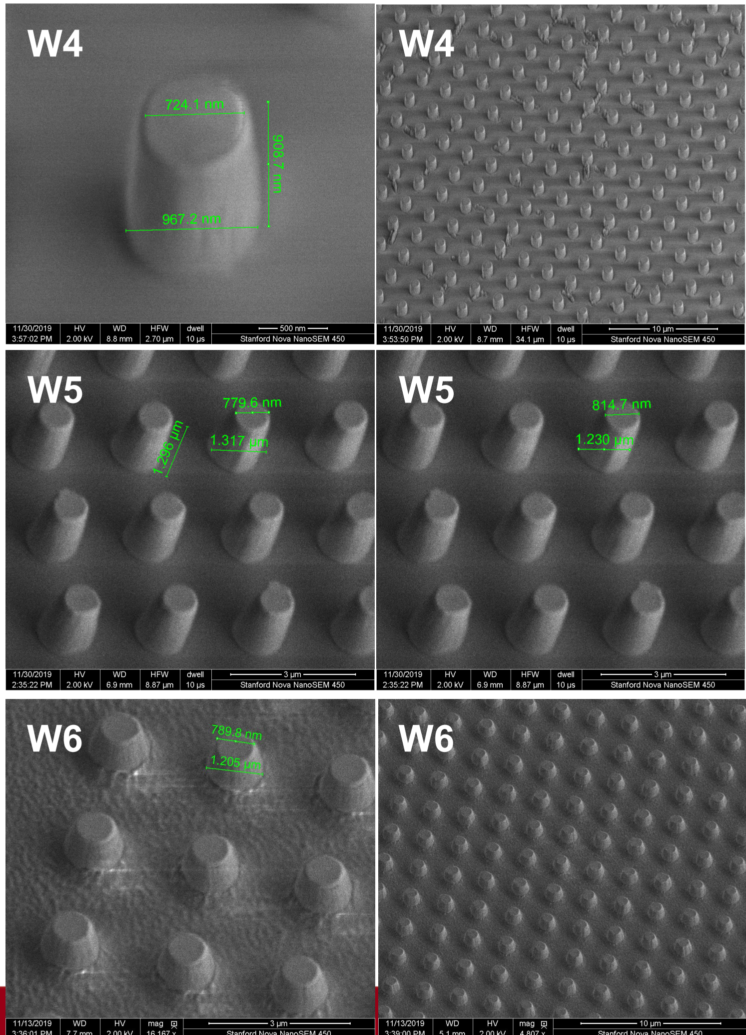
Results (Part A) –Dry Etching using PT-Ox

C_xF_y Chemistry effects



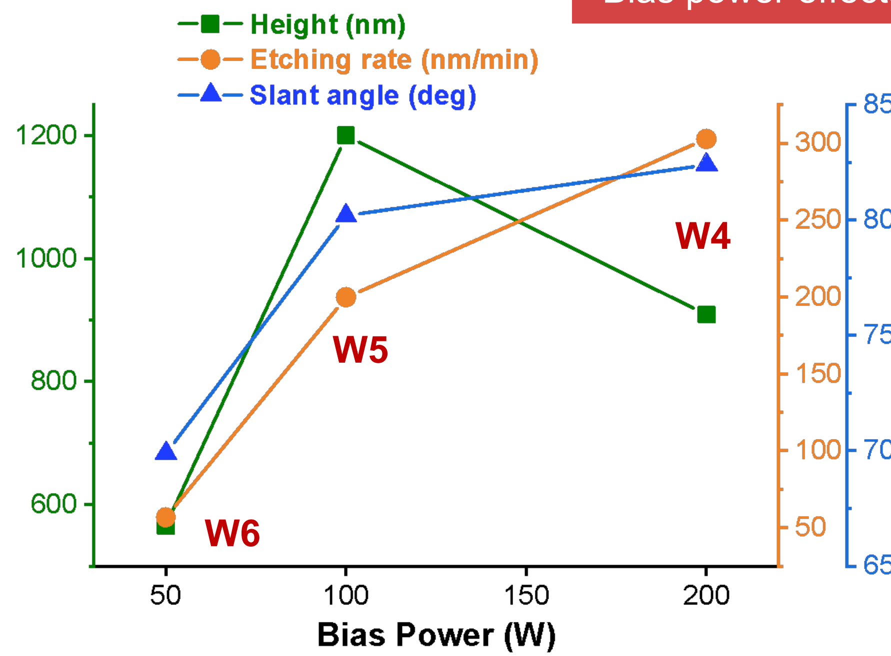


Fixed parameters:
H_2 flow rate: 40 sccm; Bias Power: 200 W; Etching time: 3 min



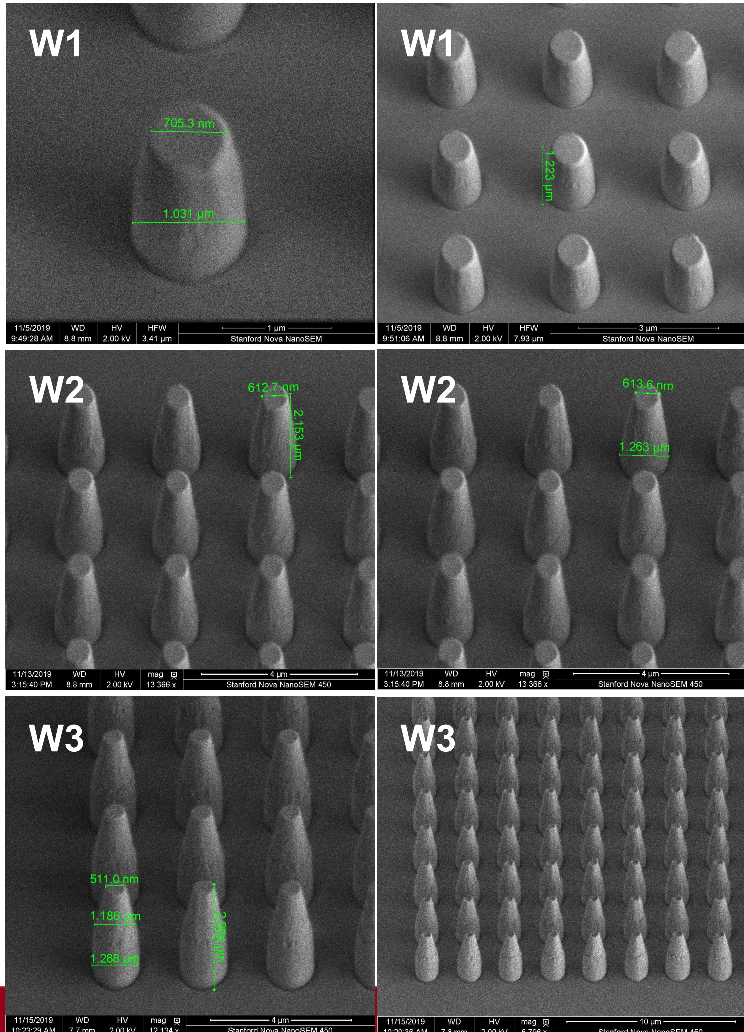
Results (Part A) –Dry Etching using PT-Ox

Bias power effects

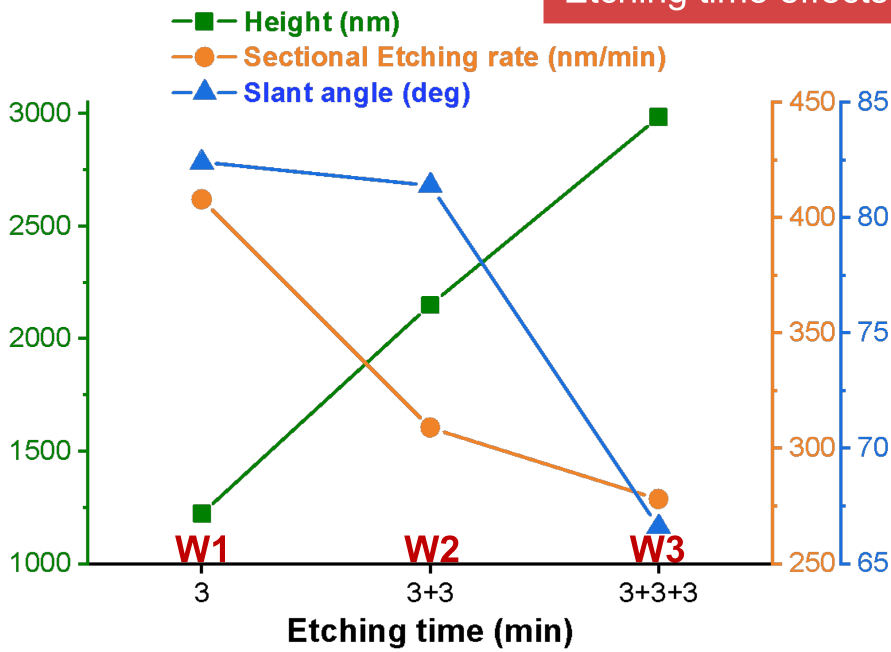


Fixed parameters:
CHF_3 flow rate: 80 sccm; H_2 flow rate: 40 sccm; No C_4F_8 & *Ar**

Results (Part A) –Dry Etching using PT-Ox



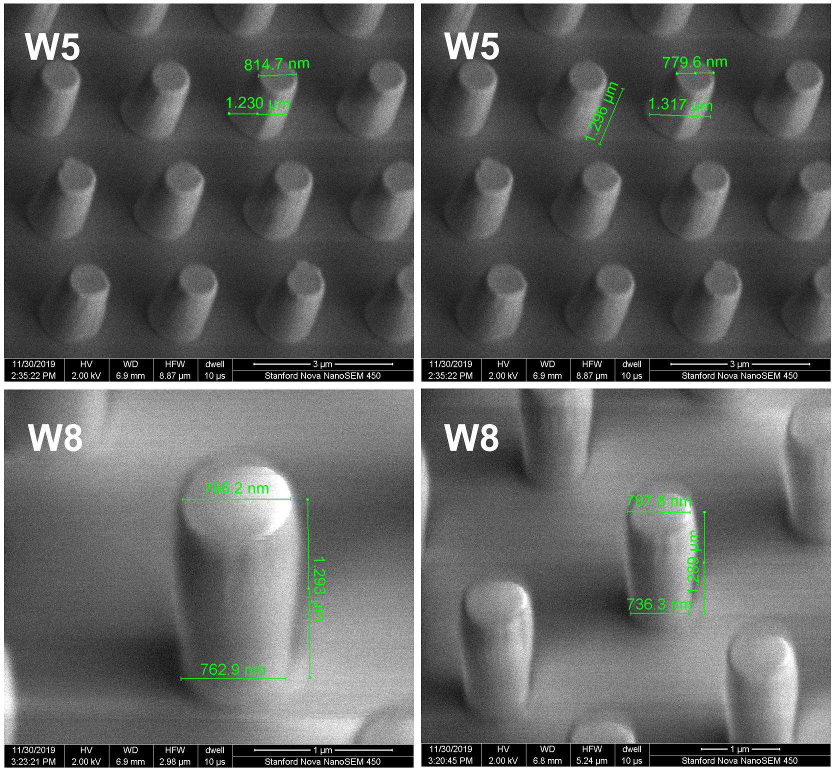
Etching time effects

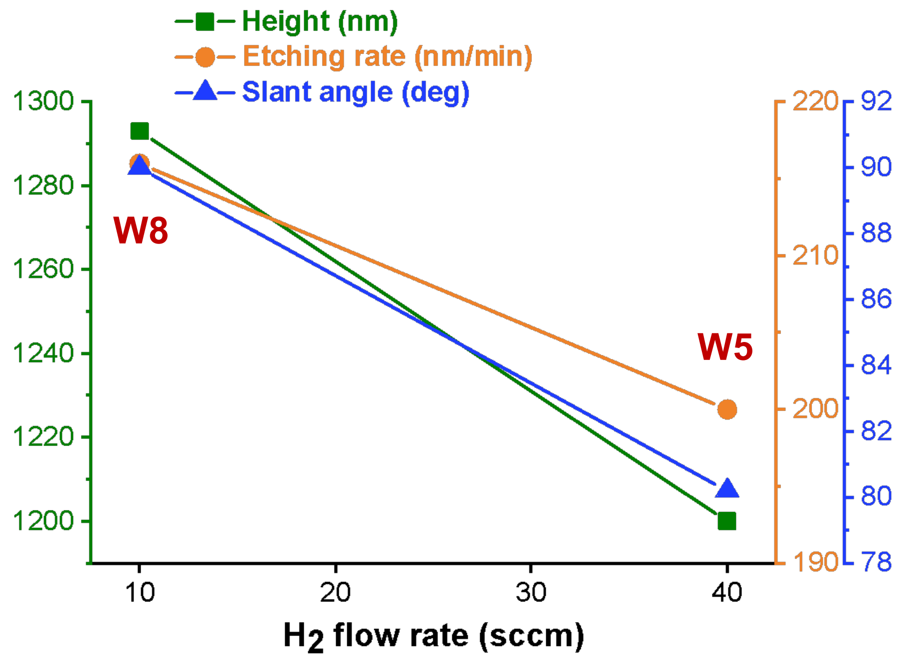


Fixed parameters:
CHF_3 flow rate: 80 sccm; C_4F_8 flow rate: 20 sccm;
H_2 flow rate: 40 sccm; Bias Power: 200 W; No Ar

Results (Part A) – Results – Dry Etching using PT-Ox

H_2 effects





Fixed parameters:
CHF_3 flow rate: 80 sccm; Bias Power: 100 W;
Etching time: 6 min; No C_4F_8 & Ar

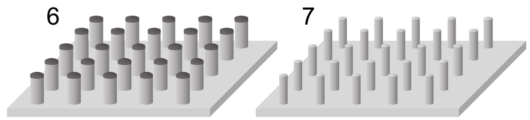
Results (Part A) – Results – Dry Etching using PT-Ox

Brief Summary

- C_4F_8-based chemistry gives straighter side walls.
- CHF_3-based chemistry gives tapered side walls (bullet-shaped).
- High H_2 level leads to slower etching rates and the formation of more tapered side walls.
- High Bias power results in faster etching rates and the creation of straighter nanopillars.

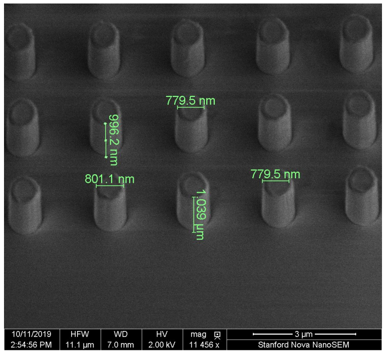
Results (Part A) – Wet Etching

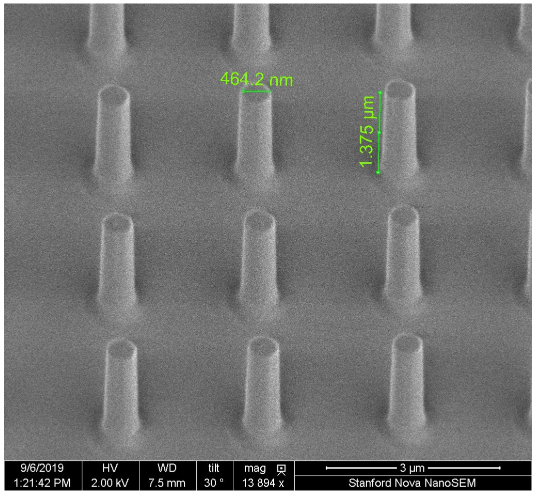
Isotropic Shrinkage of the Pillars by Wet Etching



- **Dry etching using PT-Ox to create nanostructures of various shapes**
- Wet etching using 20:1 BOE to shrink vertical nanostructures isotropically

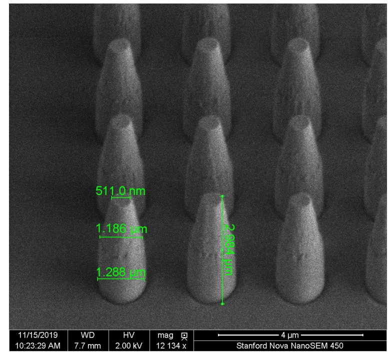
Wet etching can also give us some interesting shapes

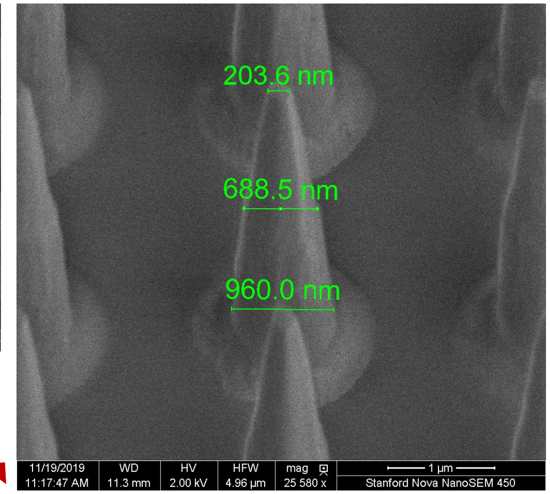




1) Cr Etching for 20 min
2) 20:1 BOE for 5 min

- Height: raised by ~330 nm
- Diameter: shrunk by ~320 nm





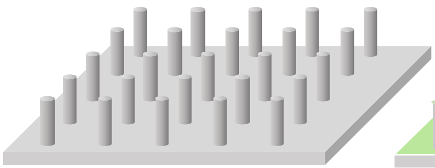
1) No Cr Etching
2) 20:1 BOE for 4 min

- Top Diameter: shrunk by ~307 nm
- Middle Diameter: shrunk by ~498 nm
- Bottom Diameter: shrunk by ~320 nm

Materials & Methods

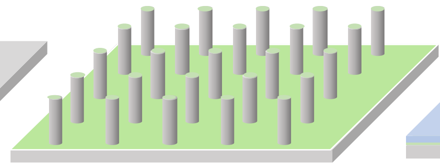
Part B - Bioprinting using PRIMO

1



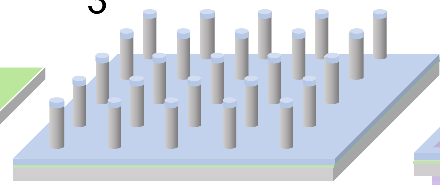
- Nanostructured chips cleaning using plasma cleaner

2



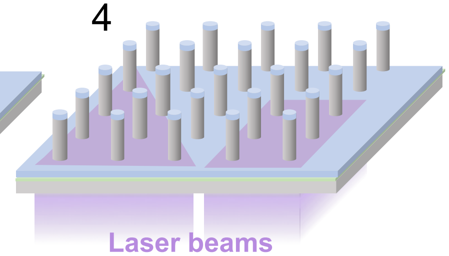
- Poly-L-lysine coating
- Anti-fouling polymer (e.g. mPEG-SVA) coating & incorporation

3



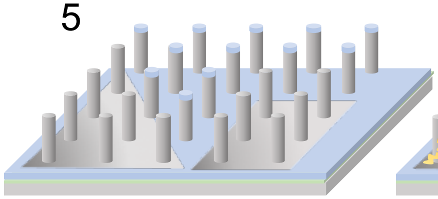
- PLPP (photoactivatable gel) gelation on the chips

4



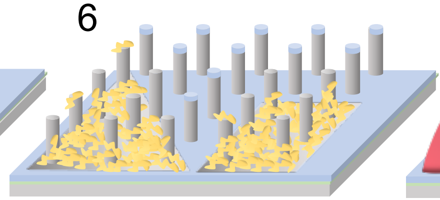
- Photopatterning with PRIMO

5



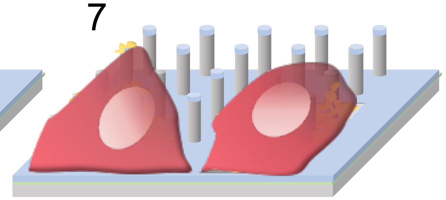
- PLPP gel removal
- Chips rehydration

6



- Cell adhesion proteins (e.g. fibronectin, gelatin) coating in the patterned regions

7

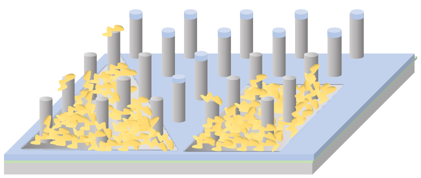


- Cell culturing on the patterned area

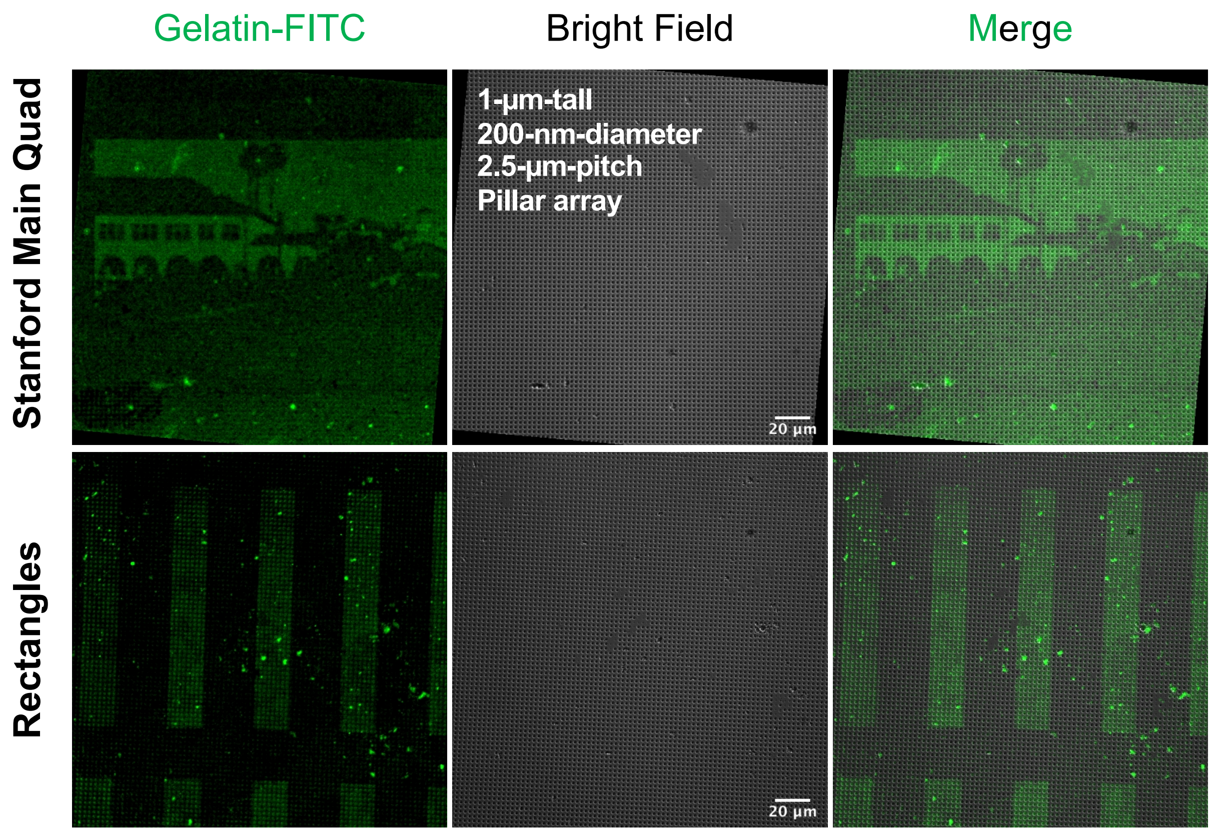
Cells are adhered on the nanostructured chip and confined in given regions.
- Vertical nanostructures induce cell membrane curvature.
- Cell are shaped differently.

Results (Part B) – Bioprinting using PRIMO

PRIMO Patterning



- Nanopillar chips are coated with **F**luorescein **I**so**T**hio**C**yanate-tagged gelatin.
- Images are resolved using confocal microscope (60X).



Results (Part B) – Bioprinting using PRIMO

Mammalian Cells Culture on PRIMO-patterned Nanopillar Chip

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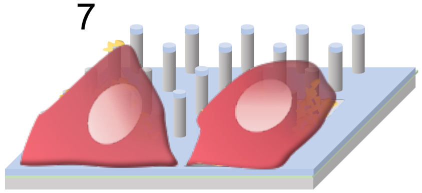
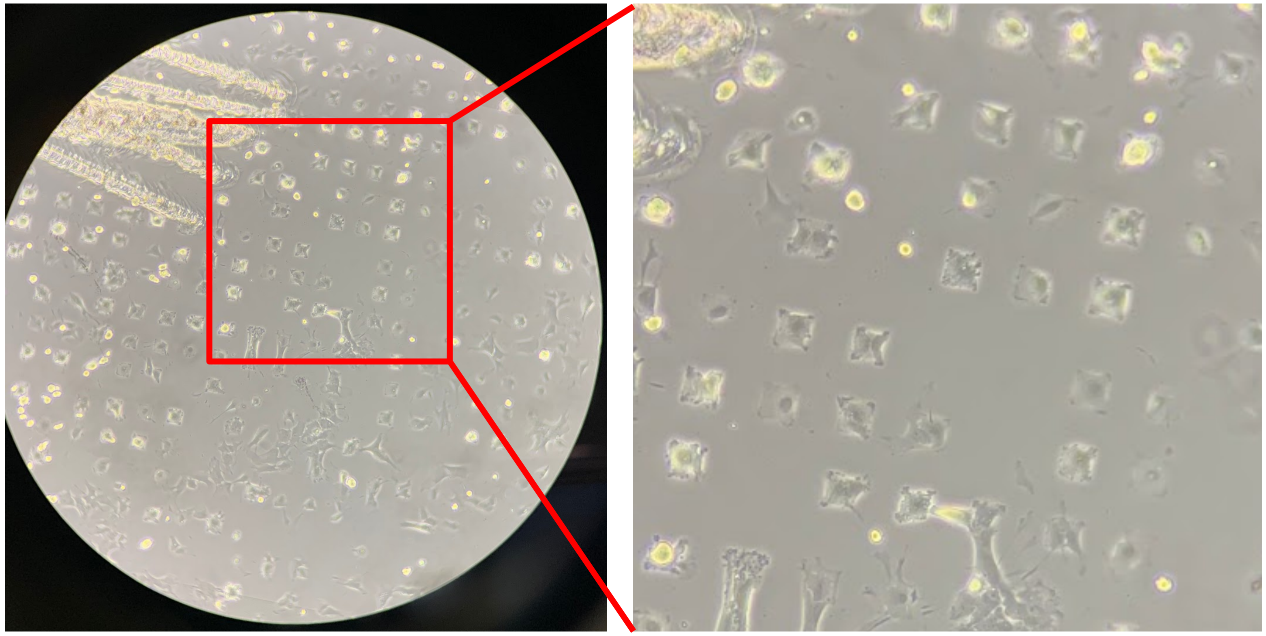


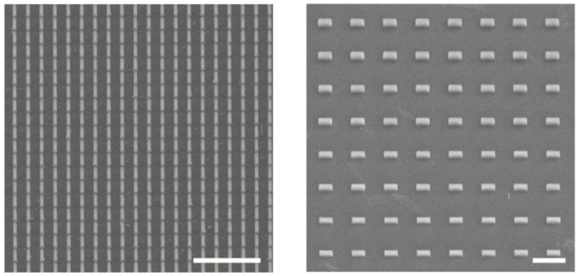
- Cell culturing on the patterned area

- Nanopillar chips are patterned with **square** array.
- Nanopillar chips are then coated with unlabeled gelatin.
- Cells are adhered on patterned/gelatin-coated area.
- Images are resolved using bright field mode in epifluorescence microscope.

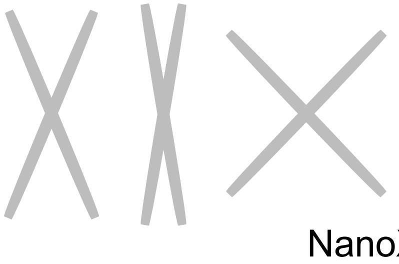


Future Plans (Winter Quarter)

- Apart from nanopillar-derived patterns, we are also going to fabricate chips decorated with **nanobar (various sizes)**, **nanoX (various angles)** to study how cells respond to various physical perturbations and investigate cell membrane curvature-dependent biological pathways.



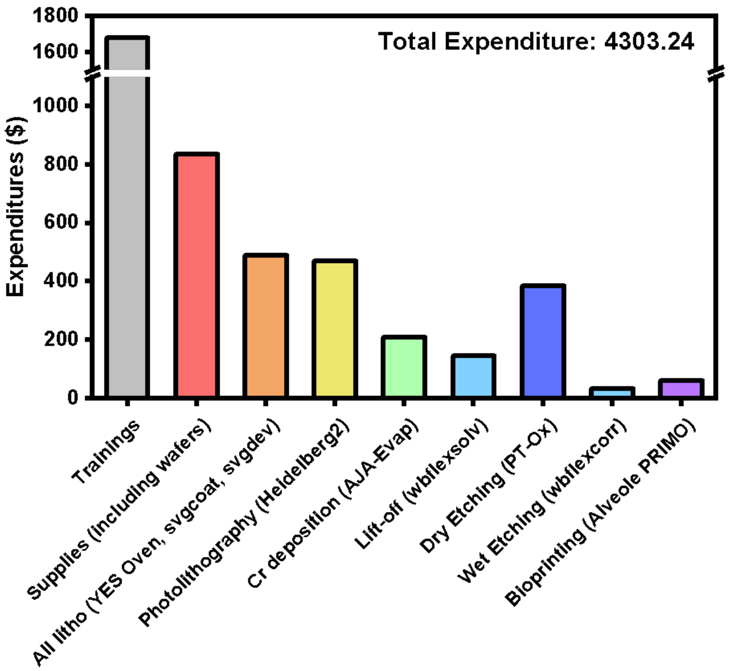
Zhao et al., 2017, ***Nat. Nanotechnol.*** 12
Li et al., 2019, ***Nat. Protoc.*** 14



- Aside from using 4' quartz wafer, we plan to fabricate vertical nanostructures and print biomolecules on **thinner chips**, which can be used for **super-resolution cell imaging** experiments.

- Design PRIMO patterns and Optimize PRIMO experimental conditions, such as PLL-mPEG coating density, PLPP gelation protocol, and UV dose, etc.

Quarterly Budget



Acknowledgements

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- Stanford Nanofabrication Facility (SNF)
- Stanford Nano Shared Facilities (SNSF)
- Stanford University Department of Chemistry
- Prof. Jonathan Fan
- Prof. Roger T. Howe
- Charmaine Chia (Course TA)

Stanford University