

Use of Novel Surfaces to Reduce Bioadhesion on Infrastructure



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Overview

- **Research context: DoD relevance**
- **Recent technology history**
- **Novel surface properties employed**
- **Methods**
 - **Material science**
 - **Biological**
- **Bioadhesion resistance efficacy**
- **Conclusions**
- **Future Directions**





Research context: DoD relevance

- **Problem: invasive species stowaways**
 - Snails, plant propagules, microbes
 - Biodiversity and agricultural
- **Current solution**
 - Executive Order 13112
 - Army Forces Washdown Guidance
 - Chemical control: methaldehyde
 - Physical inspection / removal



Armed Forces Pest Management Board

Technical Guide No. 31

RETROGRADE WASHDOWNS:
Cleaning and Inspection Procedures

Published and Distributed by the
EST MANAGEMENT INFORMATION ANALYSIS CENTER
Forest Glen Station
Walter Reed Army Medical Center
Washington, DC 20307-5001
November 2004

Table 6. Estimated cost for vehicle cleaning in Kuwait.

(Assume labor cost of \$5-10/hr and 2 persons per vehicle)

Month	Total Vehicle Cleaning Time, hr	Labor Cost, 2 Pers @ \$5/hr	Labor Cost, 2 Pers @ \$10/hr
Nov	14,322	\$143,220	\$286,440
Dec	10,465	\$104,650	\$209,300
Jan	50,175	\$501,750	\$1,003,500
Feb	84,502	\$845,020	\$1,690,040
Mar	179,784	\$1,797,840	\$3,595,680
Apr	69,894	\$698,940	\$1,397,880
May	17,116	\$171,160	\$342,320
Jun	7,333	\$73,330	\$146,660
Jul	47,043	\$470,430	\$940,860
Total (9 months)	480,634	\$4,806,340	\$9,612,680



CoFrancesco et al. TR-07-8 (2007)
Natural Selections volume 6, issue 2 (2010)



Model species selection

- Terrestrial gastropod
 - Danger to crops
 - Intermediate host to pathogens
 - Nocturnal – vehicles parked



Otala lactea

Scientific Name	Number of Interceptions	Countries of Origin
<i>Theba pisana</i>	376	Spain-Israel-Portugal
<i>Helicella spp</i>	307	Italy-Israel-Greece
<i>Helix aspera</i>	192	Italy-Mexico-Spain
<i>Succinea horticola</i>	146	Italy-Japan-Greece
<i>Helicella conspurcata</i>	119	Italy-Spain-Greece
<i>Cochlicella barbara</i>	109	Italy-Spain-Greece
<i>Cochlicella spp</i>	108	Israel-Portugal-Italy
<i>Monacha spp</i>	54	Israel-Italy-Greece
<i>Helicella maritima</i>	53	Italy-Spain-France
<i>Monacha syrlaca</i>	48	Greece-Italy-Turkey
<i>Helicella cretica</i>	48	Greece-Italy-Turkey
<i>Helicella virgata</i>	47	Italy-Spain-France
<i>Monacha carthusiana</i>	47	Italy-France-Israel
<i>Helicella gigaxii</i>	46	Italy-Spain-Greece
<i>Otala spp</i>	45	Italy-Greece-Spain
<i>Cepaea spp.</i>	42	Hawaii-Brazil-France
<i>Cochlicella conoidea</i>	35	Italy-Germany
<i>Helicella protea</i>	31	Turkey-Italy-Israel
<i>Achatina fulica</i>	31	Hawaii-Taiwan-Hong Kong
<i>Helicella derbentina</i>	28	Turkey-Italy-Greece

Source: Interception Records (1974-1987) USDA-APHIS

Armed Forces Pest Management Board

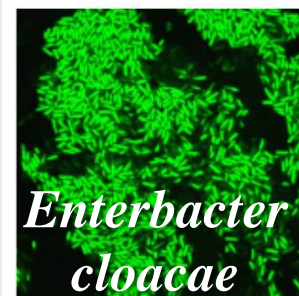
Technical Guide No. 31

RETROGRADE WASHDOWNS:

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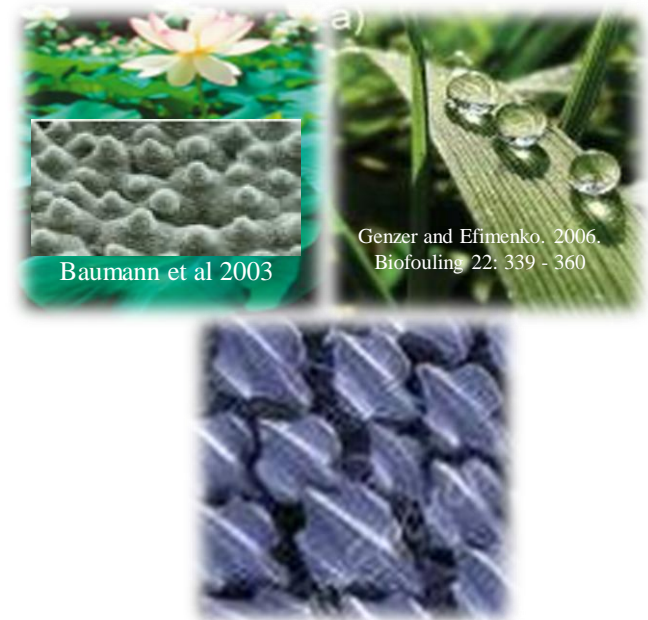
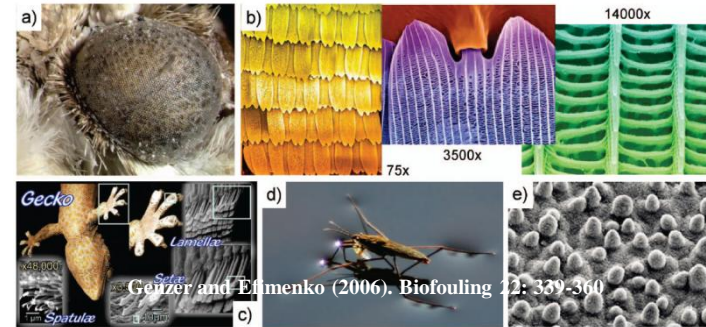
- Biofilm forming bacteria
 - Disease transfer
 - Troop respiratory and urinary infections in hospitals



Enterobacter cloacae

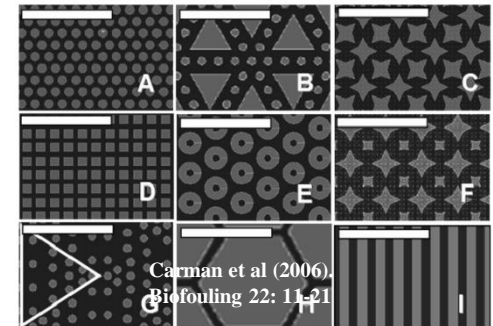
Rationale: Environmentally benign surfaces

- Chemical / physical controls undesirable
 - Bottom paints: copper, TBT
 - Non-target species, legacy contamination
 - Laborious
- Bio-inspiration: leverage biological surfaces that have adapted unique properties
 - Adapted over 1000s of years
- Hypothesis: microstructured pattern tessellations, hydrophobicity, surface properties or hybridizations will reduce bioadhesion via:
 - Behavioral cues (lack of surface recognition)
 - Mechanistic properties (compromised adhesion or micro-fluid layer interfacial interactions)

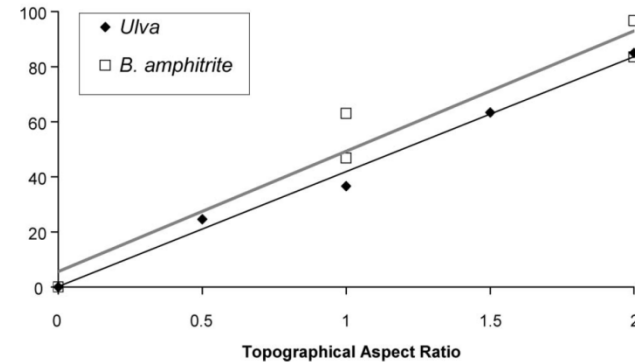


Background: Textured surfaces

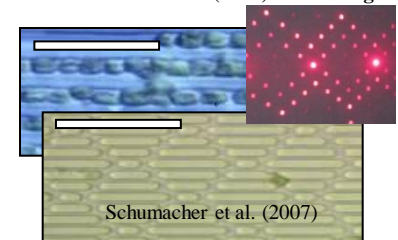
- Leverage hierarchical structures on biological surfaces
- Surface feature height and aspect ratio affects bioadhesion
- Sharklet® technology: Navy
- Greater than 80% reduction in *Ulva* spore and barnacle adhesion
- Not investigated for terrestrial systems



Percent Reduction Relative to the Smooth PDMSe Surface (%)

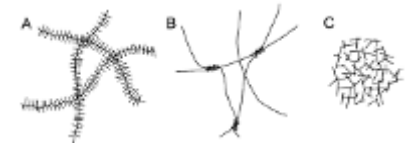
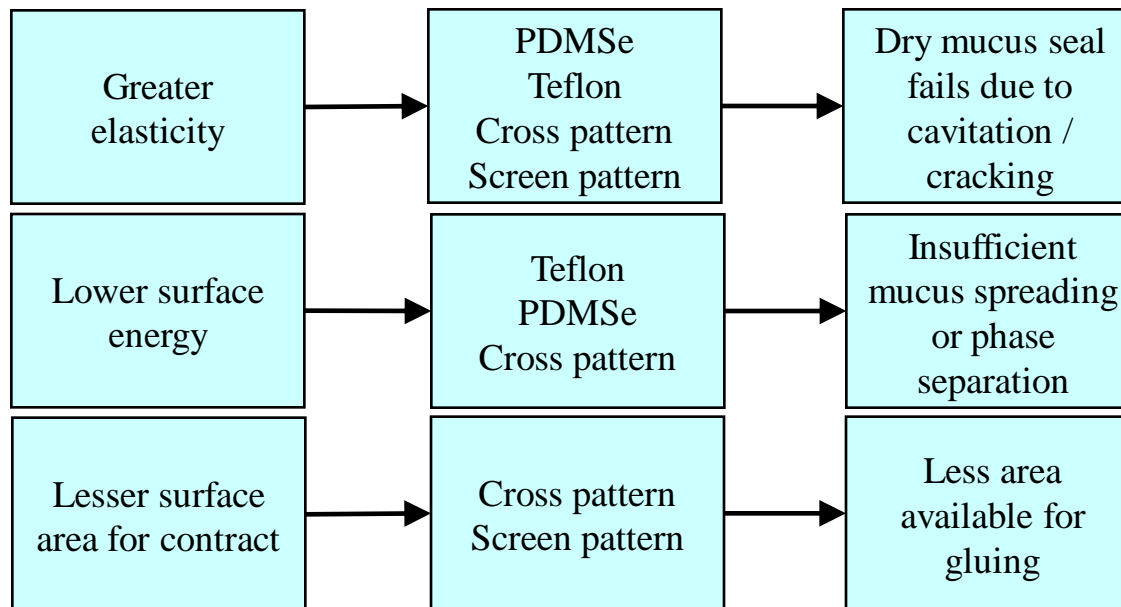


Schumacher et al (2007). Biofouling

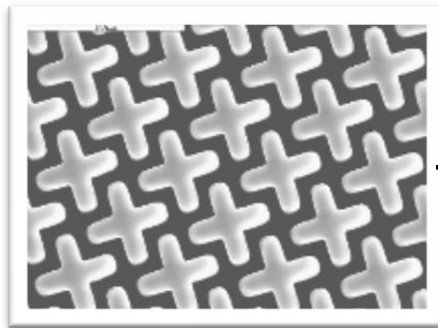
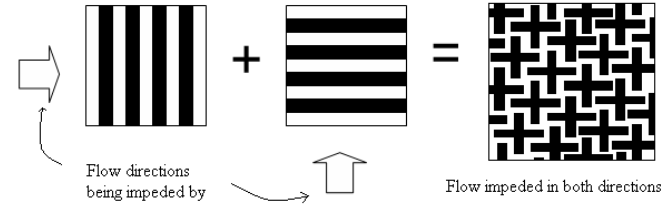
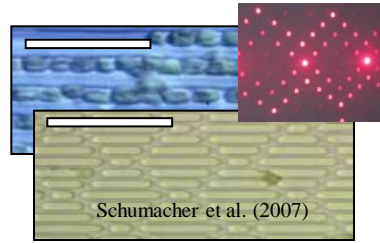


Surface properties and the bio-interface

Novel strategy		Parameters	Description
1	Mechanical	Elastic modulus Hardness	Ability to return to original dimensions after deformation
2	Hydrodynamic	Surface pattern, Dimension, roughness	Patterns of micro-sized dimensions
		Hydrophobic	Water fearing surface
		Hydrophilic	Water loving surface
3	Chemical	Biocide release pH, ionic strength	Toxic / aversion to model species

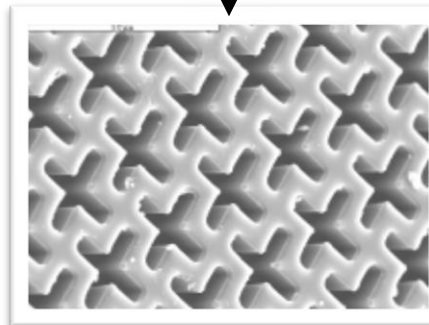


Methods: Topographical surfaces

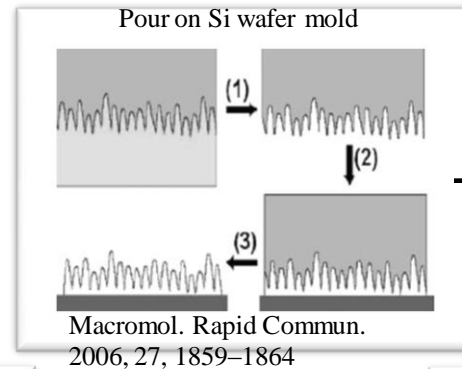


Concept

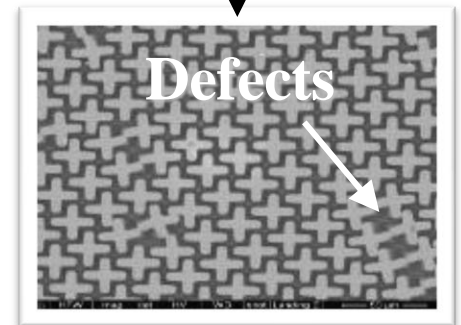
photolithography



Mask: Si wafer



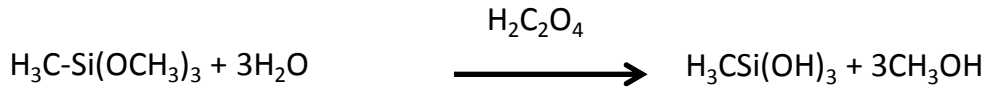
Peel off PDMS



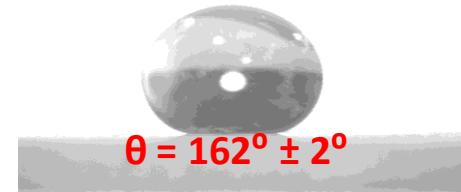
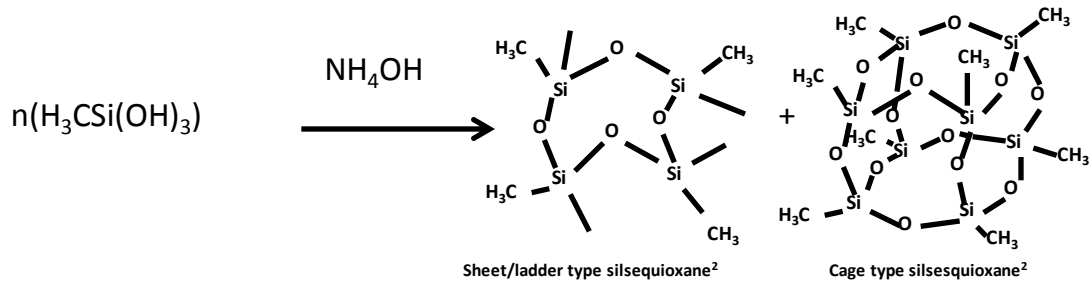
Silastic T2
polydimethyl siloxane
resin + polymethyl
silane curing agent,
mix, degas to remove
bubbles

Methods: superhydrophobic surface

Hydrolysis: catalyzed by oxalic acid¹



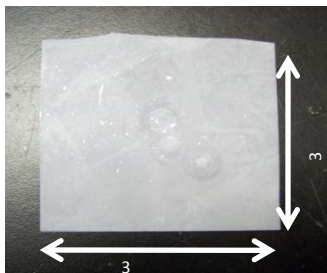
Condensation: catalyzed by ammonium hydroxide¹



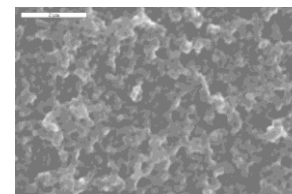
Sessile Drop Contact Angle with De-ionized water (16MΩ)

Piranha treatment: silicon wafer and glass substrates to be coated, were boiled for 10 minutes in a 1:1 solution by volume of 50% H₂O₂ and 96% H₂SO₄

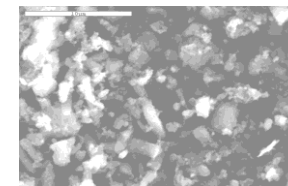
Drying: The gel while in contact with the substrate is dried at 60°C for a day



Silicon wafer coated with the 3D polymerized MTMS based sol-gel. Relatively large coated area.



A



B

SEM Images A & B were taken at 15000X (SB: 2μm) and 4000X (SB: 10μm) magnifications

Methods: bioadhesion testing

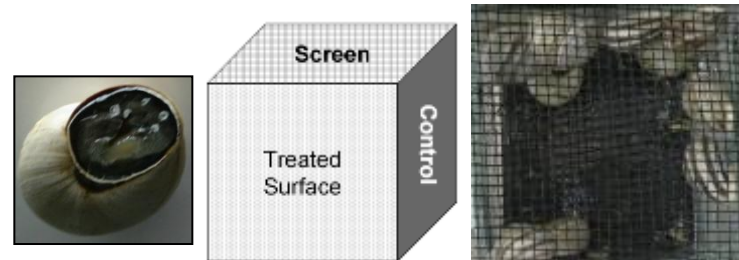
1. Real time surface interface communication

- May be important for surface selection
- Short-term (8-10 min) behavioral trials
- Noldus EthoVision® digital image tracking



2. Longer-term behavior

- Aestivation surface selection (48-h)

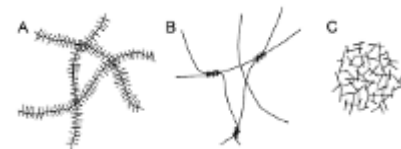


3. Bioadhesion

- Whole snail
 - Shear force for removal
 - Mark-10 digital force meter
- Fluid tensile strength
 - Mucus interaction with surface
 - Mark-10 digital force meter / test stand



ASTM D 5618-94



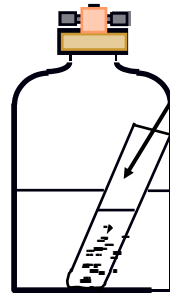
$$Pascal = \frac{F}{A} = \frac{Newtons}{1/4\pi \cdot d_a^2}$$

Methods: biofilm testing



Enterobacter cloacae (-)

Grown overnight in Nutrient broth from a colony



Slide with biofilm developing

48 h Nutrient broth incubation in the presence of surface slide



Remove slide and stain



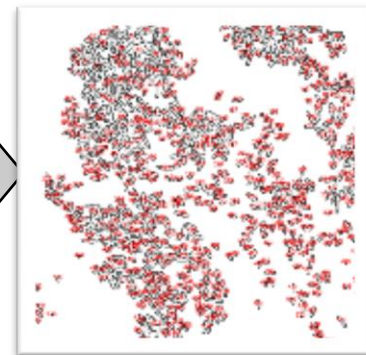
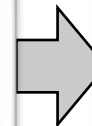
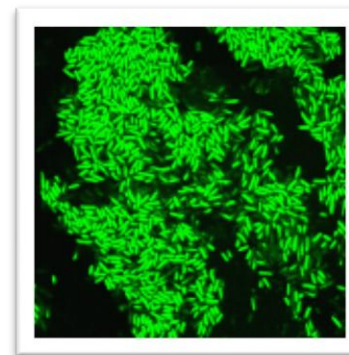
Extent of coverage assessment

1. Confocal microscopy
2. Qualitative: +/- biofilm growth
3. Quantitative: % area covered
- Image J

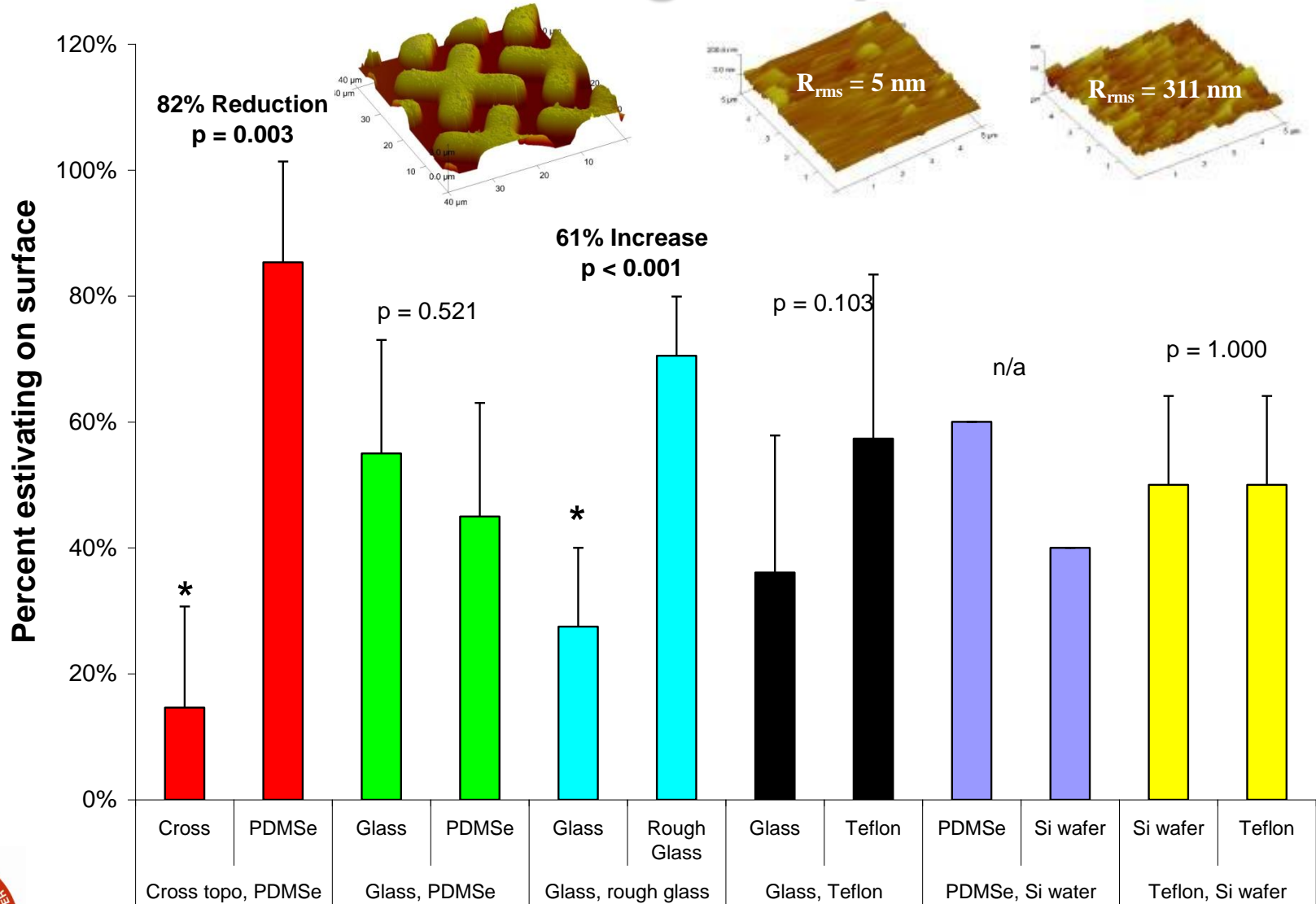
Biofilm of *Enterobacter cloacae* after 48 h incubation at 22°C

Glass surface

Enterobacter cloacae is associated with respiratory and urinary tract infections in hospitals increasing and has exhibited multi-drug resistance. Has use in explosives biodegradation.

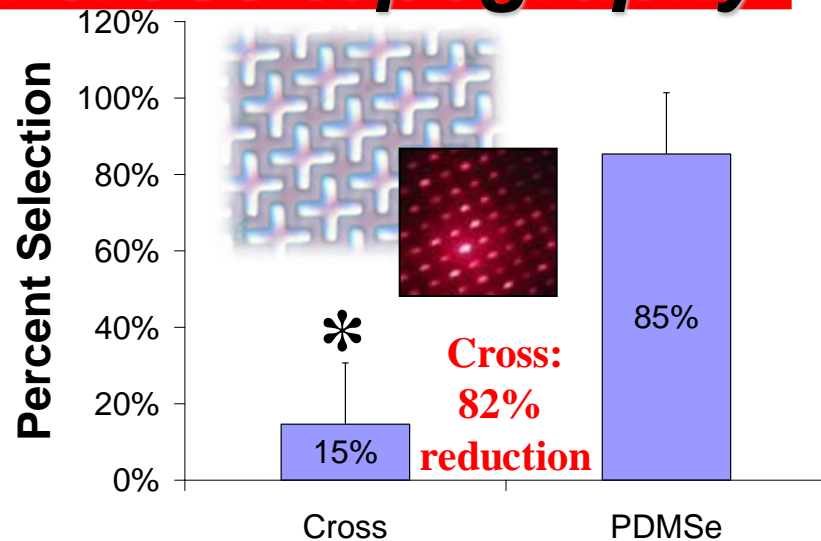
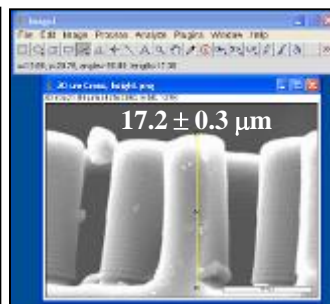
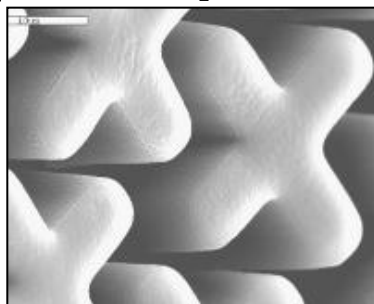


Surface selection bioadhesion: gastropod aestivation

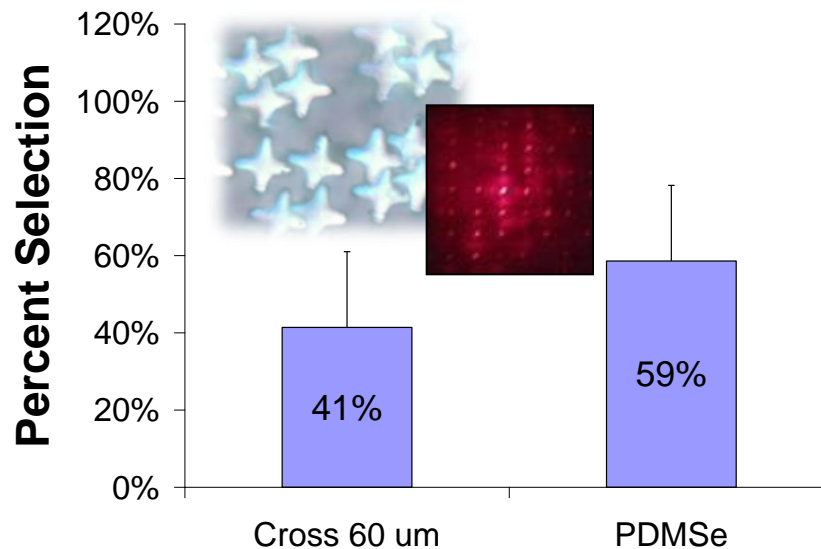
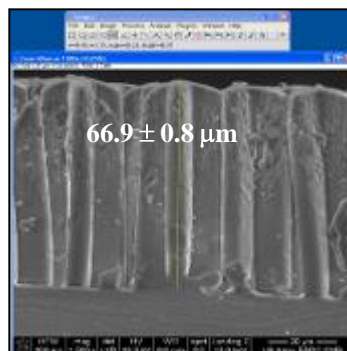
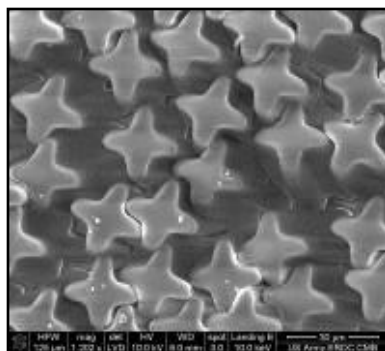


Gastropod surface selection: aestivation on cross topography

- 25 μm cross
 - 20 μm amplitude

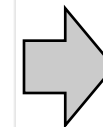
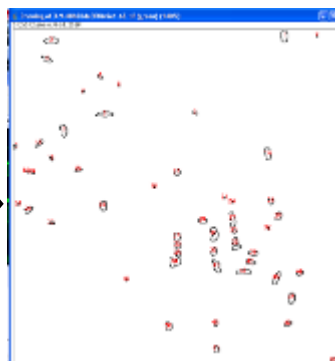
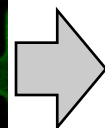
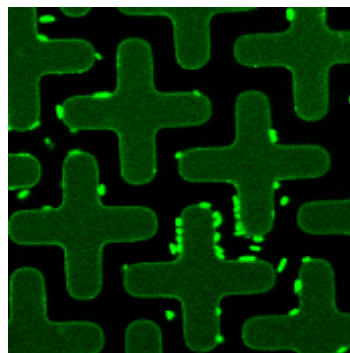
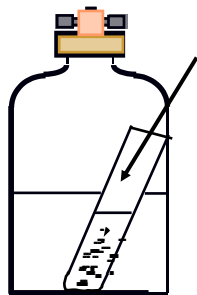


- 60 μm amplitude (clumped)



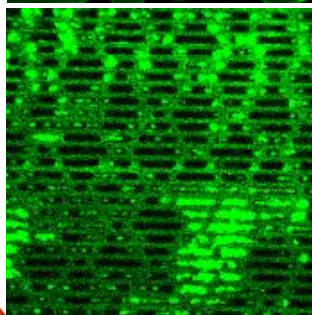
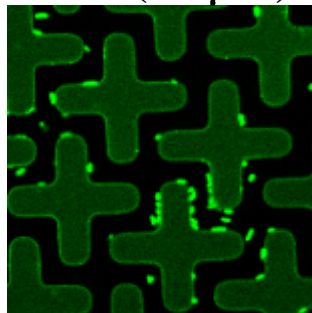
– Significance removed by sample reuse.

Biofilm bioadhesion: cross topography



1.4% of
total area
covered

Cross (20 μm)



Percent Area Covered

60
50
40
30
20
10
0

**Cross:
93%
reduction**

$p < 0.001$

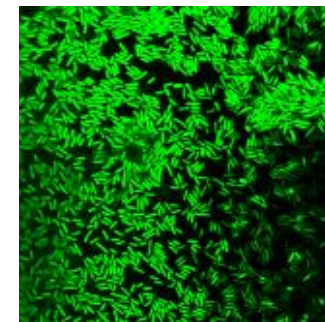
2.9

Cross (20 μm)

42.5

PDMS_e

PDMS_e



Other surfaces:

PDMS CoP: 25.3 ± 15.3

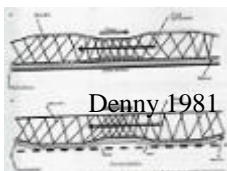
Vs.

Glass: 9.8 ± 13.6 ($p = 0.028$)

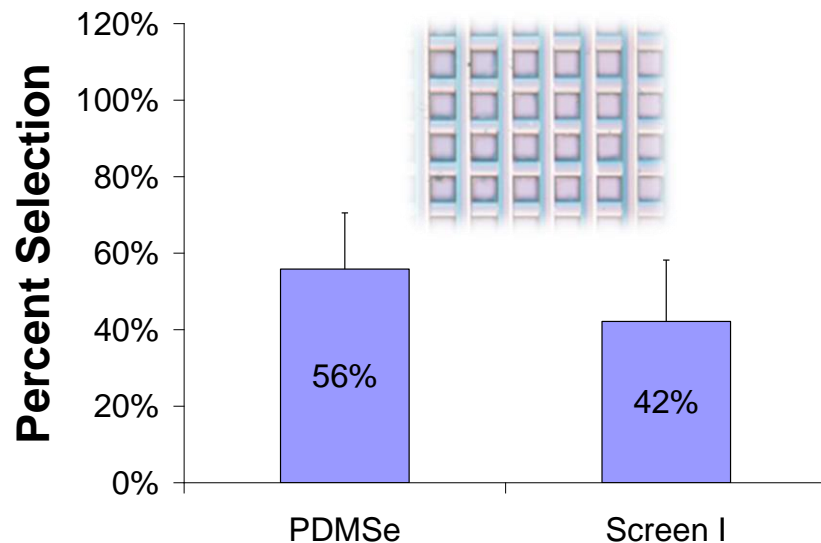
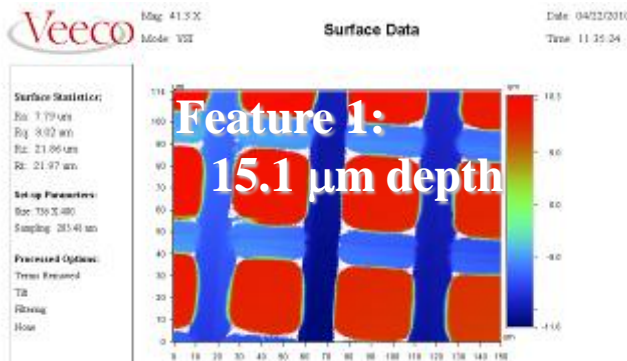
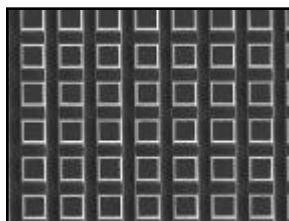
(Glass II: 7.1 ± 5.8)

Gastropod surface selection: aestivation on screen pattern

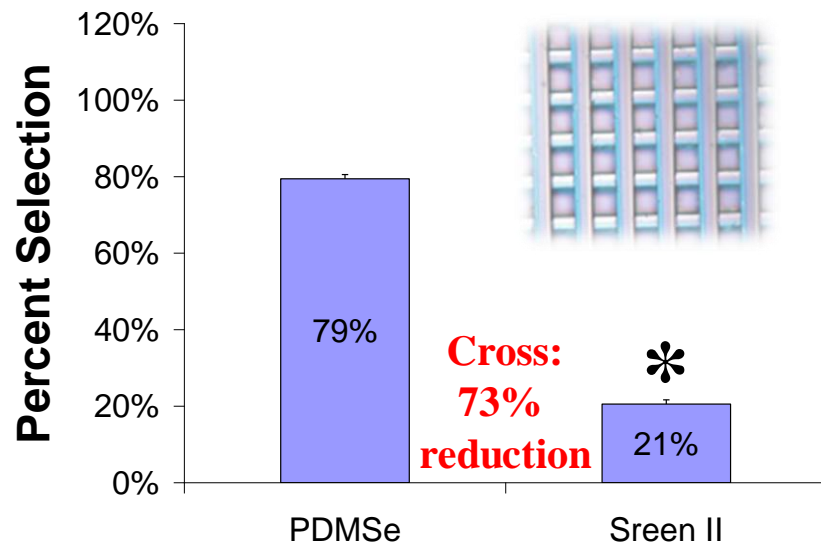
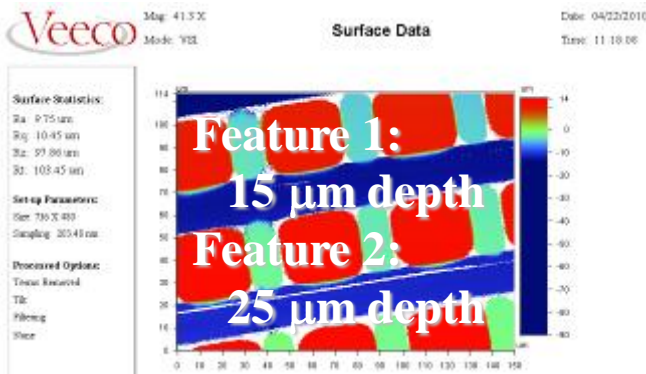
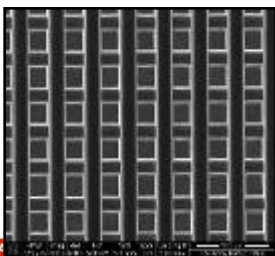
Snails can sense surfaces and change mucus properties:



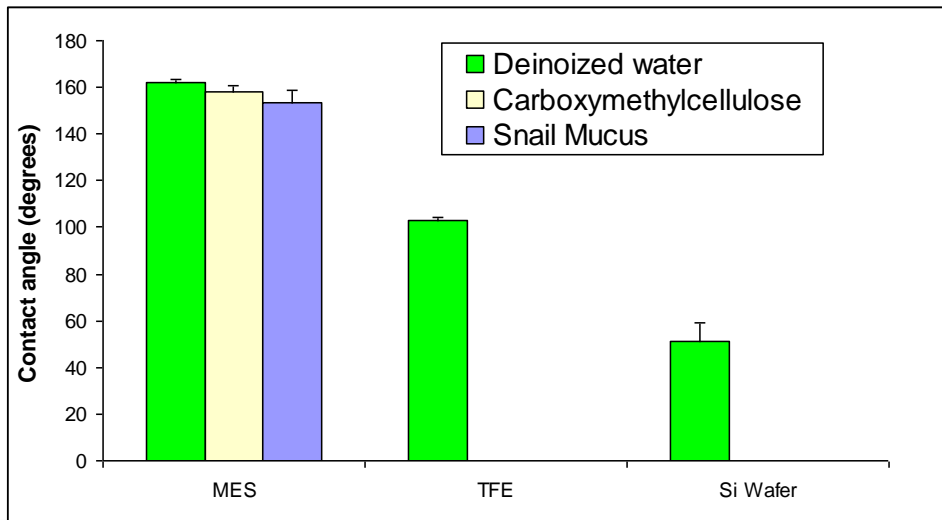
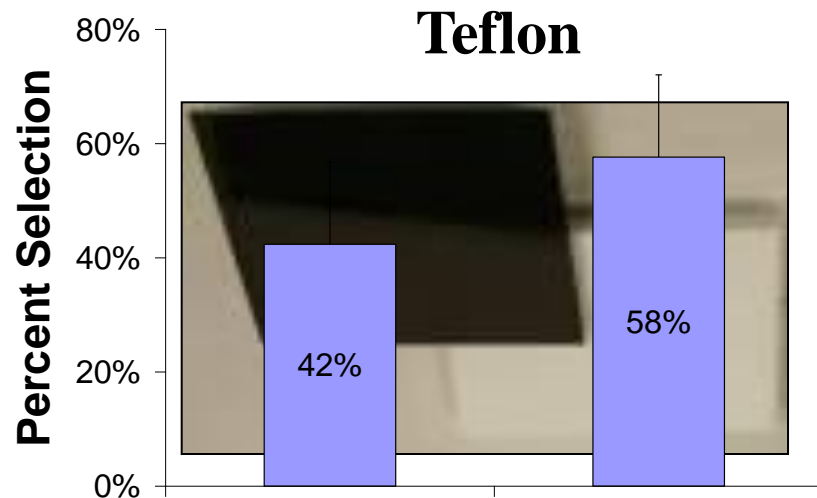
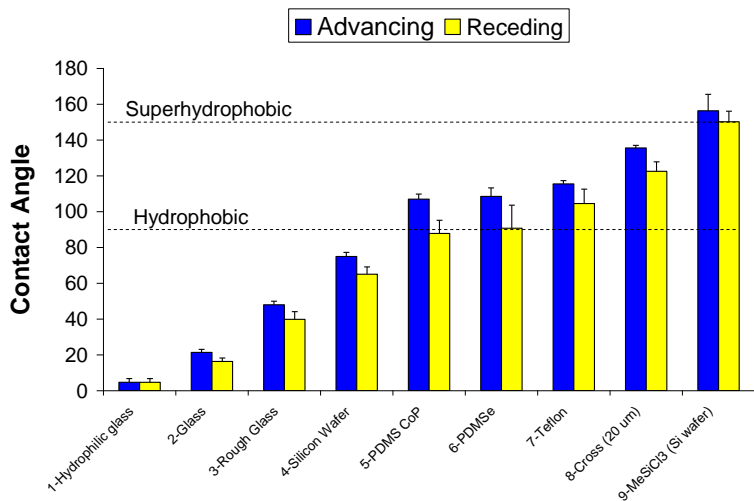
- **25 μm Screen I**



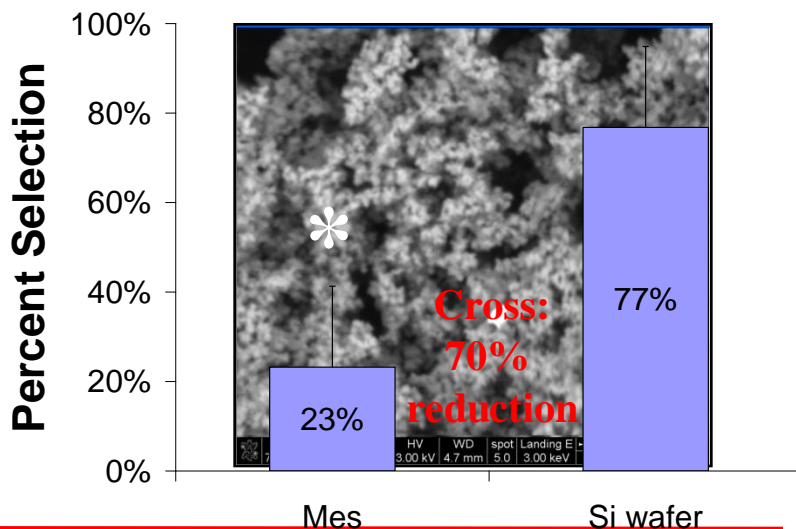
- **25 μm Screen II**



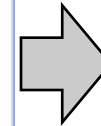
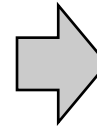
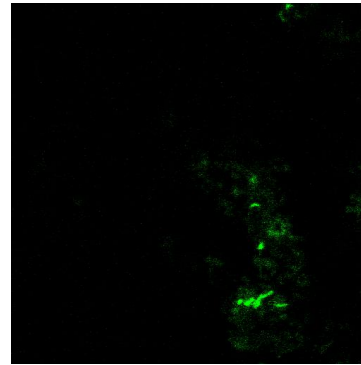
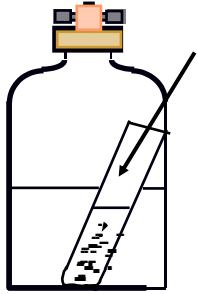
Gastropod surface selection: aestivation on hydrophobic surfaces



• **Methyl silsesquioxane (MES)**

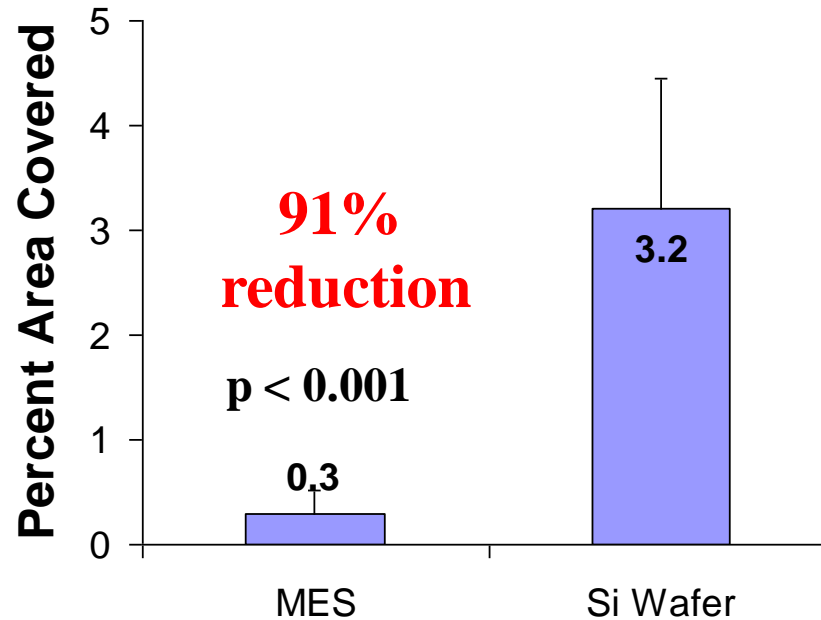
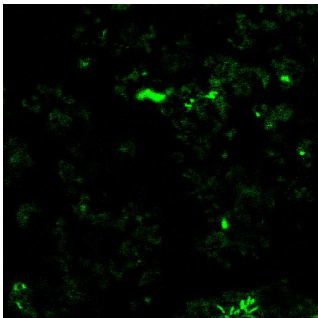


Biofilm bioadhesion: MES xerogel

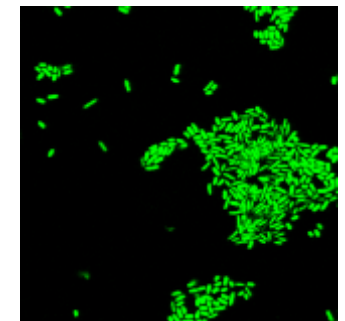


0.1% of
total area
covered

MES

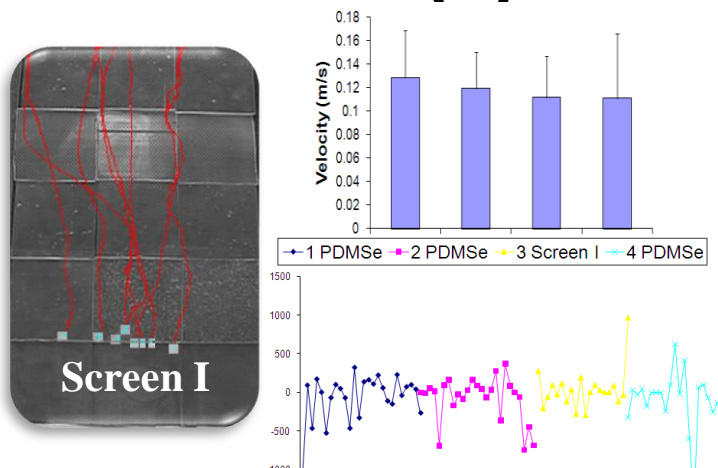


Si Wafer

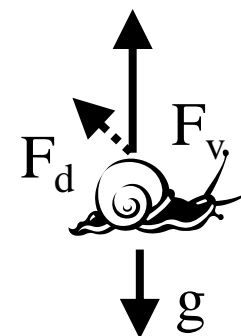


Gastropod real-time behavior at surface interface

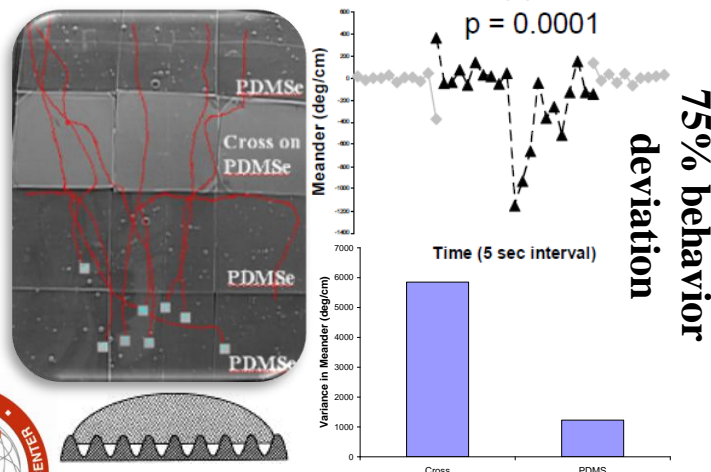
- No effect (7)



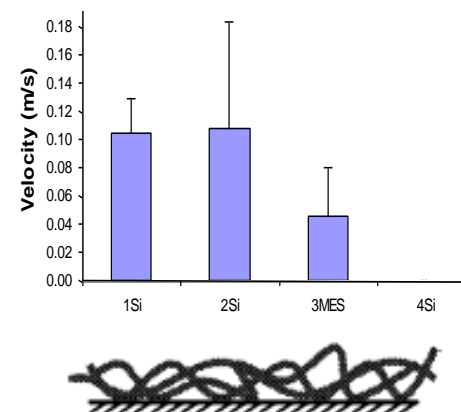
- Teflon vs. glass



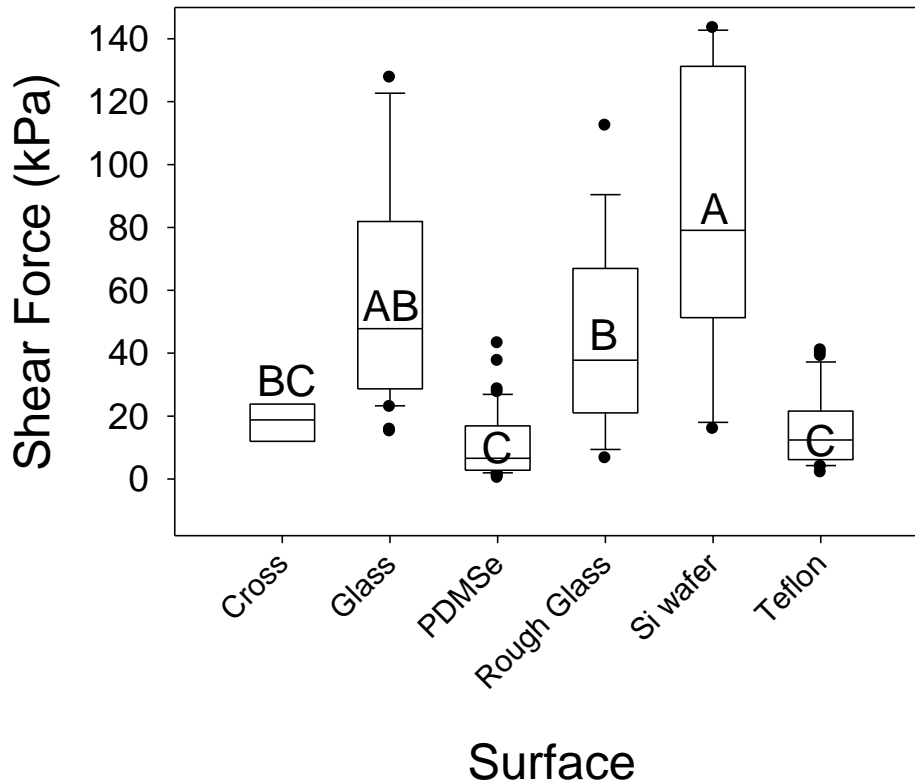
- Cross vs. PDMSe



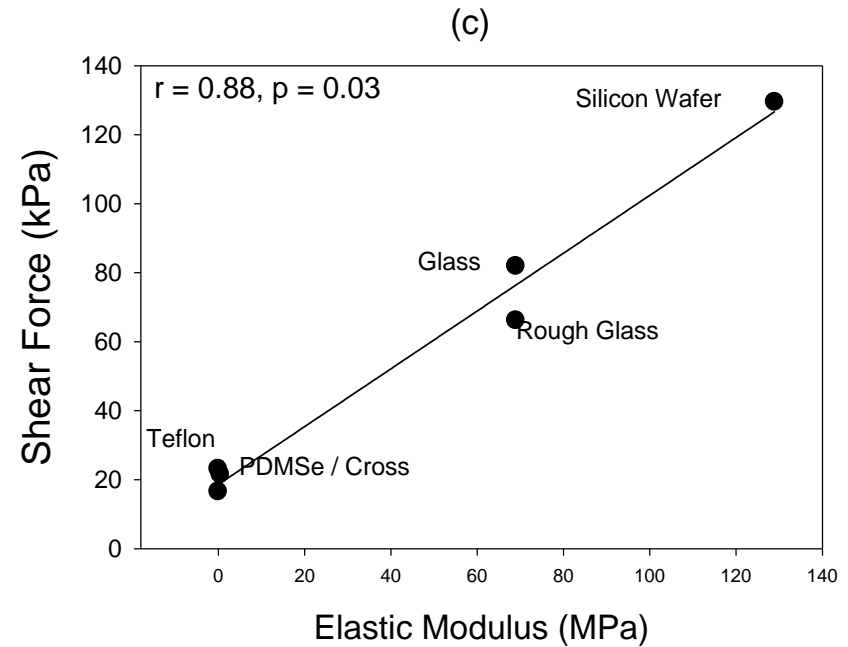
- MES vs. Si wafer



Gastropod bioadhesion strength: shear force for removal



75th percentile

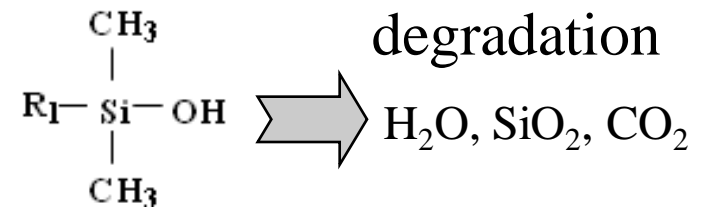


$$\text{Adhesion Strength } (\tau) = \frac{F(\text{in N})}{A(\text{in m}^2)}$$



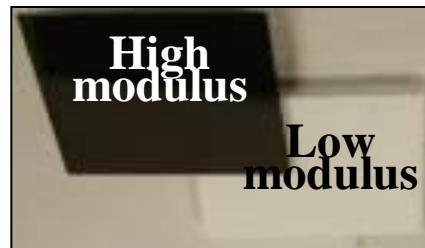
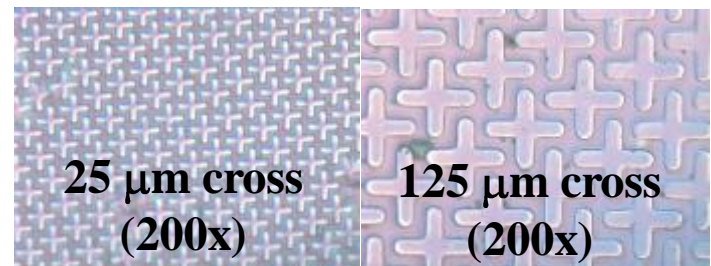
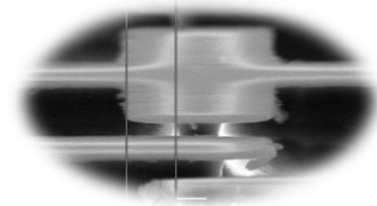
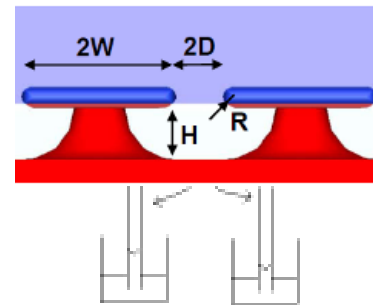
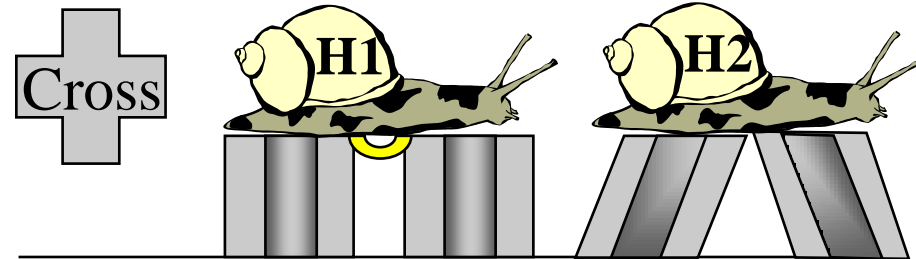
Conclusions

- **Surface roughness controls behavior**
 - Non-patterned roughness facilitates gastropod selection preference
 - Cross pattern tessellation reduces gastropod selection preference
 - 20 μm feature magnitude superior to 3 and 60 μm
- **Material elastic modulus controls bioadhesion strength**
 - Low modulus materials reduce shear adherence
 - High modulus materials increase shear adherence
- **Cross tessellation on PDMS_e provides**
 - Behavioral deterrence for aestivation
 - Mechanical property to reduce adhesion strength for ease of removal
- **Triethylsilanol**
 - Initially toxic
 - Breaks down rapidly, nontoxic



Future Research

- Why successful?
 1. H1: Mucus breakthrough pressure
 2. H2: Feature modulus
- Silanized Micro hoo doo
 - Tuteja et al, Science, 2007
 - High breakthrough pressure (> 1400 Pa)
- Different sizes (25, 125 μm) and 25 μm cross spacings (4, 10 μm)
- Cross: PDMSe vs. Si wafer





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