A Primer on Planetary Protection

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Outline

- Introduction
- Taking a Census: the NASA Standard Assay
- Size Matters
- Square Peg , Round Hole
- House of Straw or Brick?
- Blow This House Down!

Outline

What this is:

- An orientation guide to the world of biology for planetary protection missions
- A different way to think about the materials selection process for planetary science missions.

What this isn't:

- A review of planetary protection policy
- A Headquarters-directed list of what can or cannot/should or should not be used.

Terminology

Eukaryote

Spore

Virus

Cell

Archaea

Microbe

BACTERIA

microorganism

Terminology

microorganism

A microscopic organism, such as a bacterium, virus or fungus, unable to be seen with the naked eye and typically composed of a single cell.

Microbe

A minute organism,
A microorganism

Cell

The basic organizational unit of an Earth organism; some are composed of many cells, together

Eukaryotes
BACTERIA
Archaea

Types of organisms: most are single-cell microorganisms, but Eukaryotes can be LARGE and multicellular and bacteria make films and aggregations

Smaller

Spore

A dormant form of a bacterial cell – smaller than the cell itself

Virus

A parasitic entity composed of RNA and/or DNA, and proteins

Terminology

	Bacteria	Spore	Virus
Size Range	~0.2-10 µm	~2-5 µm	~100-10 nm
Unique Features	DNA in a circular molecule; cell wall; ribosomes make proteins	Can exist in a state of dormancy for long periods; bacterial machinery in storage	Needs a host; some can persist almost indefinitely in the environment
Examples	*Staphylococcus aureus* *Streptococcus mitis*	*Bacillus subtilis* *Anthrax* *NOT S. mitis*	*Influenza* *Ebola* *Bacteriophage T-4*

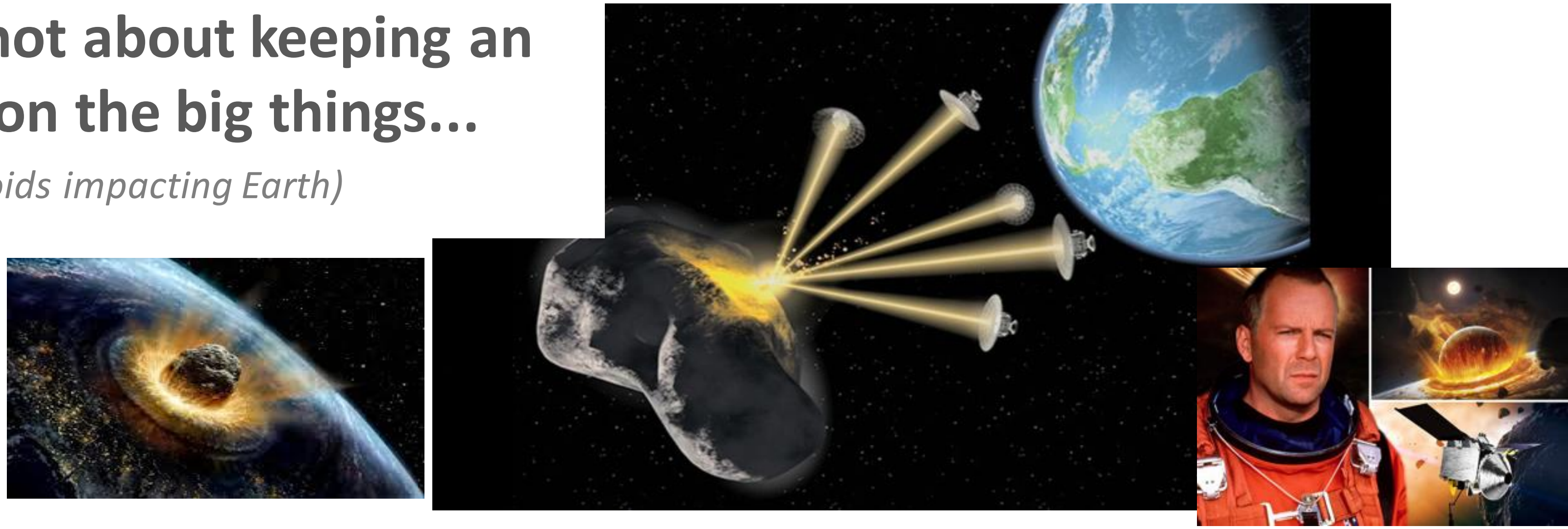
Introduction

NASA planetary science missions are required to comply with requirements that protect the science and protect the planet, depending on the mission's objectives.

What Planetary Protection **isn't**

It's not about keeping an eye on the big things...

(asteroids impacting Earth)

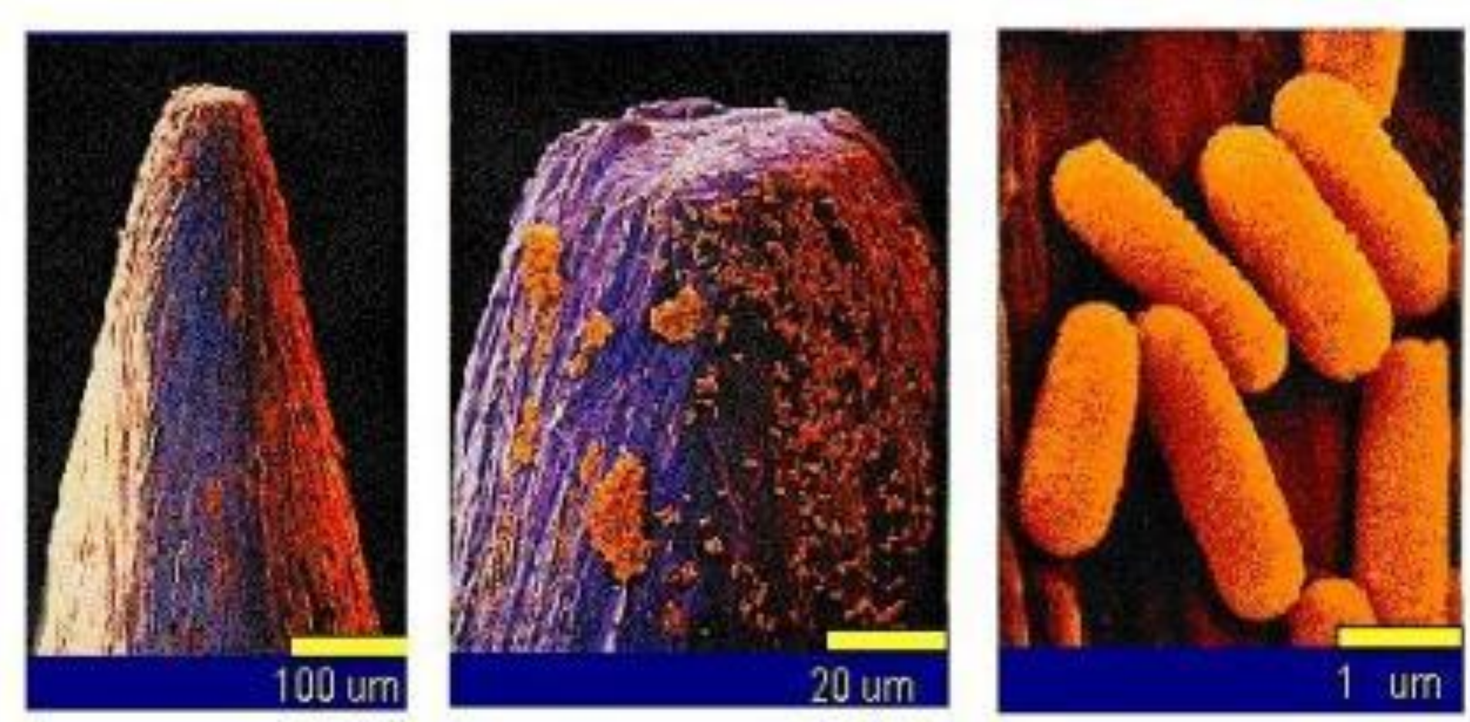


We don't carry ray guns...

What Planetary Protection *is*

It's about keeping an eye on the little things…

Purple = Head of a Pin
Orange = bacteria on the head



What Planetary Protection *is*

It's about keeping opportunities open for future exploration science when we visit…

Adios, Earth!
Hello, Solar System!

Greetings Earthlings!

…And not closing the door on our Earthly civilization when we bring samples back…

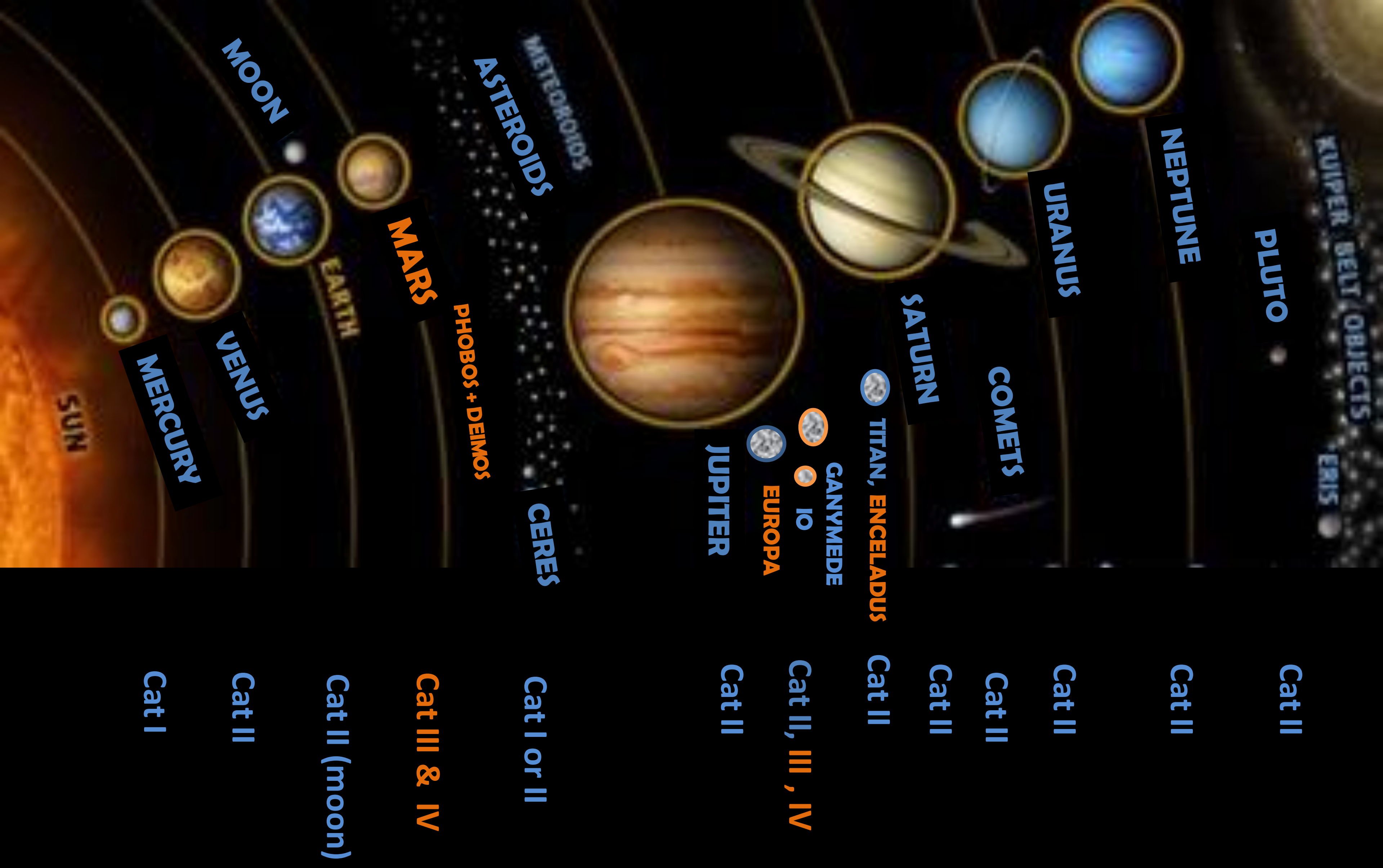
FORWARD CONTAMINATION

BACKWARD CONTAMINATION

The Central Question for Planetary Protection

What is the chance that any terrestrial contamination **NOW** will influence future biological investigations on a planetary body **LATER**?

NASA missions have different requirements depending on where they're going and what they're doing.



Blue = primarily documentation/tracking of organics

Orange = Missions to these locations have bioburden monitored and tracked

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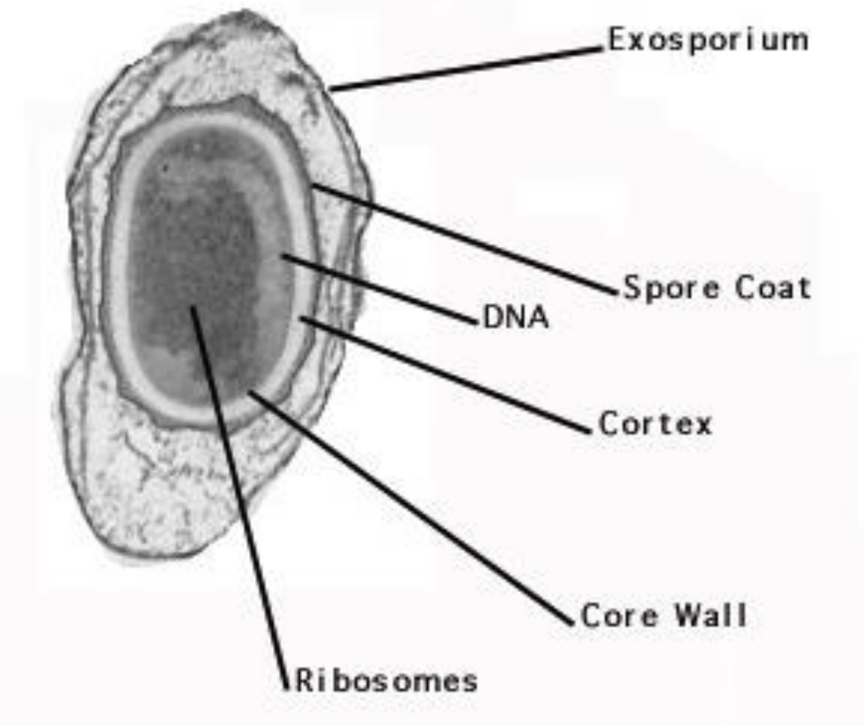
Taking A Census

NASA takes a spacecraft census by counting.

Counting what?

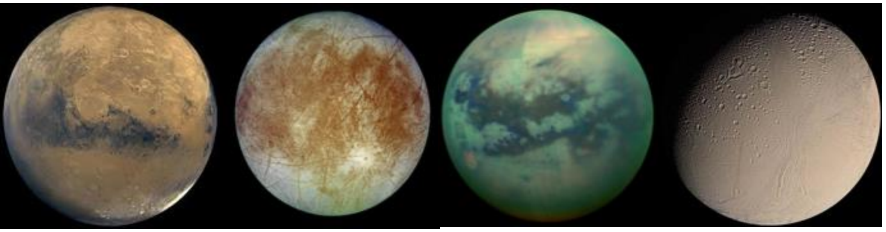
Counting spores.



Some bacteria form a metabolically inactive state when environmental conditions are harsh.

When we take a census, we heat shock the collected bacteria to drive spore formation – looking for the hardiest critters, those that could potentially survive the trip to another place in our solar system.

NASA Standard Assay



Mars, Europa, Titan, Enceladus

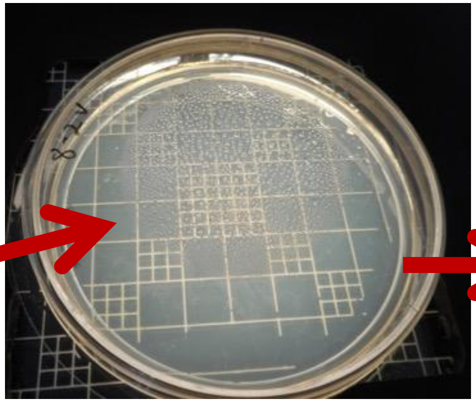
1. Sample spaceflight hardware

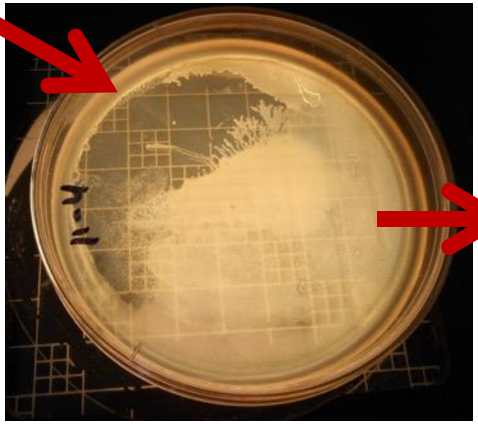
2. Process sampling matrices

- *Sonication*
- *Heat shock (80C, 15 min)*

and plate onto TSA

3. Check growth after 72 hours at 32°C

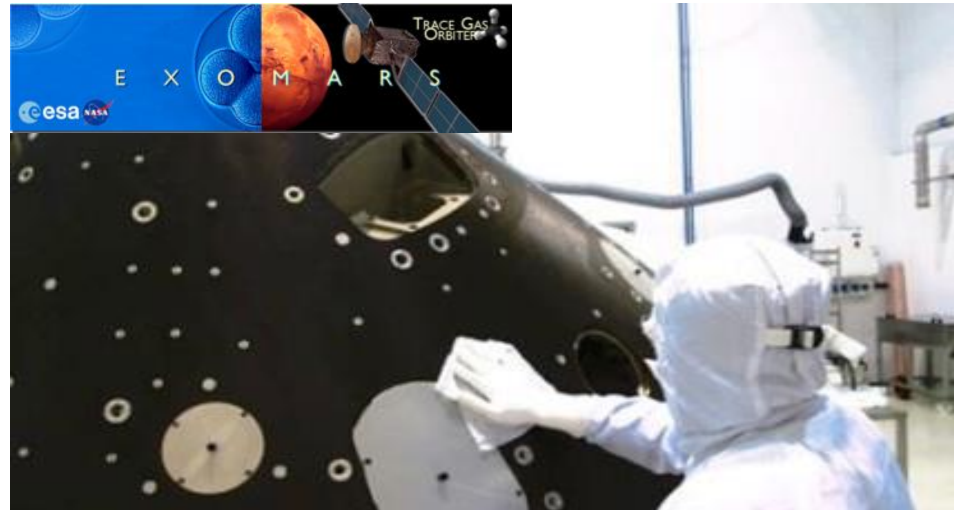




Reclean!

Assaying in Real Life

Heatshield

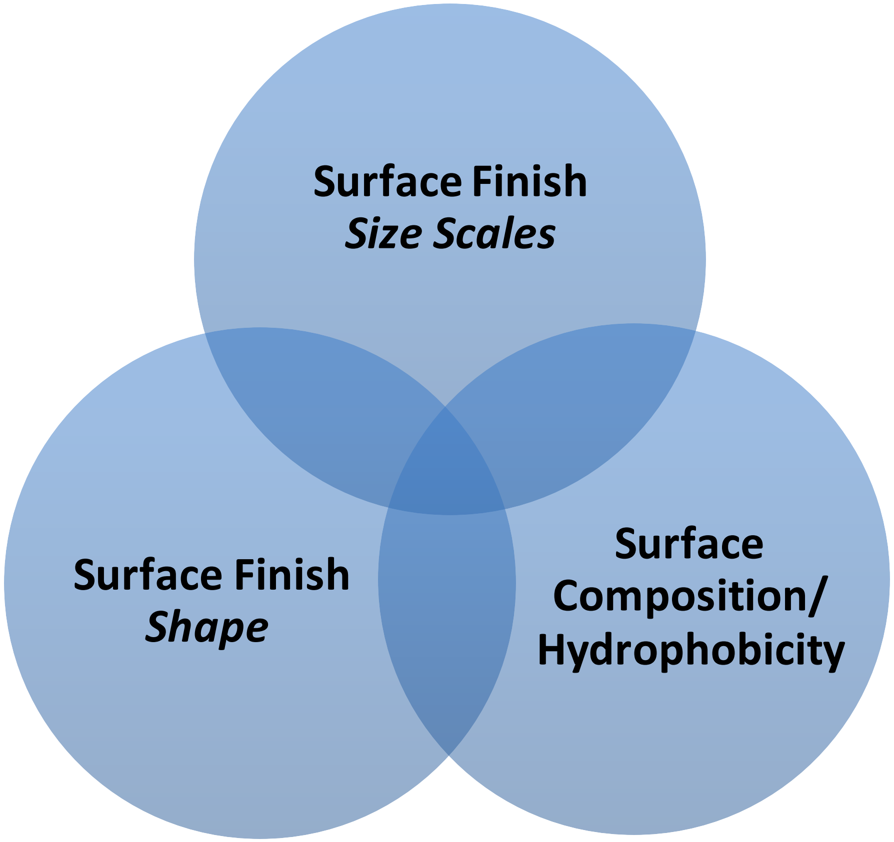


Capsule Exterior

Solar Array Hardware

Rover Exterior

Key Parameters Influencing Tiny House Habitability



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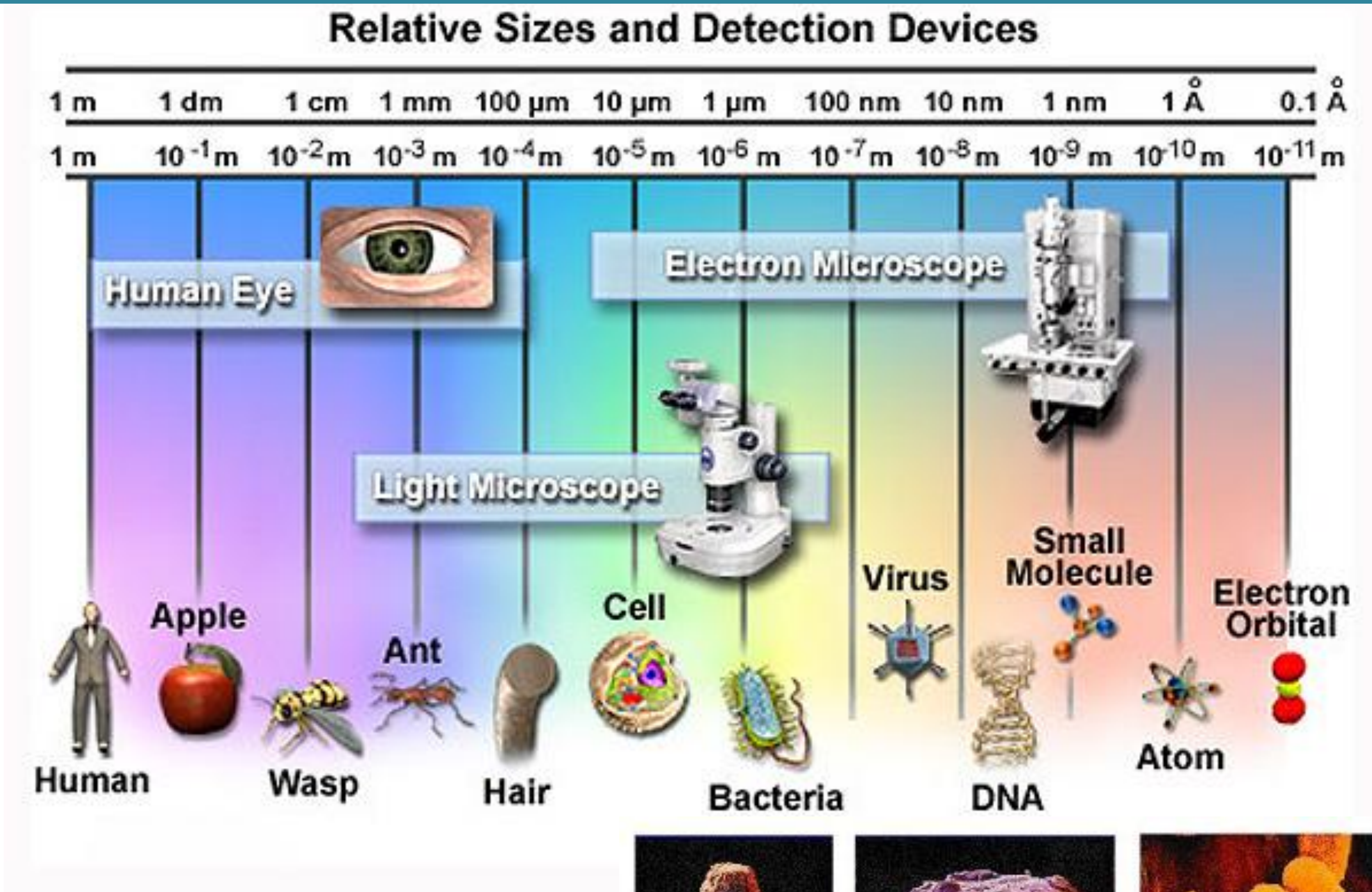
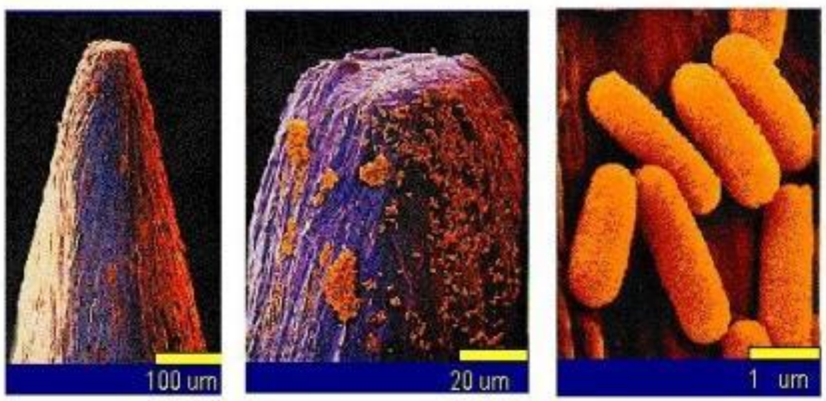
Relative Size Scales of Microbes

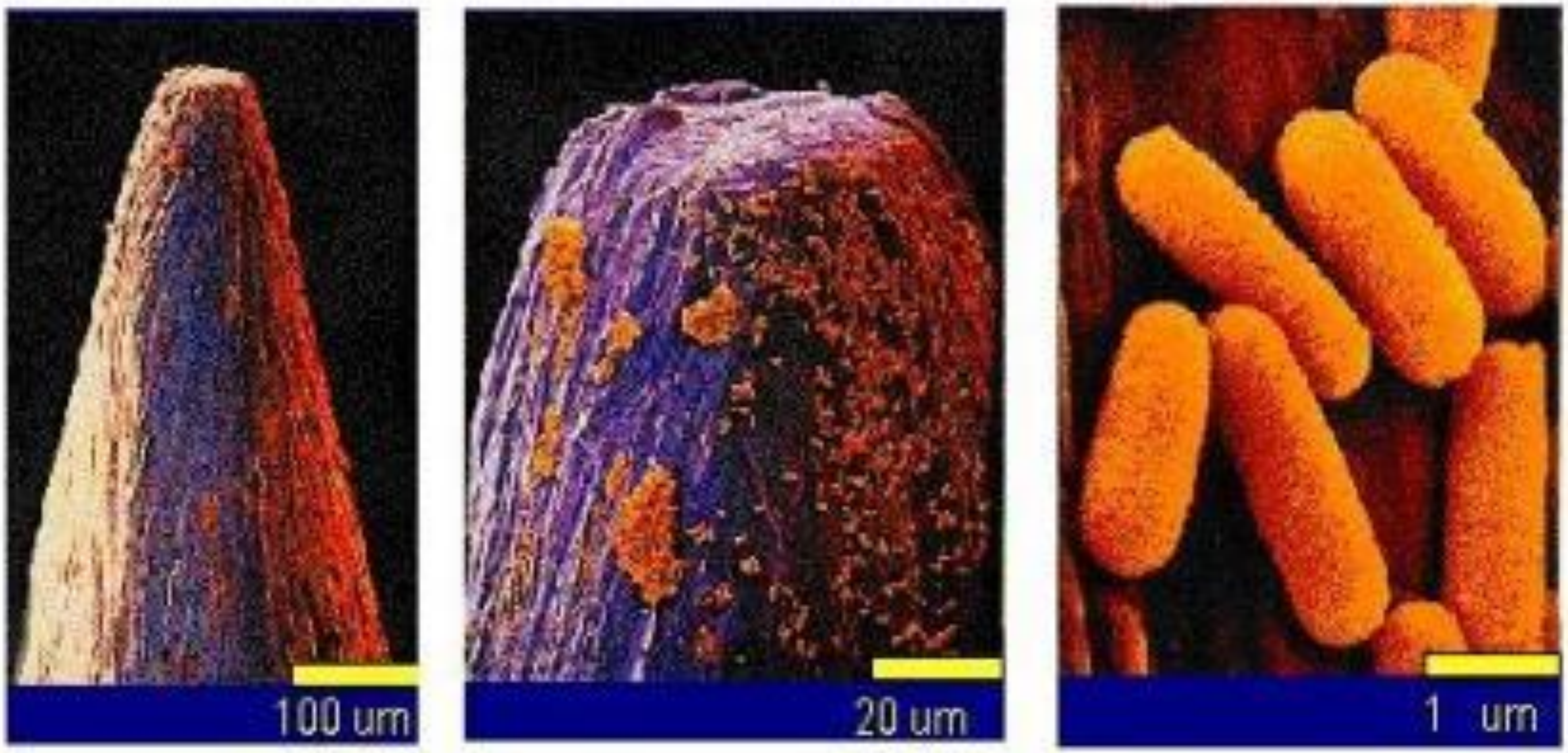


Bacteria on the head of a pin
—there are lots there!
—and they are not angels…

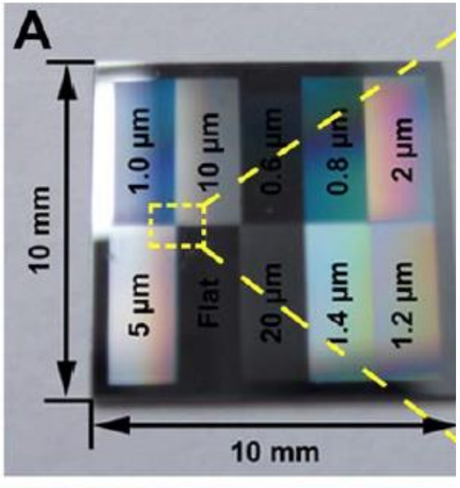


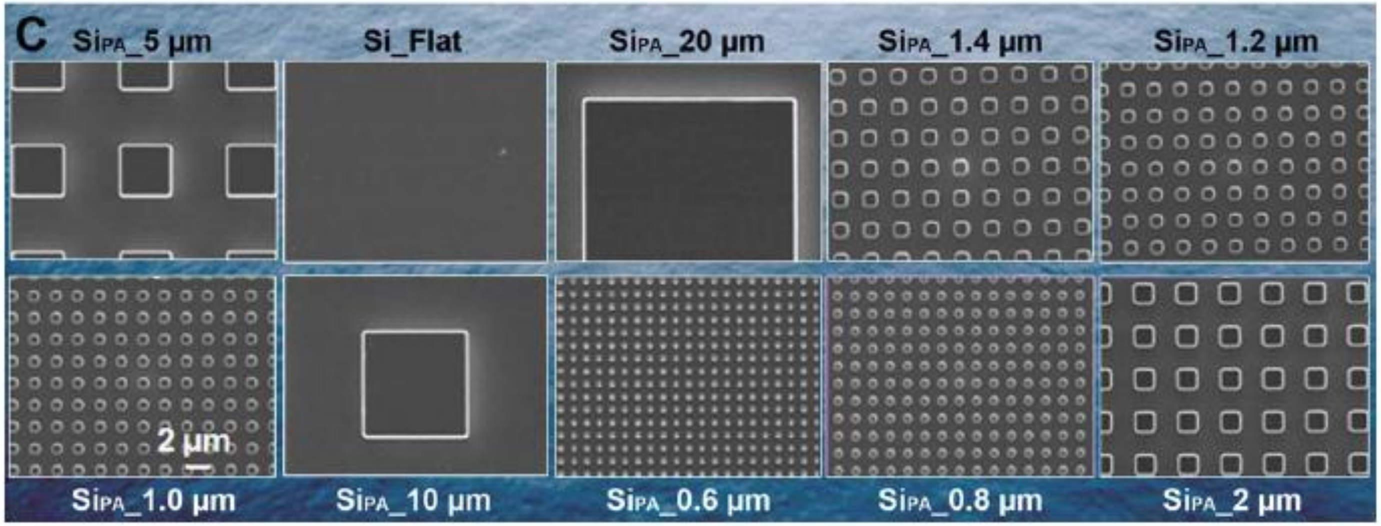
How does surface finish play a role in retention?

Consider the head of a pin…
There can be lots of bacteria there and they may not be angels dancing...



Does size matter?





S. aureus
Spherical ∅ ~0.6 um

E. coli
Rod
W × L 0.5 µm × 2 µm

Micropillars patterned on Si exposed to bacteria of **different sizes & geometries to test retention**

X. Ge et al., J. Biomedical Materials Research A ***103A*** *384 (2015)*

Size Matters!

Reduced growth when periodic spacing of engineering pillars is reduced to < 0.8 um

- Pillar sizes smaller than the size-scale of the bacterium result in less bacterial retention.
- Large sizes and flat pillars favor retention (after 12, 24 hour examination)

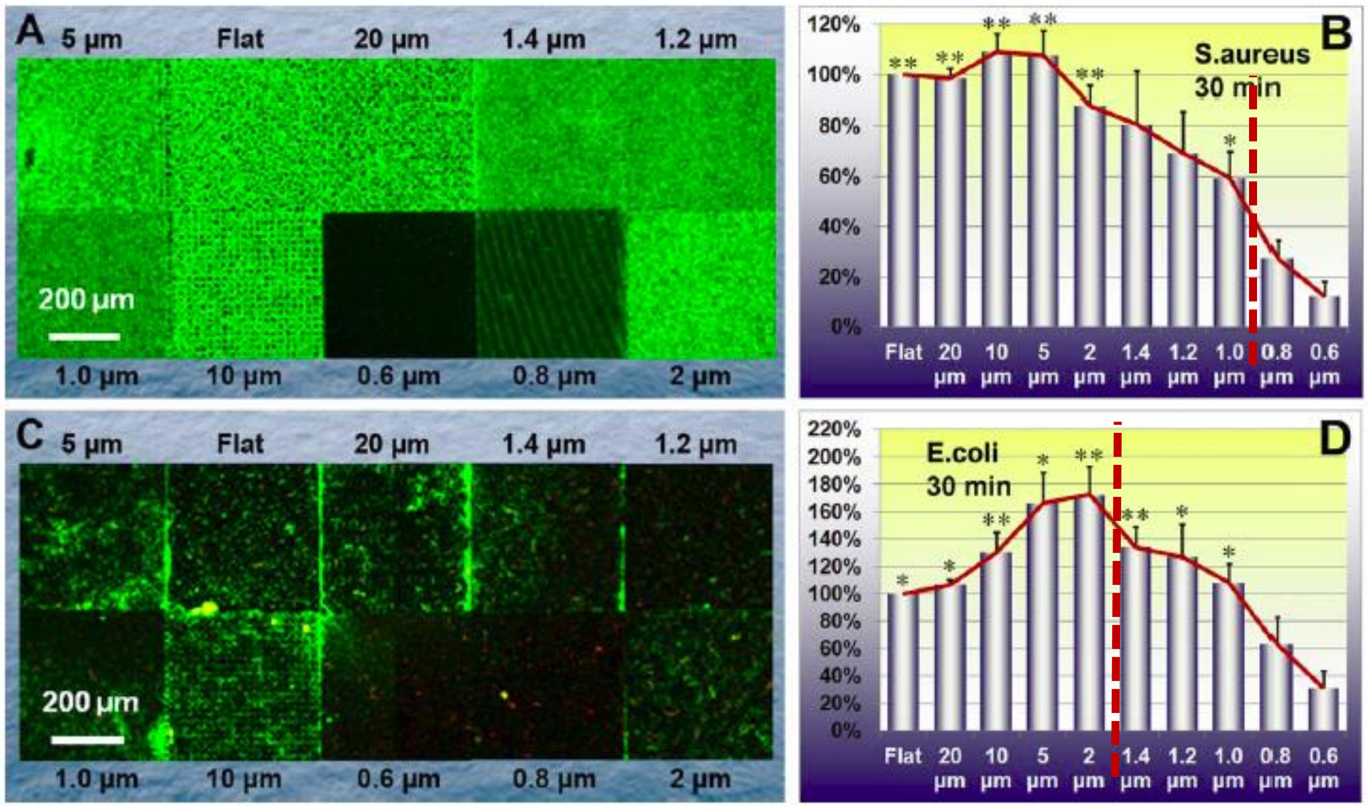
S. aureus

Spherical ∅ ~0.6 um

E. coli

Rod

W × L 0.5 µm × 2 µm



= Threshold Of Organism SIze

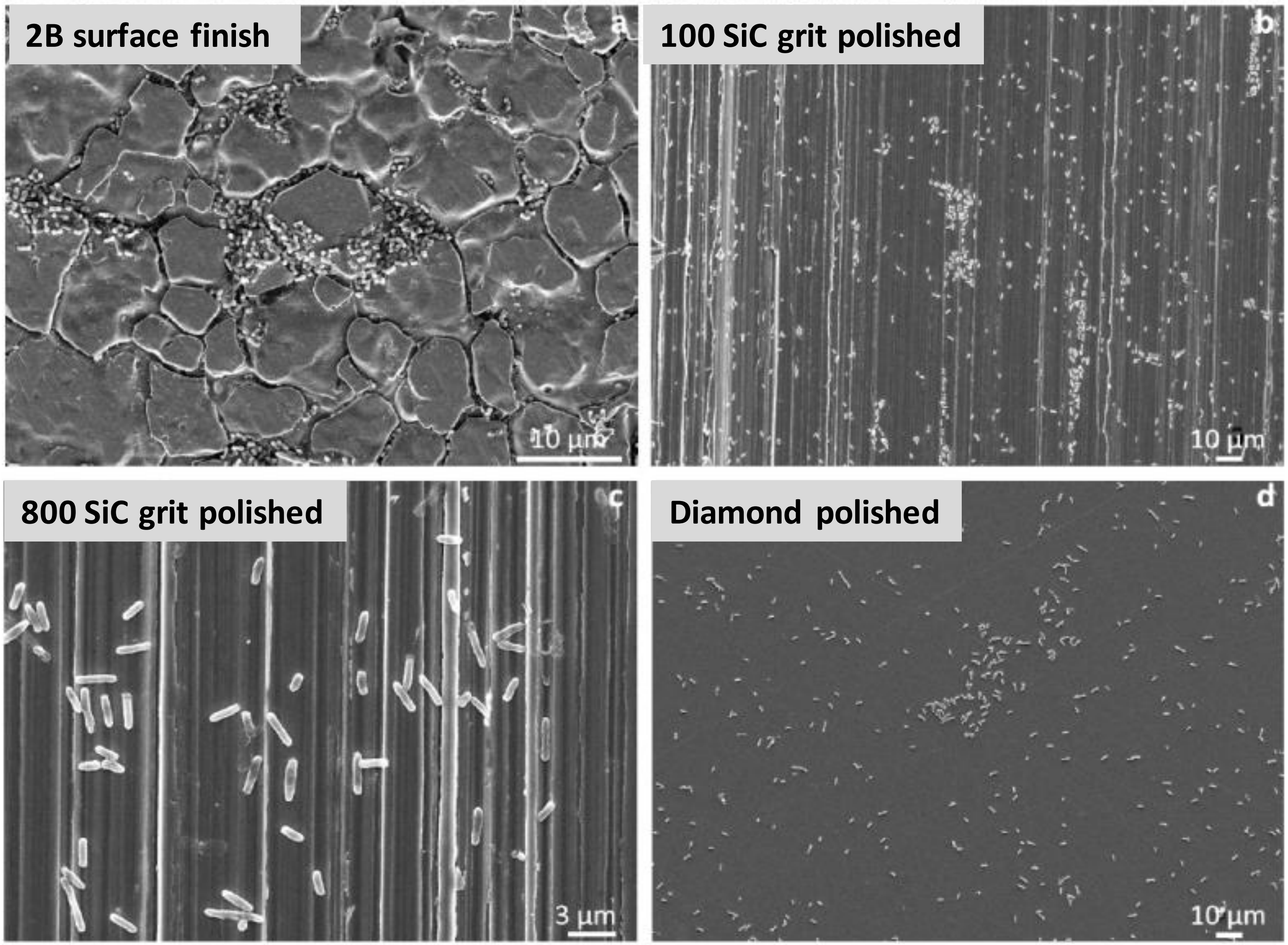
Does this translate to common materials used to build spacecraft?

*X. Ge et al., J. Biomedical Materials Research A **103A** 384 (2015)*

316 Stainless Steel
With different surface finishes

+

E. coli
Rod W × L 0.5 µm × 2 µm



M. Hocevar et al, poster and in, Materials and Technology ***48****, 609–617 (2014)*

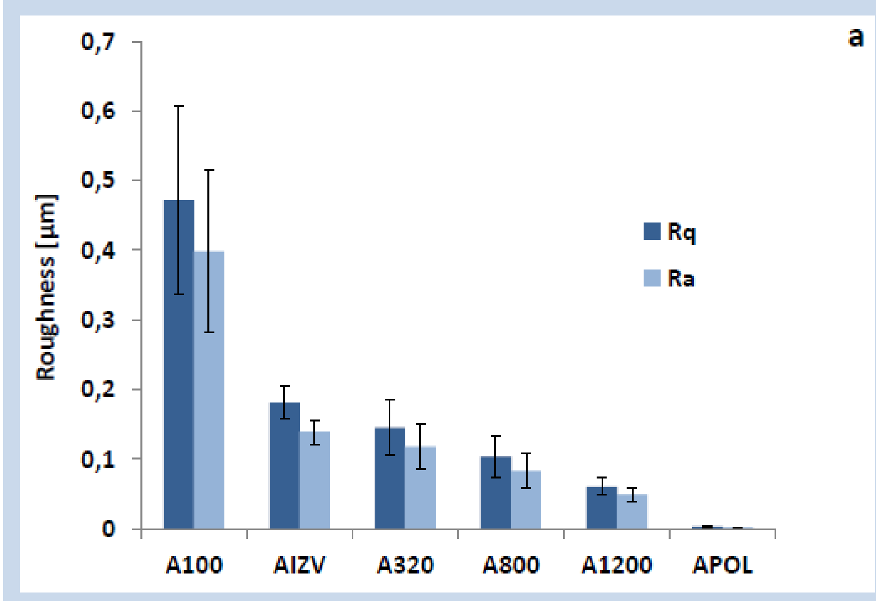
316 Stainless Steel

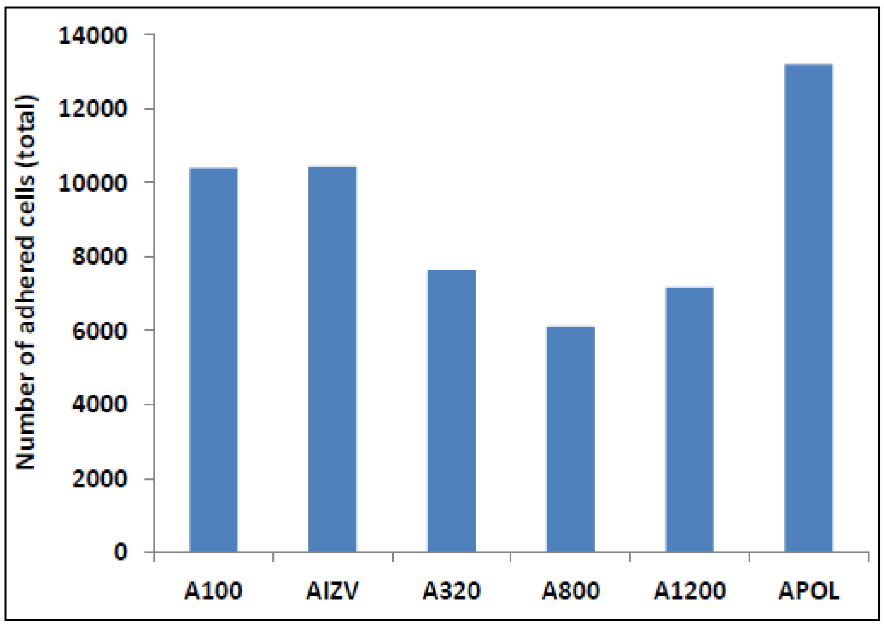
With different surface finishes

E. coli

Rod W × L 0.5 µm × 2 µm

Finish	Treatment
AIZV	As received
A100	100 grit SiC paper
A800	800 grit SiC paper
A1200	1200 grit SiC paper
APOL	Polished with diamond paste





Upshot:
Rougher surfaces contain a larger number of cells

(because they can!)

In the APOL "ultrasmooth" limit for 316 SS, there may be chemical composition effects, influencing surface energy and hence, adhesion.

M. Hocevar et al, poster and in, Materials and Technology ***48****, 609–617 (2014)*

Titanium

- The behavior of titanium, titanium nitride (TiN) and titanium dioxide have shown contradicting results with regards to the degree of microbial adhesion
- The most common titanium used for spaceflight applications, Ti 6AL-4V, has shown that when inoculated with *Bacillus subtilis* spores, efforts to clean Ti are not successful when the surface is cleaned with isopropyl alcohol or with water
- When that particular method of cleaning is used, the solution breaks spores open and serves as a culturing medium for *B. subtilis* spores on a titanium surface.
- Additional work examining the surface roughness and the compositional variation is needed, given the co-existence of Ti, and both anatase and rutile TiO_2 on most Ti surfaces.

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- **Square Peg , Round Hole**
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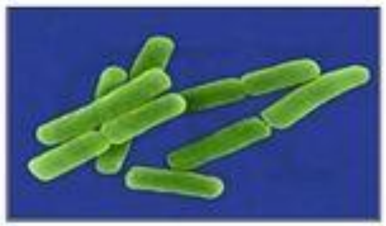
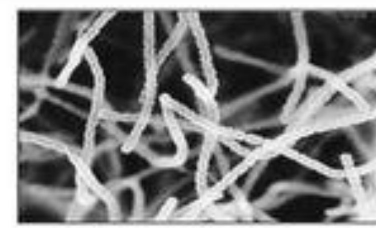
"You're not from around here are you?"

The Many Shapes of Microbes

Circular (Coccus)	Rod-shaped (Bacillus)	Curved Forms	Other Shapes
 Diplo- (in pairs)	 Coccobacilli (oval)	 Vibrio (curved rod)	 Helicobacter (helical)
 Strepto- (in chains)	 Streptobacilli	 Spirilla (coil)	 Corynebacterium (club)
 Staphylo- (in clusters)	 Mycobacteria	 Spirochete (spiral)	 Streptomyces (filaments)

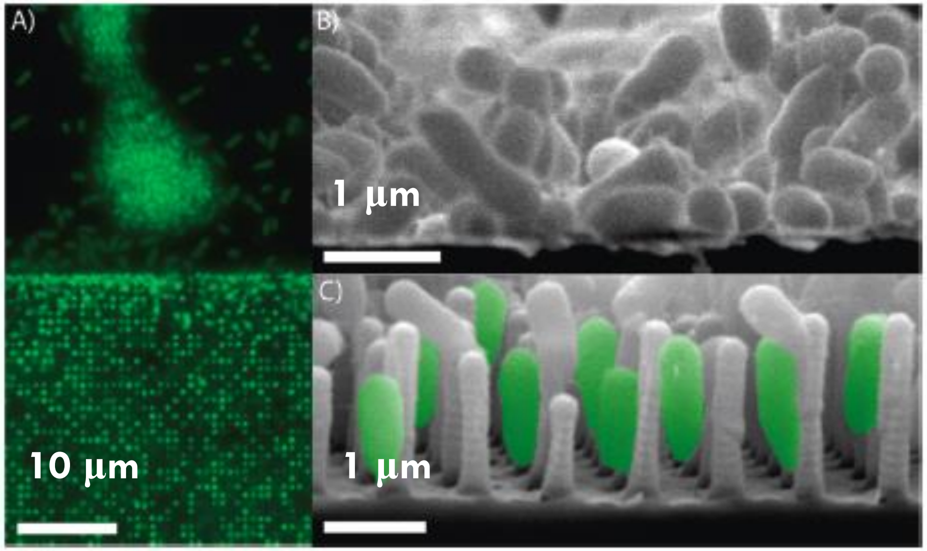
Cocci	*Sphere-shaped*	*~ 1 um*	
Baciili	*Rod-shaped*	*~ 0.5 -1 um wide*	*~3 um long*
Spiral	*Spiral-Shaped*	*~1-3 um wide*	*0.3-0.6 um long*

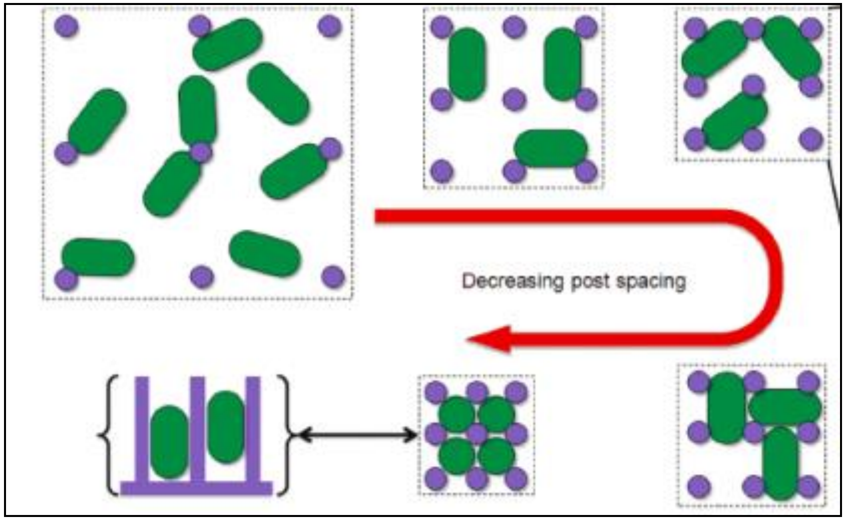
Home Sweet-Shaped Home!

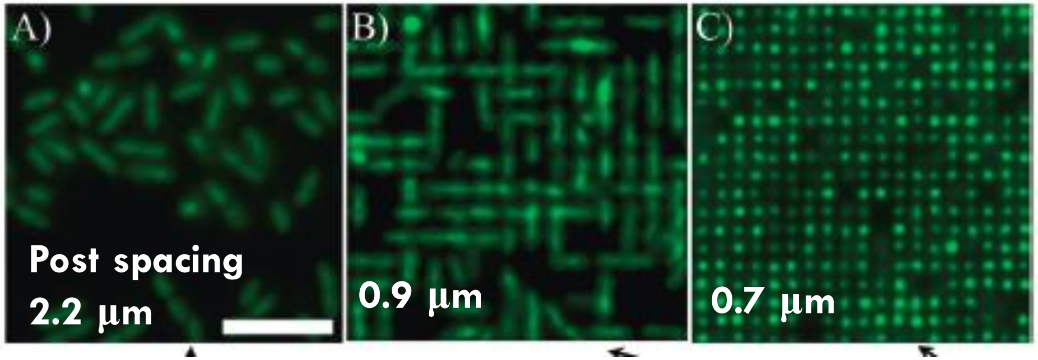
Pseudomonas aeruginosa

Rod-shaped bacteria 1-5 µm long x 0.5-1.0 µm wide

Organizes in 1.0 µm –spaced Si posts, and smaller







Surface scratches and materials defects on the size-scale of a spore or bacteria become optimal homes, as ***microorganisms tend to align with surface features of similar size in a way that maximizes surface area.***

Nano Lett ***10****, 3171-3721 (2010)*

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How to select materials?

Non-Metals:

- Polymers

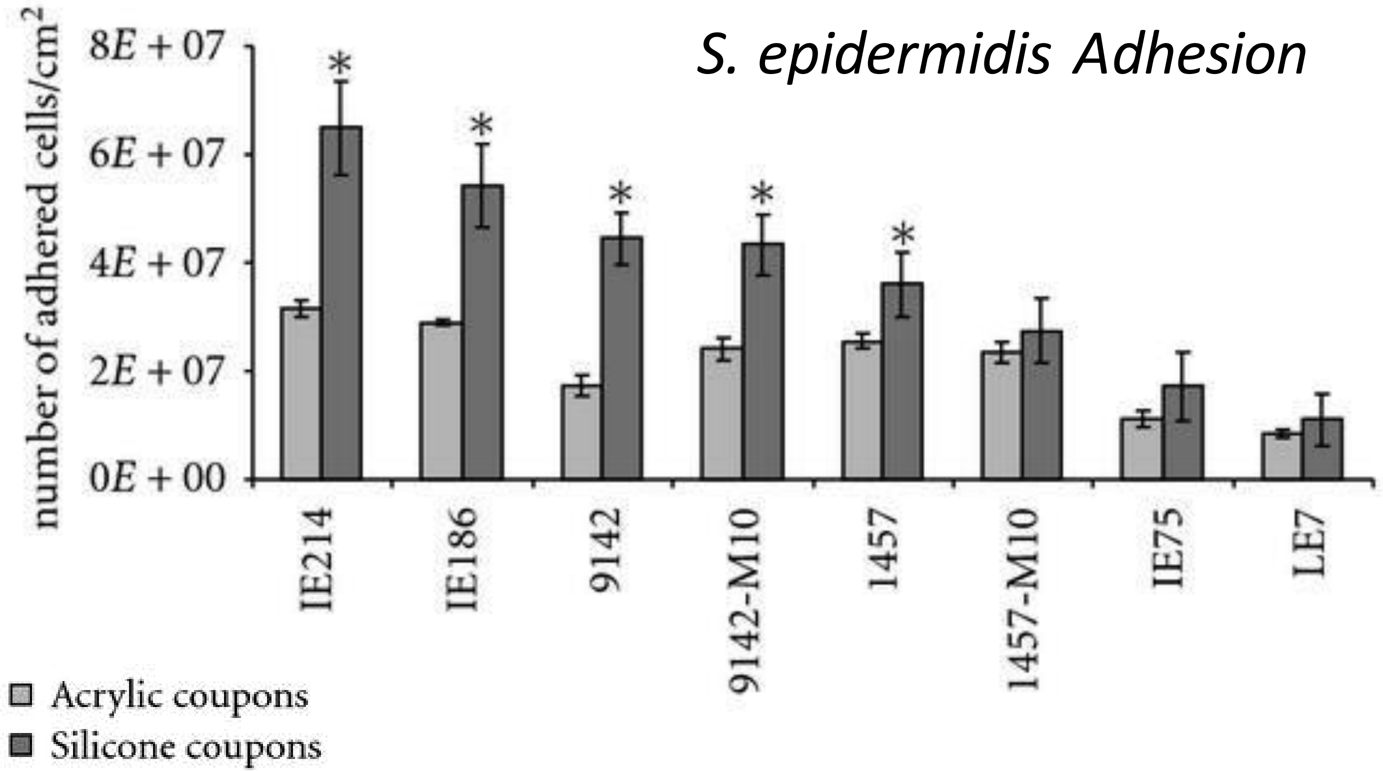
  Silicones, Acrylics, Kapton, Ultem, G-10, Vespel, Teflon
- Composites
- Oxides (Metal oxides, in particular)

  Ti-oxides, Zn-oxides (e.g. thermal paint)

Metals:

- Stainless Steel
- Titanium
- Aluminum
- Au, Ag, Cu

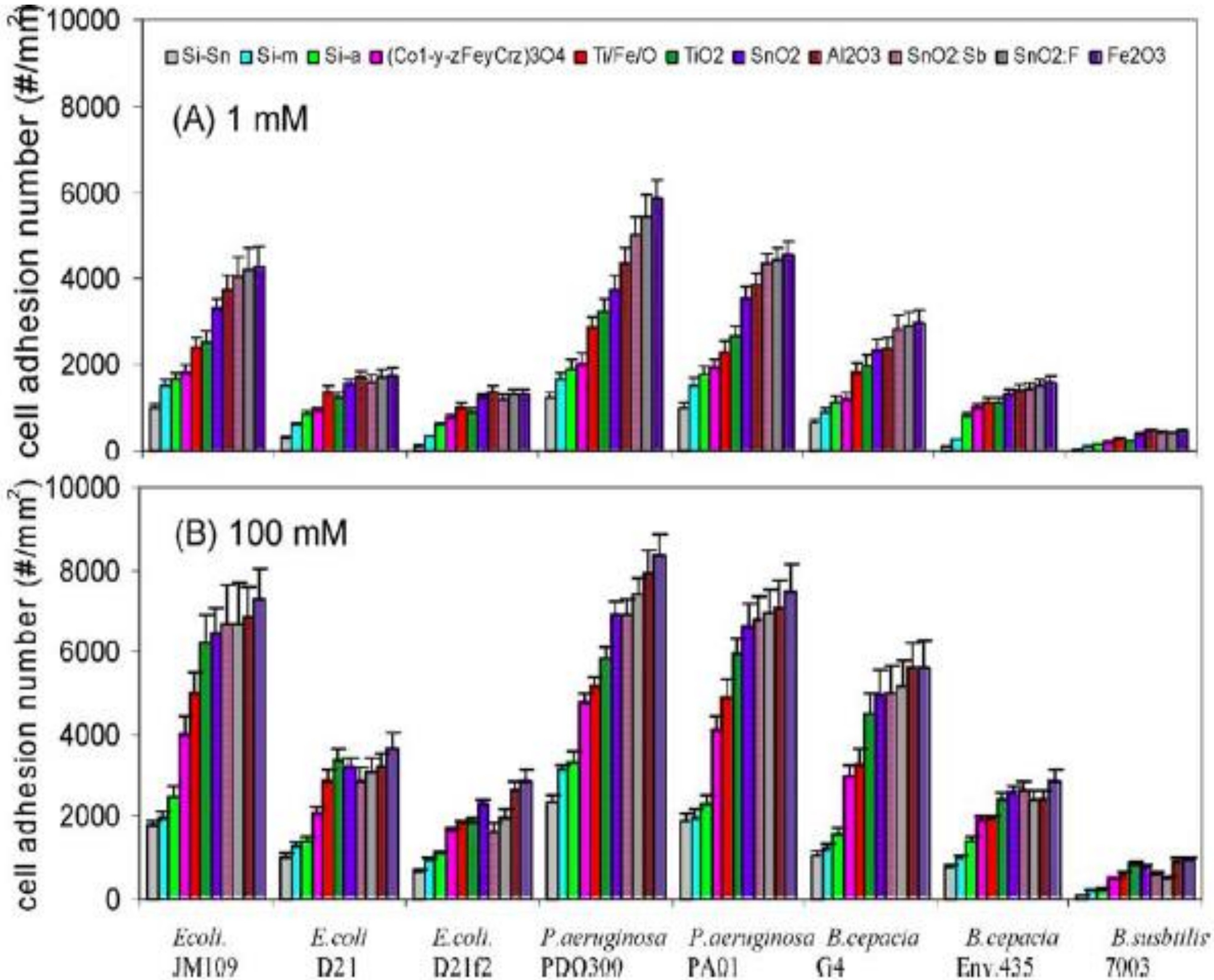
Polymers



- **Most polymers have hydrophobic surfaces, so hydrophilic strains tend to adhere better than hydrophobic strains.**
  - In the above plot, various strains of ***S. epidermidis*** were applied to silicone and acrylic, overall, the **strains adhered more to silicone vs. acrylic**
  - ***Candida, Streptococci, Pseudomonas species, and Staphylococci*** have also been seen to have **increased adhesion on silicone**

International Journal of Biomaterials Volume Article ID 718017 (2009)

Metal Oxides



Metal-oxide coatings show larger numbers of adhered cells compared to uncoated glass surfaces.

An aside: Across the board, Fe-oxides showed the greatest adhesion…could this matter for more than materials selection--Martian soil?

B. Li, B.E. Logan / Colloids and Surfaces B: Biointerfaces 36 (2004) 81–90

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Cleaning Hardware

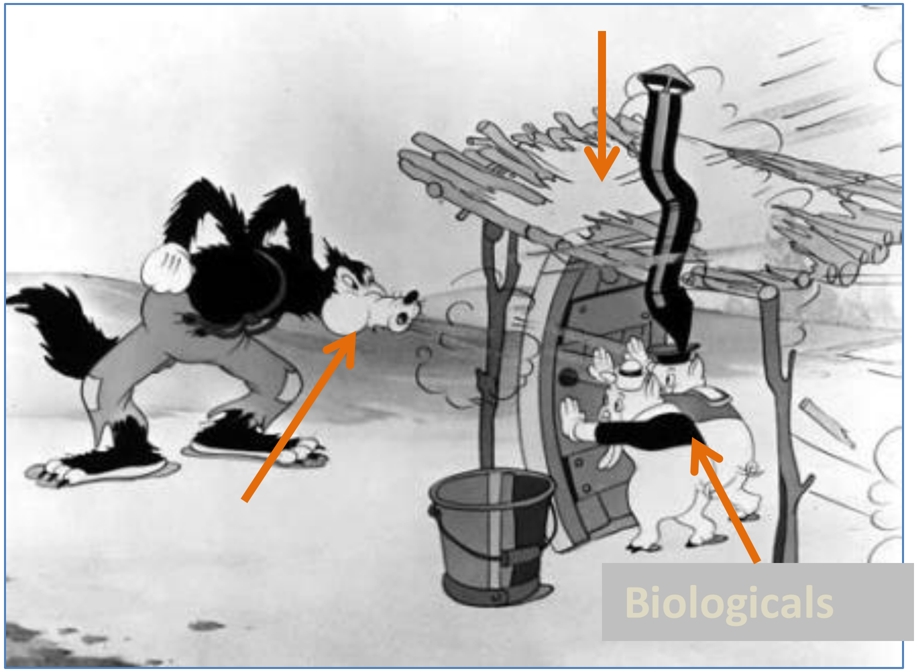
Understanding that relative differences between the shape of a microbe and the surface properties will influence removal efficiency.

Surface Wiping

- Direction of wiping
- Number of passes
- Size scale of features on the wipe relative to the surface finish

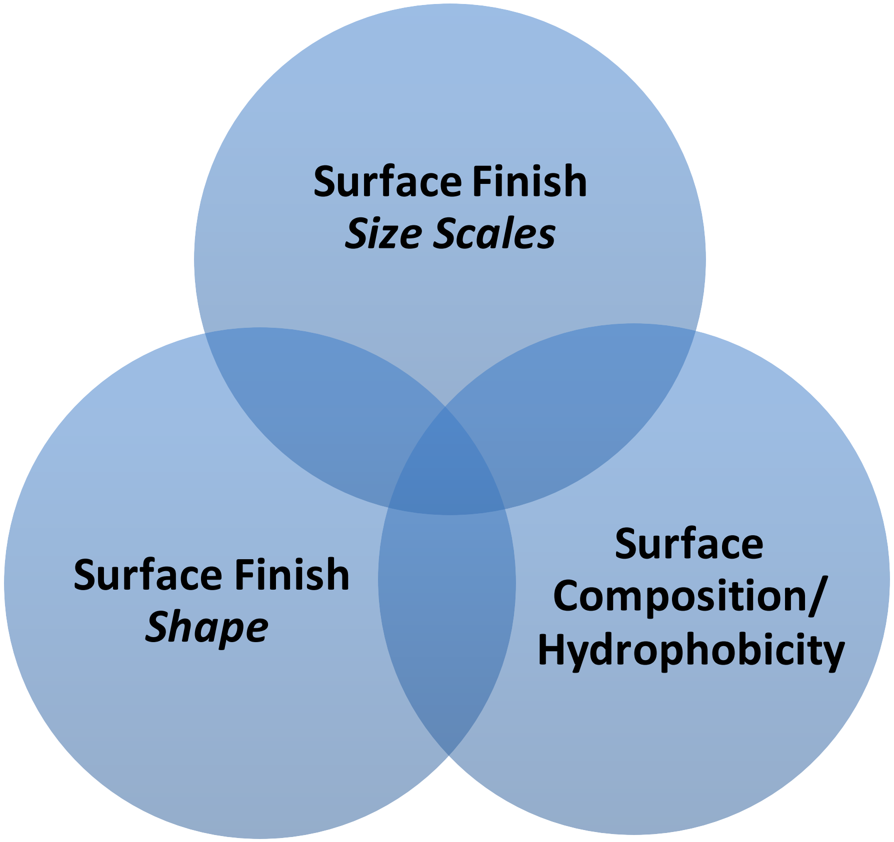
Other Cleaning Methods

- Pick the method to match the size scale of the feature s to insure removal
- For example, CO_2 snow cleans based on high-pressure particulates, for which the size scale ranges from 1-100 um, depending on thermal and flow conditions, that can be dynamically changed during the cleaning operation



***"You can huff and puff at a surface, but if the materials properties are not factored in, the biologicals may still remain!"* – B. B. Wolf**

Key Parameters Influencing Tiny House Habitability




a
b
10 µm
d
TIS BUT A SCRATCH
10 µm
10 µm


a
10 µm
But some call it a home.

Keep Our Solar System Weird

www.planetaryprotection.nasa.gov

Backup Slides

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Other Common Metals: Al, Cu

Aluminum

- limited investigations of the use of aluminum for biological, medical and dental applications, since aluminum has been observed to be supportive of biological activity
- *B. subtilis* in a simulated martian environment: Iridite-coated Al 6061 (Mars Exploration Rover Wheels) show growth

Copper

- Microorganism-dependent responses
    - *E.coli:* population increases, associated with copper tolerance
    - *Endospores:* more resilient to contact killing by copper than vegetative cells, killing may still occur *(e.g. strategic use of copper to curb spreading of C. difficile)*
- What other agencies think: EPA employs protocols for surface testing of copper for antimicrobial applications: http://www.epa.gov/pesticide-registration/updated-draft-protocol-evaluation-bactericidal-activity-hard-non-porous

Rensing *et al.* FEMS Microbiol Rev. **27,** 197-213 (2003)

Precious Metals: Au, Ag

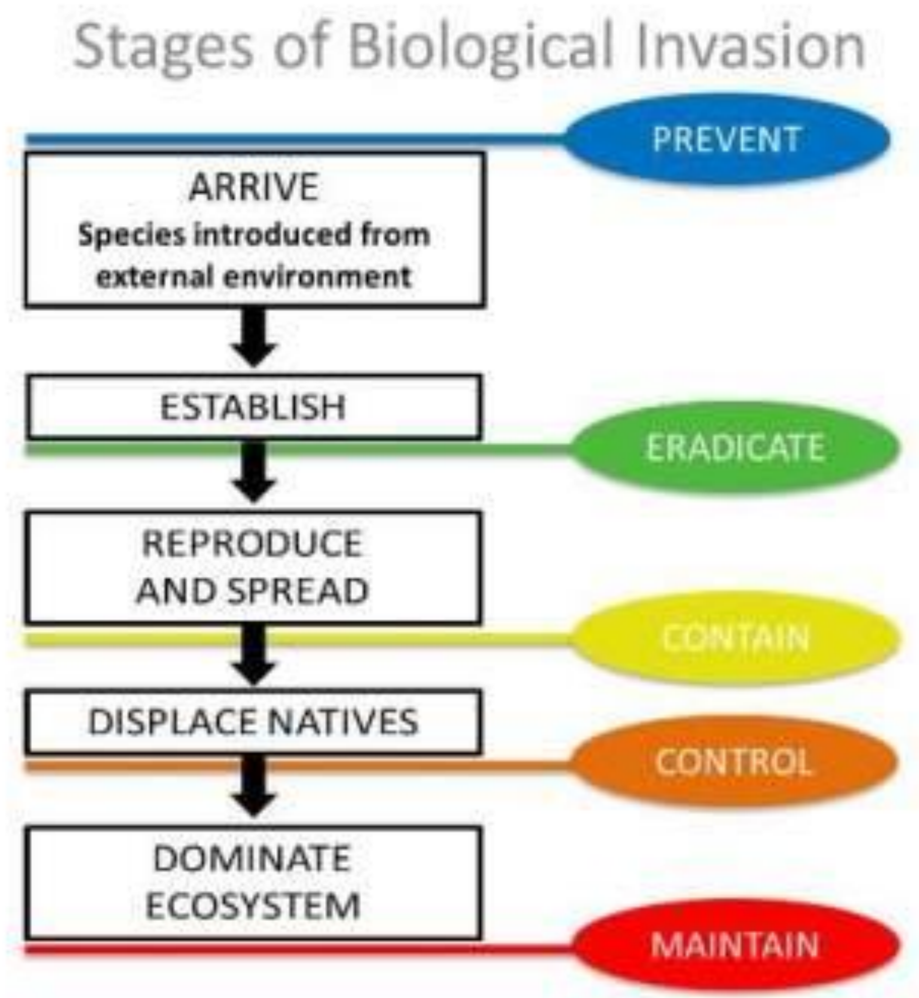
Silver

- Has been used as a way to the minimize the number of vegetative cells able to adhere to a surface
- When it comes to spores, silver surfaces of a wide range of roughnesses have not shown any effect

Gold

- Non-toxic to terrestrial microbes
- There is some recent evidence that nanoparticle gold may reduce the number of colonies of certain bacteria

How invasive species invade…



California Wildlife Foundation/Council of Invasive Species
https://www.wildlife.ca.gov/portals/0/Images/Invasives/stages.jpg