Goodbye Hospitals and Hello Nanosensors

Thomas J. Webster, Ph.D.

The Arthur W. Zafiropoulo Professor and Department Chair

Department of Chemical Engineering

Northeastern University, Boston, MA 02115 USA

Past-President, U.S. Society For Biomaterials

Editor, *International Journal of Nanomedicine*

Associate Editor, *Nanomedicine: NBM*

Th.webster@neu.edu

Disclosures

- I have a financial interest in some of the material to be presented via my involvement in:
  - Nanovis, LLC
  - Audax, Inc.
  - Perios, Inc.
  - NanoFe, Inc.
  - NanoSeleno, Inc.
  - NanoVault, Inc.
  - Ultratech, Inc.
  - Tyber Medical, Inc.
  - Ortho-Tag
  - Amedica
  - Vexti

Pop Quiz:

Is life expectancy increasing or decreasing in the U.S. ?

<u>Pop Quiz</u>:

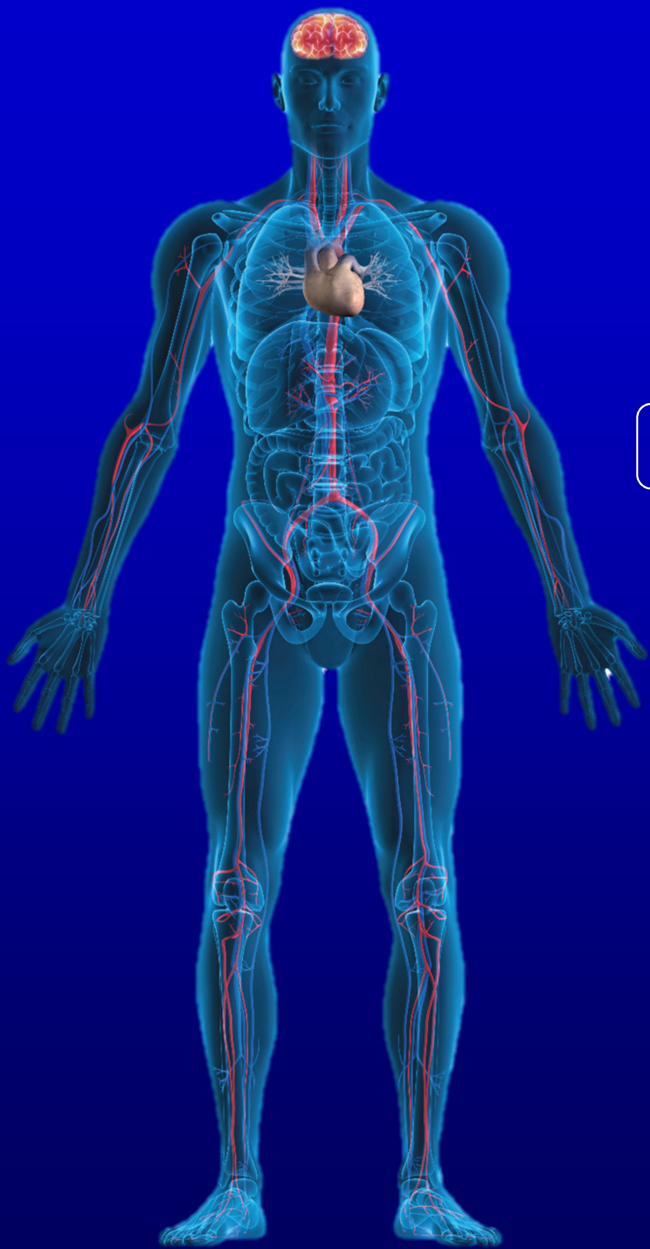
Is life expectancy <u>increasing</u> or <u>decreasing</u> in the U.S. (over the past 2 years) ?

Pop Quiz:

Do you think our approach to healthcare is working ?

Yes or No

Current Problems in Healthcare

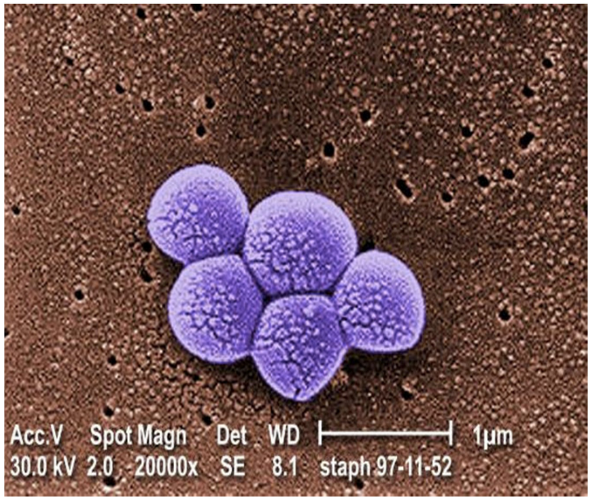


- Medical devices that fail
- Over dependency on drugs to fix everything
- Treating every patient the same
- Reactionary versus predictive
- Increasing costs
- Increasing patients
- And the list goes on…

What may be the answer ?

The Emergence of Antibiotic Resistant Bacteria

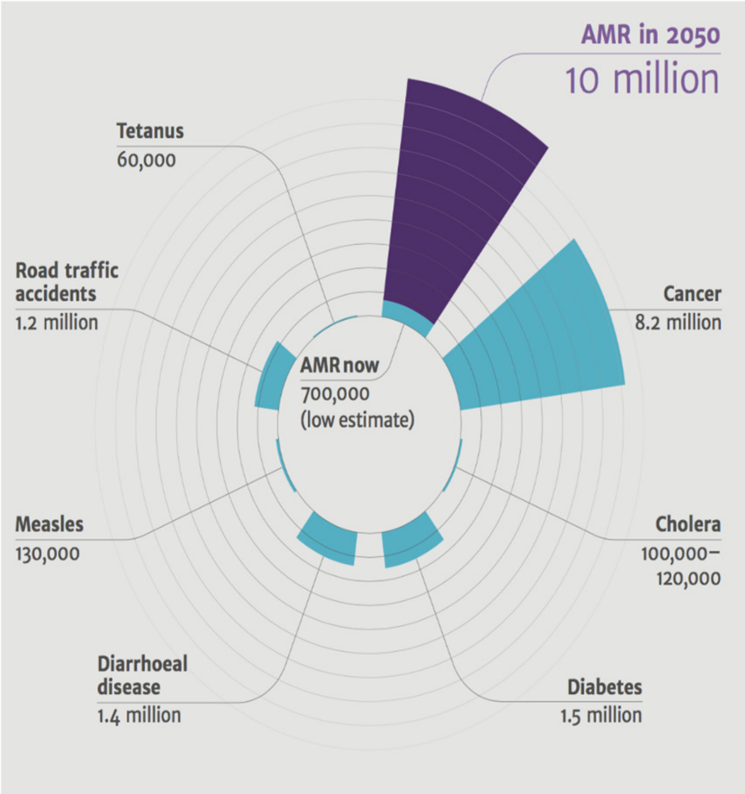
Colistin-resistant *Escherichia coil* (*E.coil*)



Methicillin-resistant *Staphylococcus aureus* (MRSA)

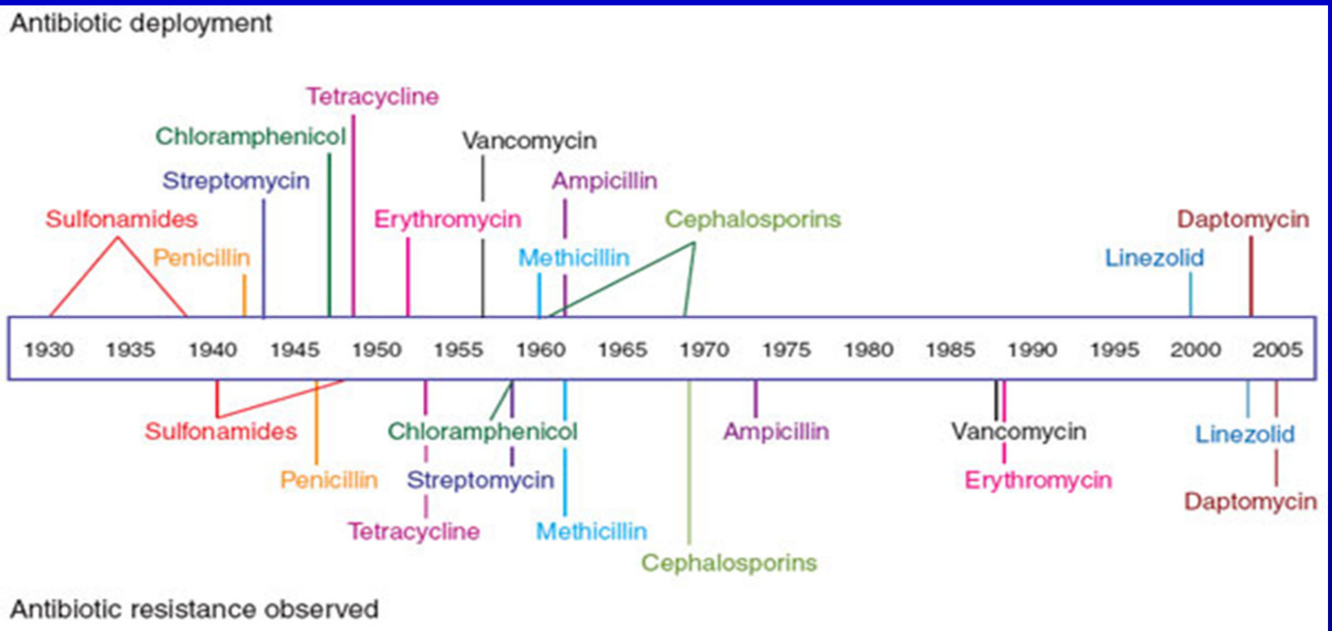
Bacterial antibiotic resistance causes

- **More than 2 million cases of illness and 23 thousand deaths annually (in the U.S. only)**
- **In 2050, about 10 million deaths and will cost 100 trillion USD annually**

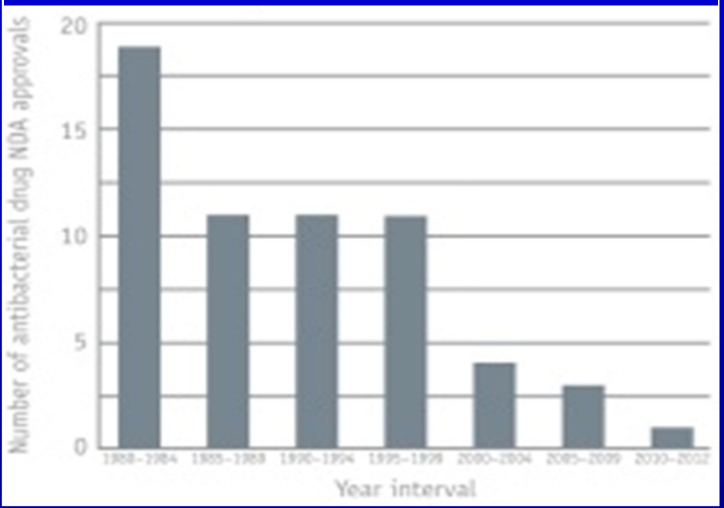


https://www.cdc.gov/drugresistance/;
https://amr-review.org/Publications.html

Problems with Infection



Number of Antibacterial New Drug Application Approvals per Year



>2 million resistant infections/yr

>23,000 deaths/yr

Longer treatment durations

Undesirable side-effects

$20 billion in excess direct healthcare costs

Immediate public health threat requiring **urgent** and **aggressive** action

Clatworthy AE, Pierson E, Hung DT. *Nat. Chem. Bio.* 2007;3:541-548

Antibiotic Resistance Threats in the United States, 2013. Center for Disease Control

Current Problems in Healthcare

- Medical devices that fail
- Over dependency on drugs to fix everything
- Treating every patient the same
- Reactionary versus predictive
- Increasing costs
- Increasing patients
- And the list goes on…

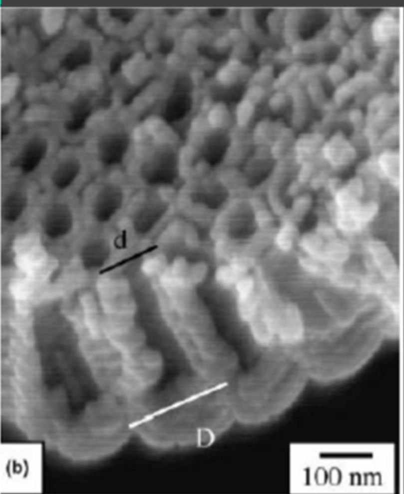
What may be the answer ?

25 Years Ago We Turned to Nanomedicine for Some Answers

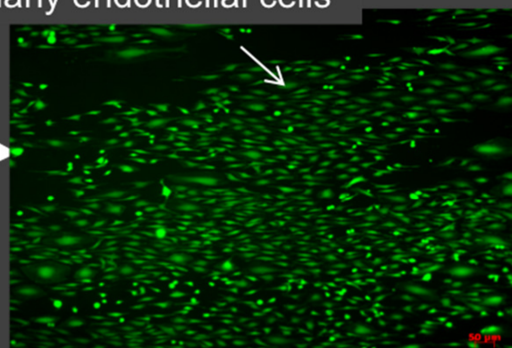
Nanotechnology: The use of materials whose components exhibit significantly changed properties by gaining control of structures at the atomic, molecular, and supramolecular levels.

Nanomedicine: Applications of nanotechnology in medicine.

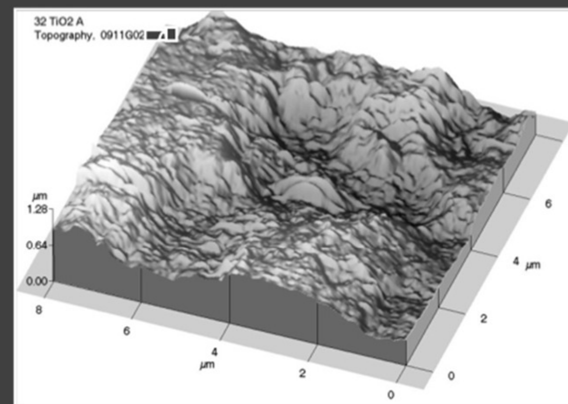
Examples: Nanostructured Surfaces



Many endothelial cells

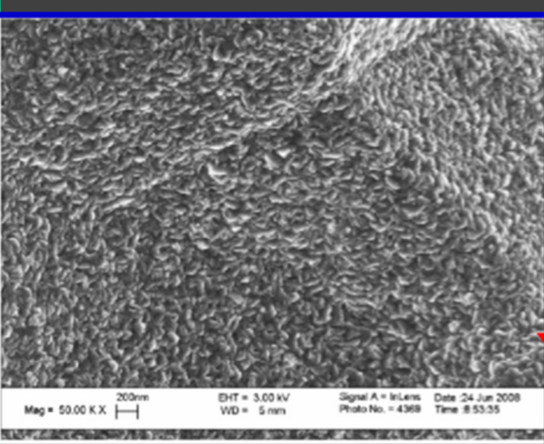


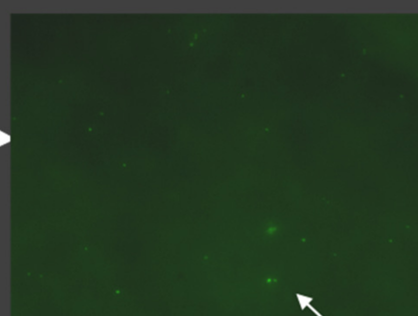
Vascular Endothelialization



Increased bone growth

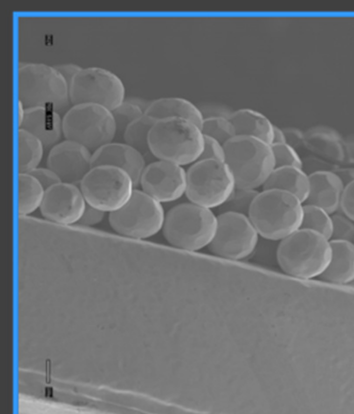
Bone Growth





Few *Staph Epi*

Antibacterial



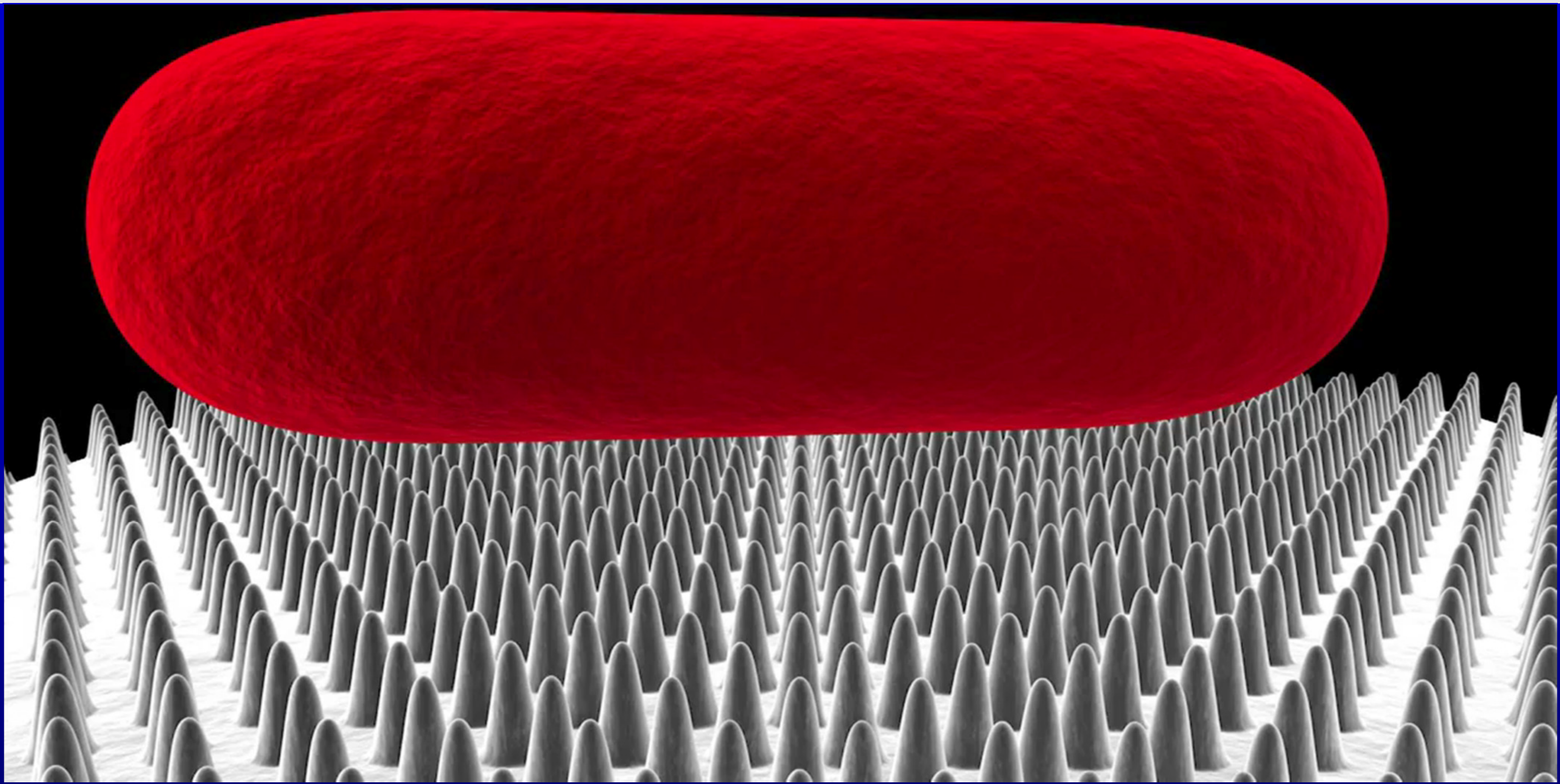
Perpendicular collagen fibers

Orthopedic Soft Tissue

Why Use Nanotechnology To Fight Bacteria ????

Part 1: Nanostructured Materials

<u>Possible Reason</u>: Biophysical model



Biophysical model of bacterial cell interactions with nanopillars

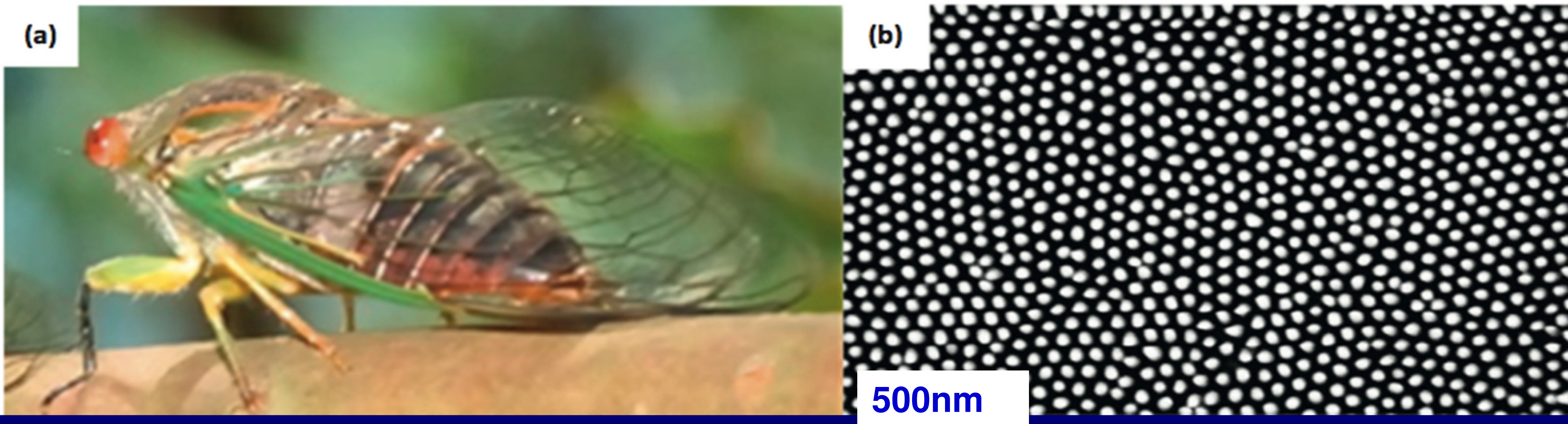
<u>Mechanism</u>: As the bacteria try to attach onto the nanopillar structures, the cell membrane stretches in the regions suspended between the pillars. If the degree of stretching is sufficient, this may lead to no attachment or cell rupture.

Pogodin at al. *Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces*. Biophysical Journal, Volume 104, pp. 835-840, 2013.

Nanostructures in Nature

It has been found that the nanopillars on cicada wings are inherently antibacterial, irrespective of surface chemistry.

- Results show that the cicada wing surface appears to be bactericidal to *Pseudomonas aeruginosa*.

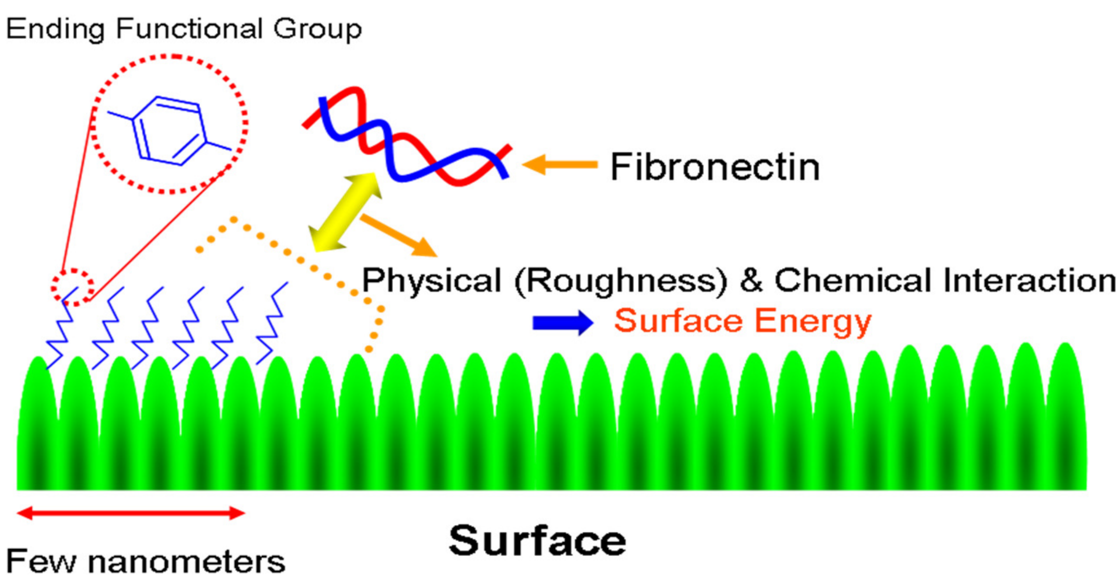


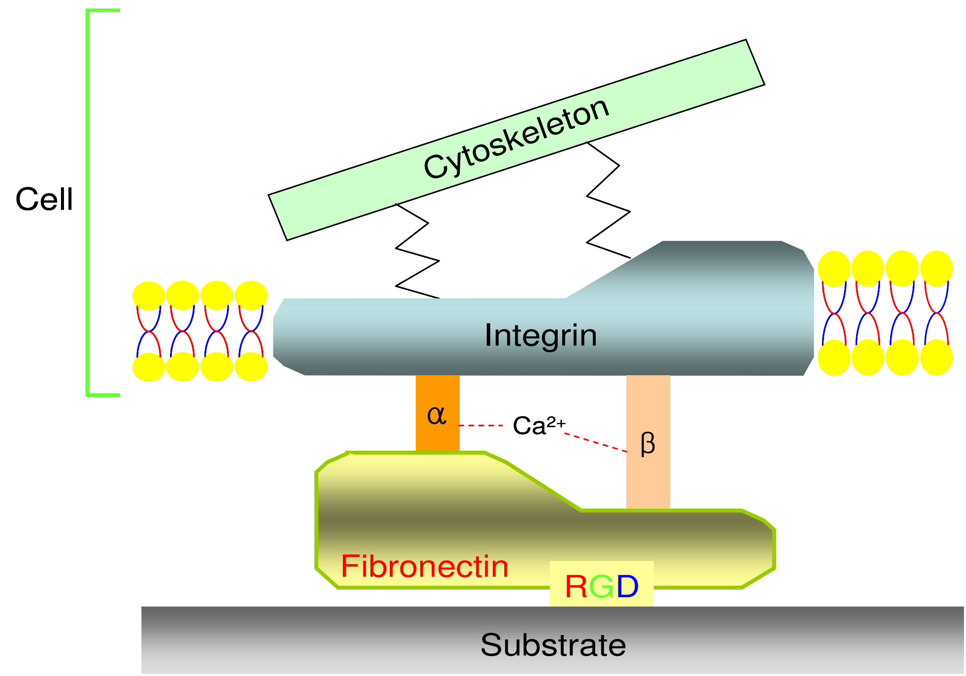
The nanopillar structures of the wing surface are spaced 170nm apart from center to center. Each pillar is ~200nm tall, with a conical shape and a spherical cap 60nm in diameter.

Pogodin et al. Biophysical model of bacterial cell interactions with nanopatterned cicada wing surfaces. *Biophys. J.* 2013, 104, 835-840.

The Cellular Micro and Nano-environment

Surface micro- and nano-scale topography, grain structure, chemistry, and substrate stiffness modulate cellular functions at the cell-substrate interface[1-6]



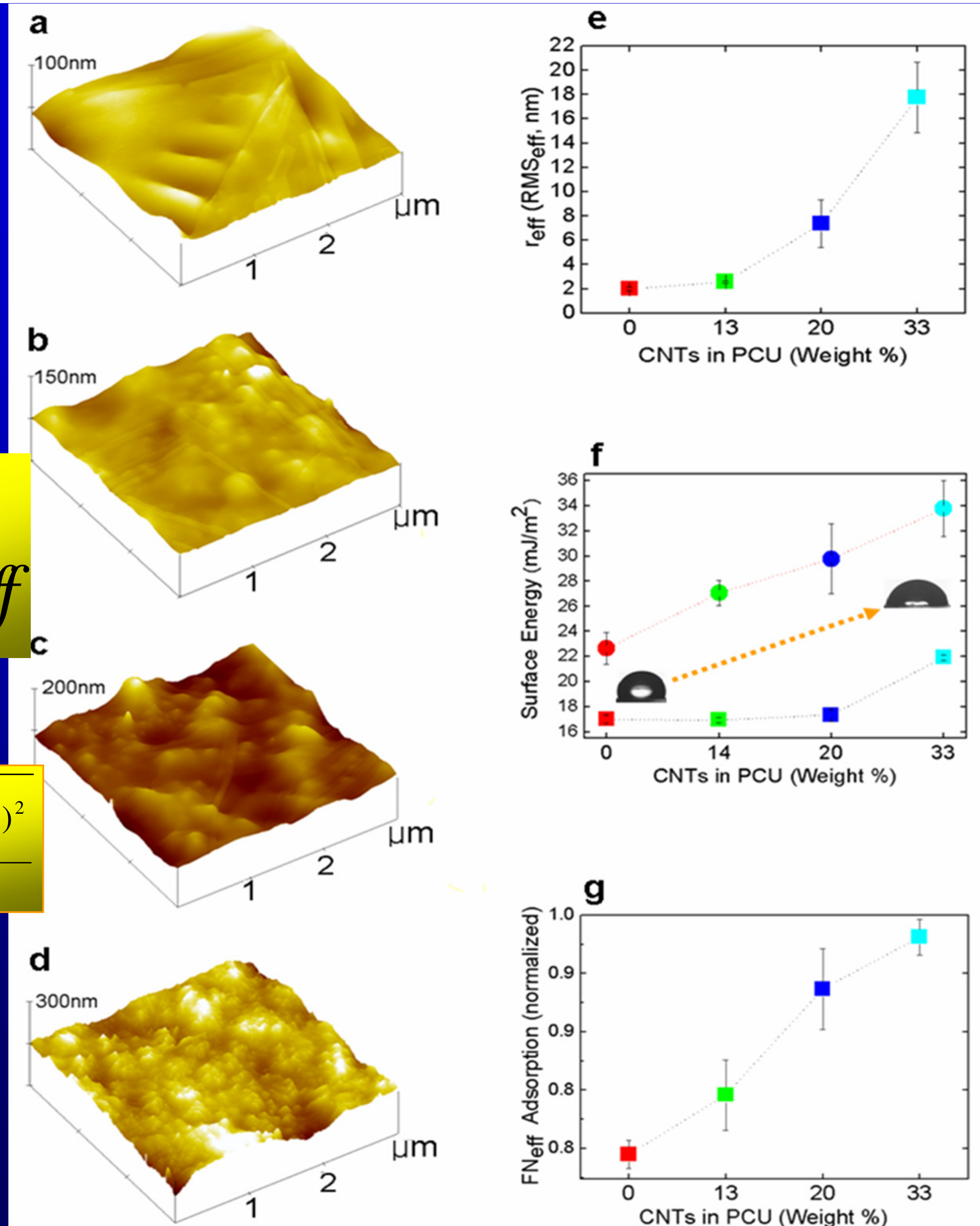


1. Webster, T. J. *et al.*, *Biomaterials* **21,** 1803–1810 (2000). **2.** Nikkhah, M. *et al.*, *Biomaterials* **33**, 5230–5246 (2012). **3.** Bagherifard, S. *et al.*, *ACS Appl. Mater Interfaces* **6**, 7963–7985 (2014). **4.** Guvendiren, M., Burdick, J. A., *Nat. Commun*. **3,** 792 (2012). **5.** Dolatshahi-Pirouz, A. *et al.*, *ACS Nano* **4**, 2874–2882 (2010). **6.** Dolatshahi-Pirouz, A. *et al.*, J. *Funct. Biomater*. **2** 88–106 (2011).

$$E_s(r_{eff}) = E_{0,s} + \rho r_{eff}$$

⇧

$$r_{eff} = \frac{S_{unit}}{S_{measured}} \cdot \sqrt{\frac{\sum_{i=1}^{N} (Z_{i,filtered} - Z_{ave,filtered})^2}{N}}$$

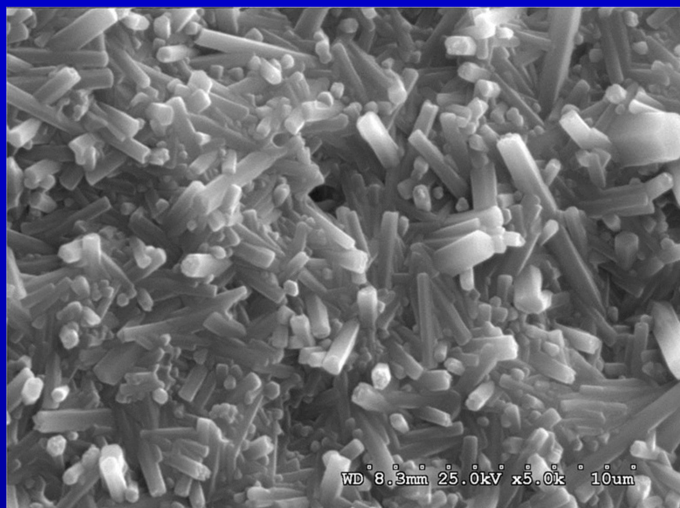


We can increase nanoscale roughness and not change chemistry to control protein adsorption

and we have taken this approach to the FDA

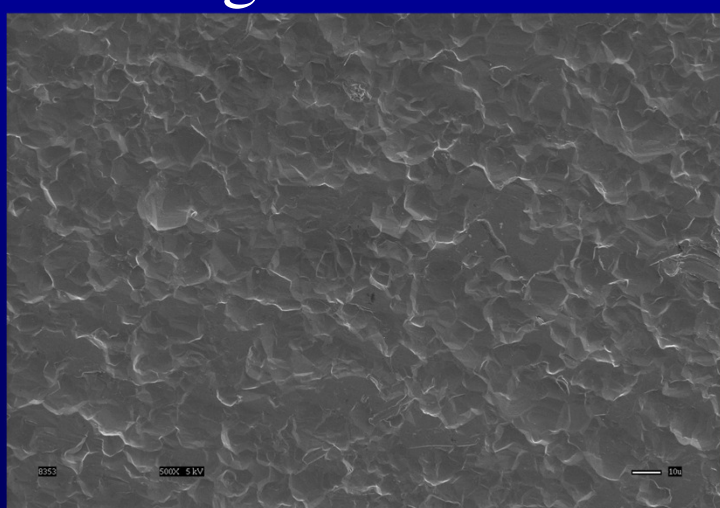
Challenge #1: We need to establish more quantitative models to predict material properties that control bacteria behavior.

Example: Commercialized by Amedica: Nanostructured Silicon Nitride

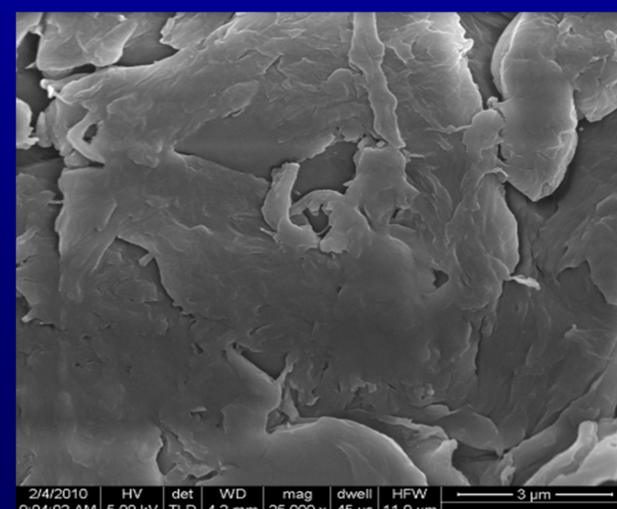


Nanorough Silicon Nitride

Smooth Silicon Nitride



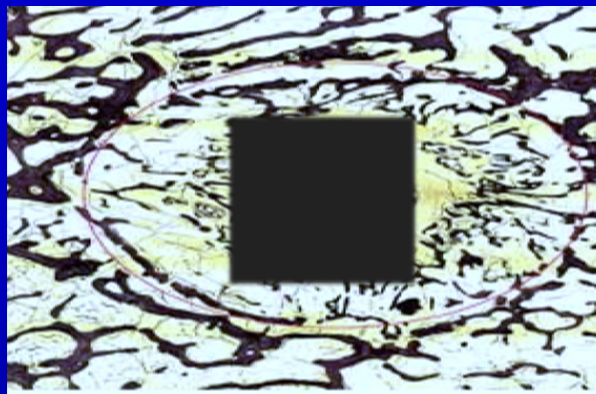
Titanium



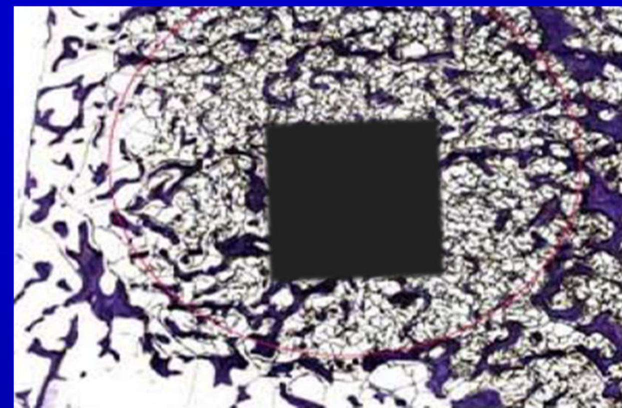
PEEK

<u>Silicon Nitride</u>: 3 Months (bacteria innoculation)

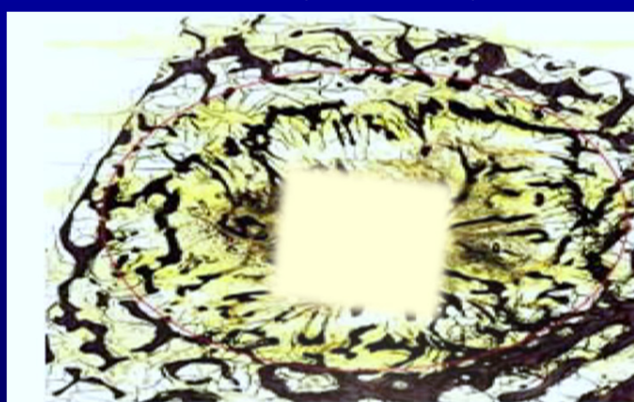
Rat calvaria model



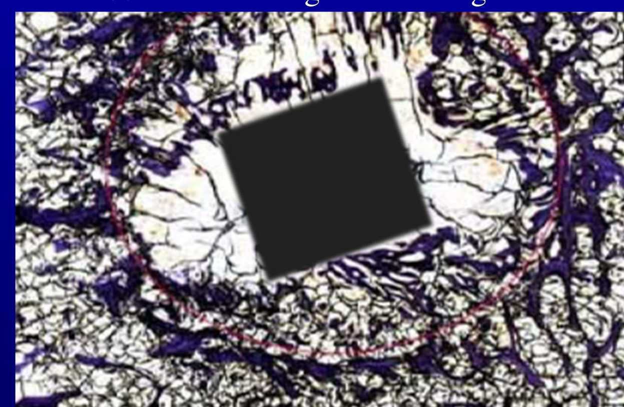
Titanium –
9% bone-implant interface
67% bacteria-implant interface
26% of new bone growth in surgical area
21% of bacteria growth in surgical area



Silicon Nitride (nano-rough) –
41% bone-implant interface
0% bacteria-implant interface
42% of new bone growth in surgical area
0% of bacteria growth in surgical area

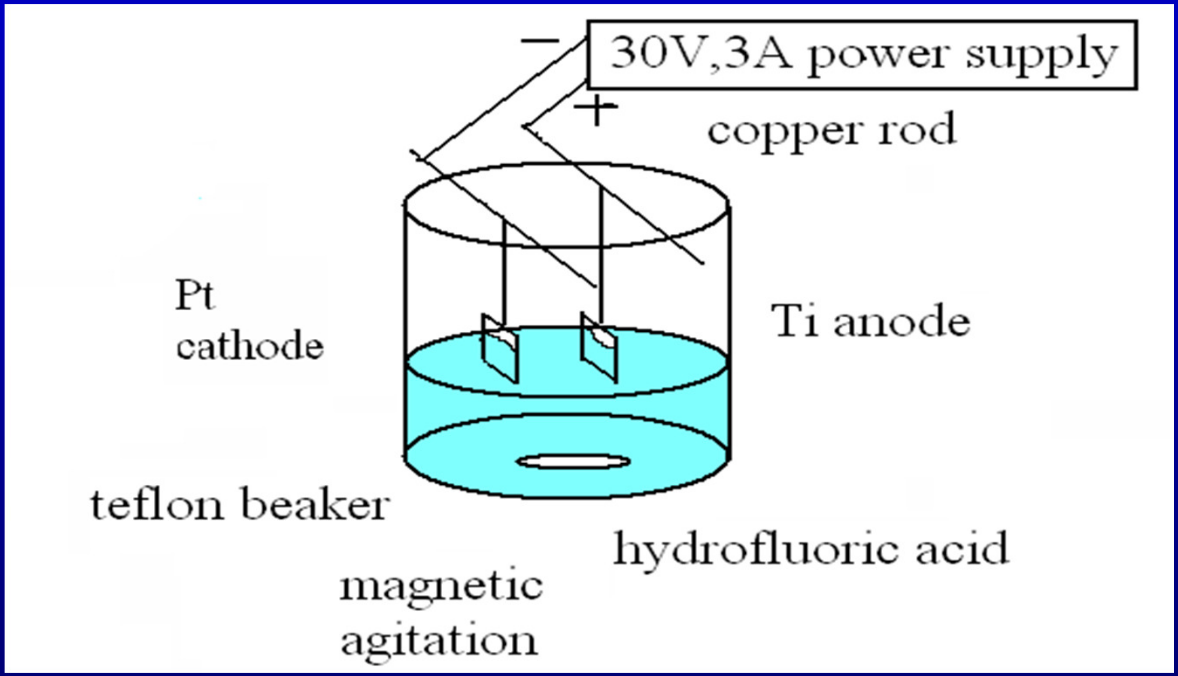


PEEK –
5% bone-implant interface
95% bacteria-implant interface
21% of new bone growth in surgical area
88% of bacteria growth in surgical area



Silicon Nitride (smooth) –
15% bone-implant interface
10% bacteria-implant interface
29% of new bone growth in surgical area
10% of bacteria growth in surgical area

Example: Commercialized by Nanovis, LLC
Anodized Titanium



Sketch map of anodization system

PROCEDURES:

Pretreatment: chemical polishing using HF/HNO_3 mixture

Anodization: 0.5 or 1.5% HF

Voltage: 20V

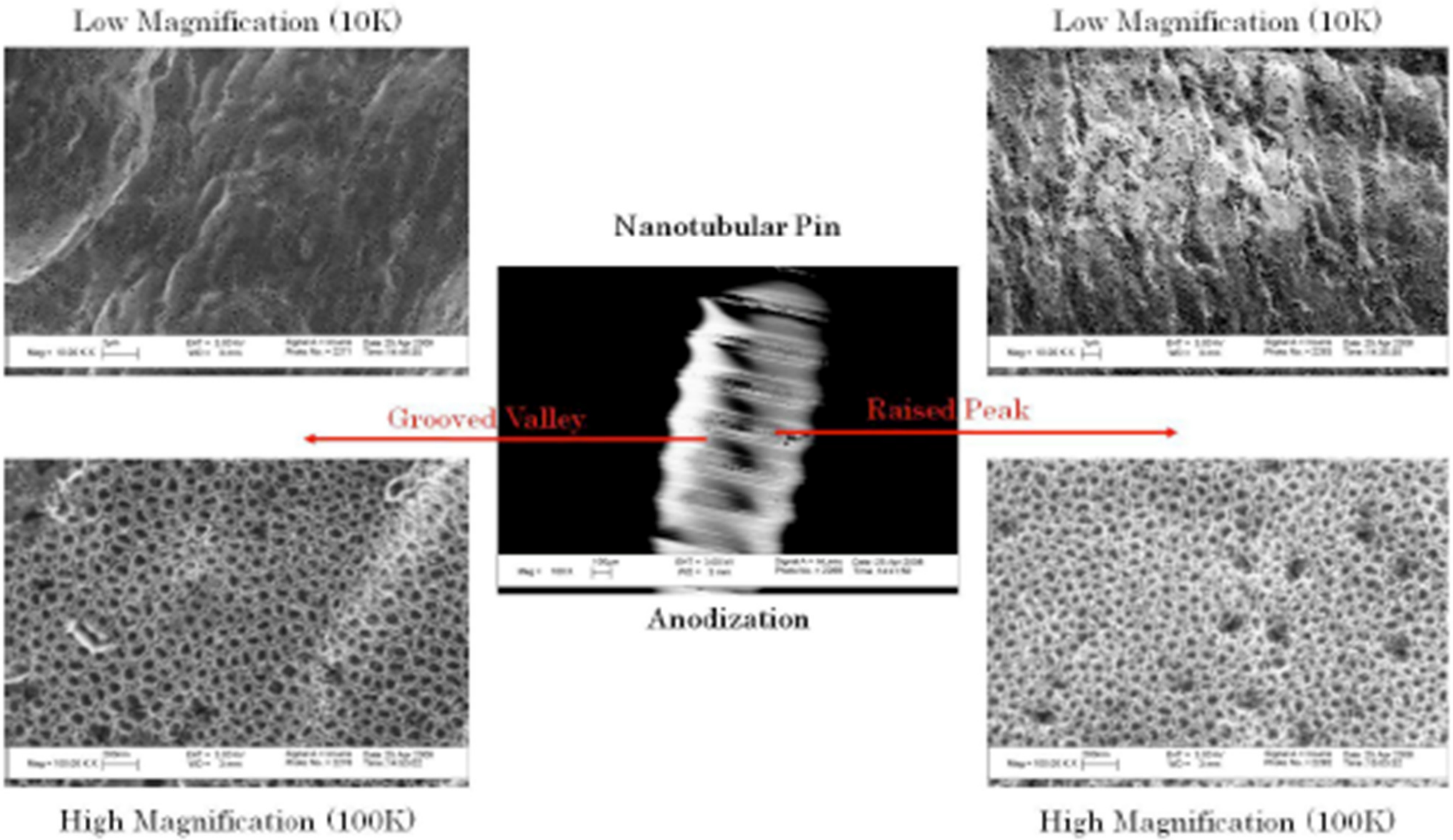
Time: 20 min

Rinse and dry

Clean: acetone and ethanol

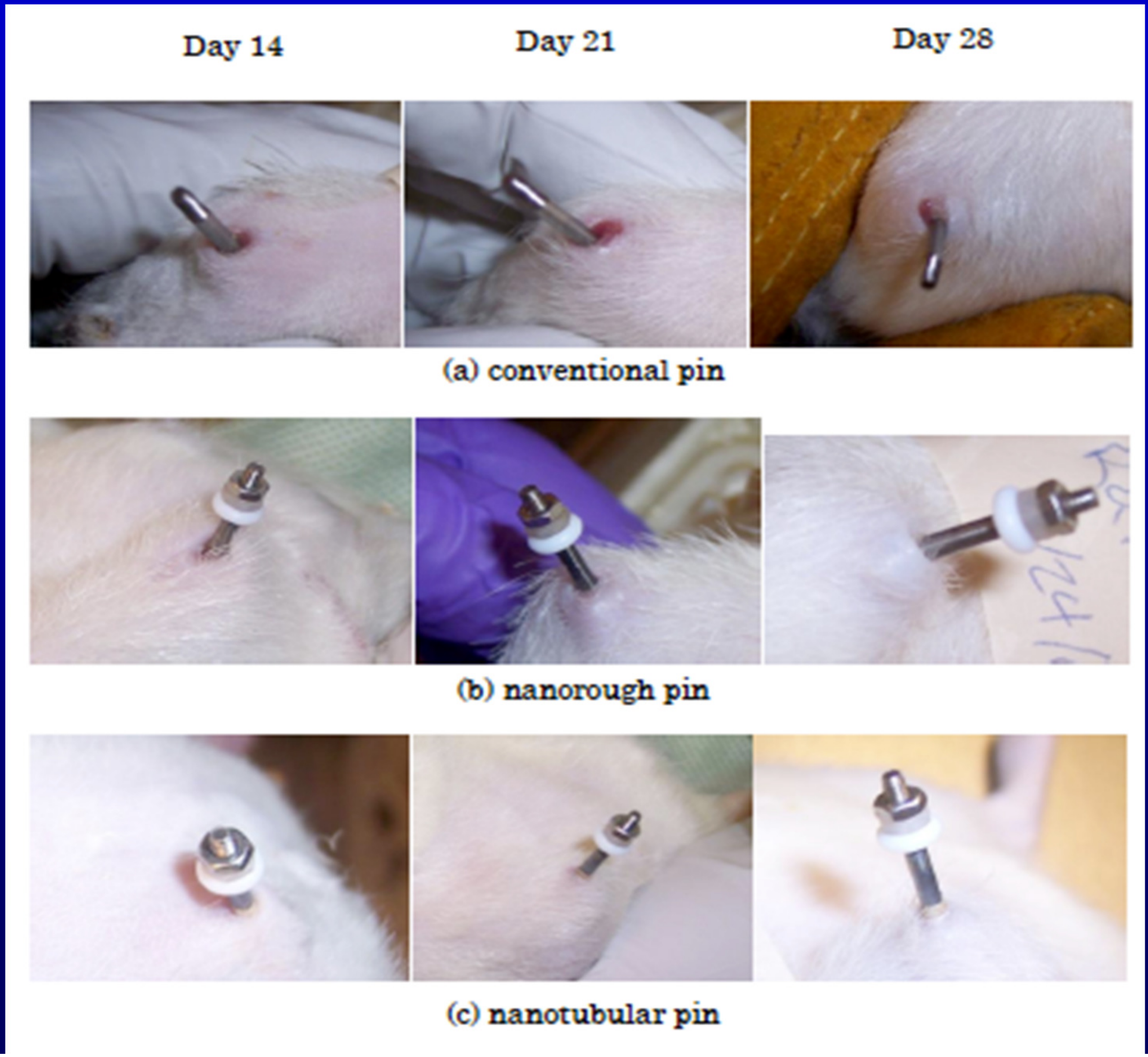
Sterilize

Anodized Ti Nanotubular Screws



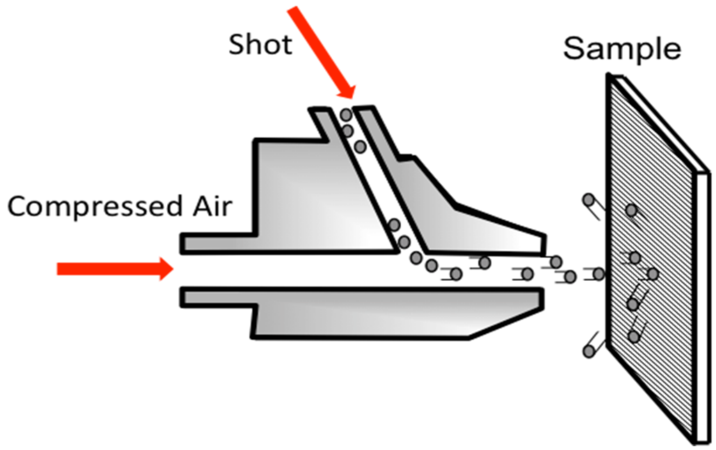
Closed Wound with No Infection Surrounding Nanotextured Screws Only

Nanovis, LLC
is now commercializing
this as a pedicle screw

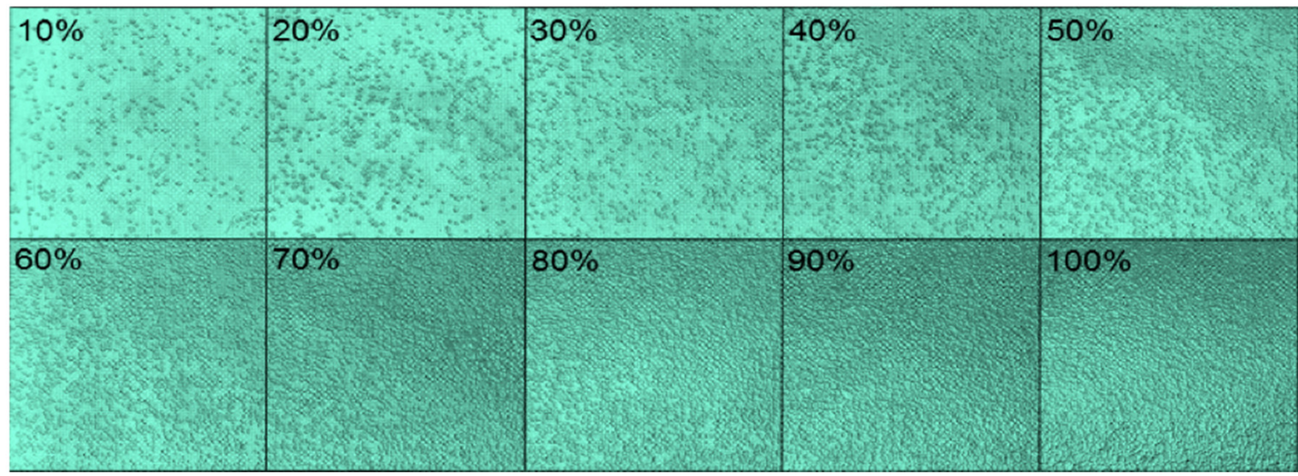


Challenge #2: Do not give up on “old” materials – we do not always need “new” materials

Example: Surface Modification Technique
Shot Peening

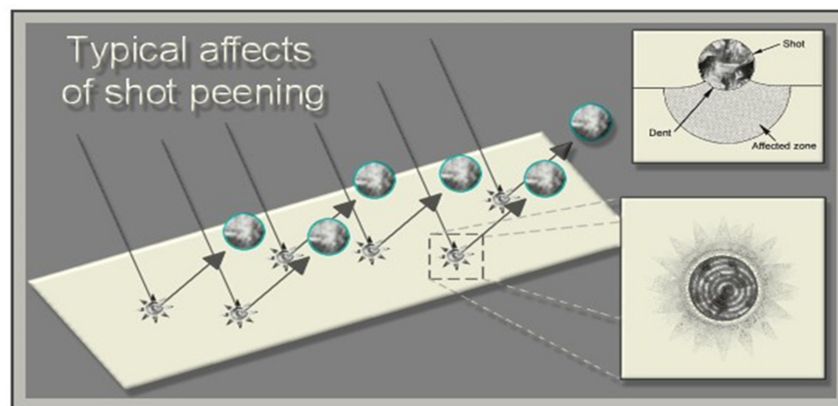


Surface coverage: is defined as the ratio of the area covered by plastic indentation to the whole treated surface area.

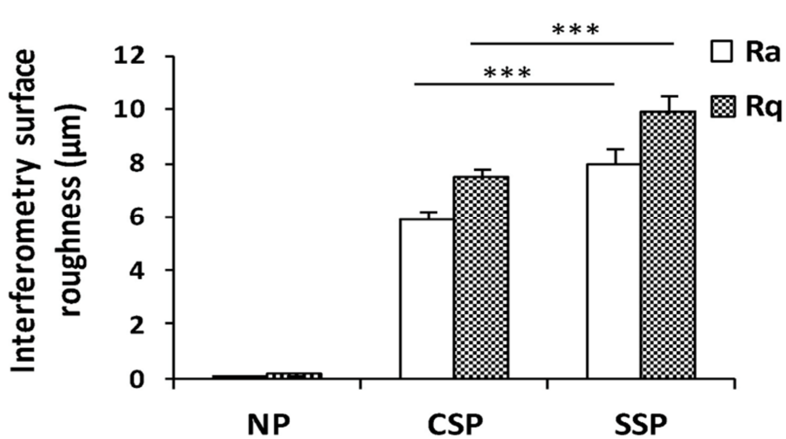


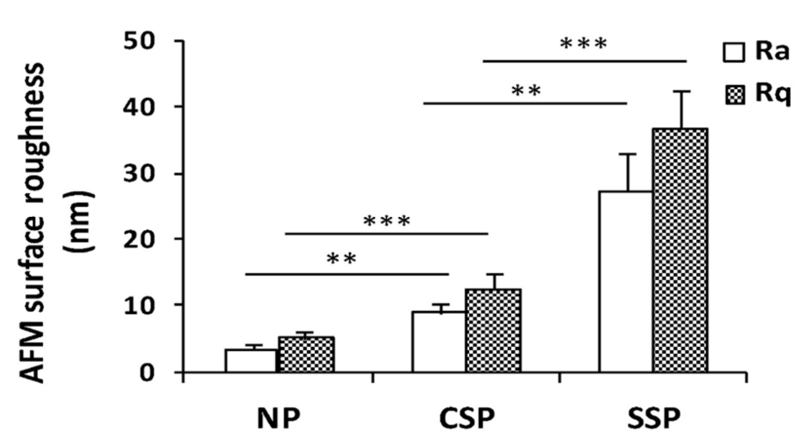
Shot peening effects:

- Residual stress
- Microstructural changes to the material
  - Dislocation density increase
  - Grain distortion
  - Phase change
- Surface roughness

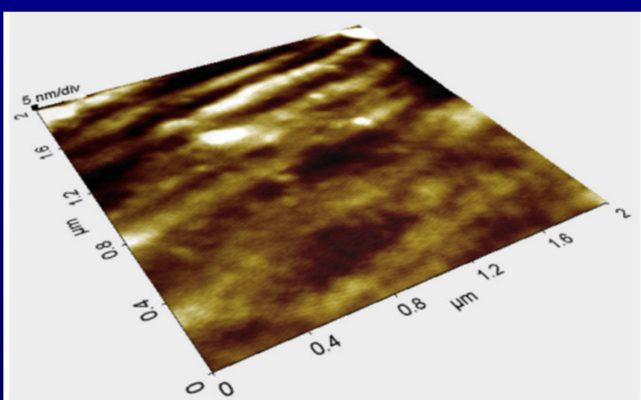


Stainless Steel: Increased Surface Roughness

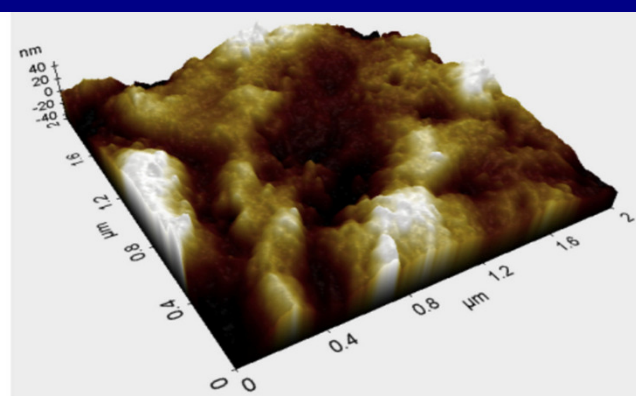




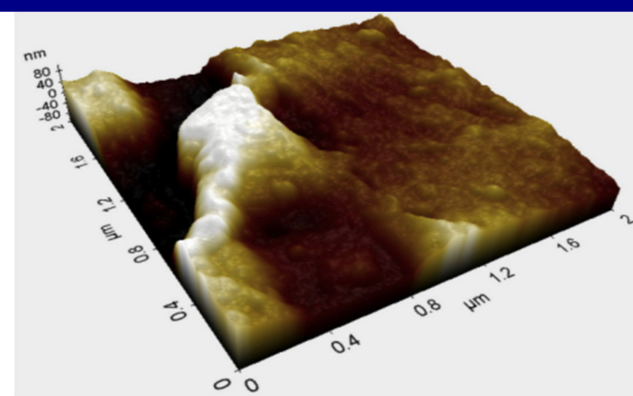
Ra = arithmetic mean, Rq = root mean square (rms) surface roughness
Data is mean ± St. Dev.; N=3, **$p<0.05$, ***$p<0.005$



NP



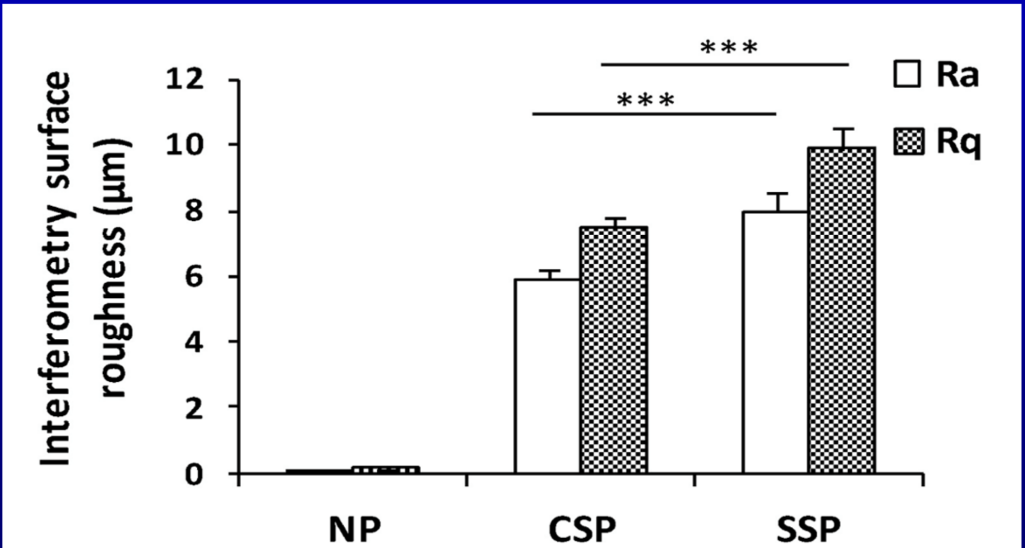
CSP



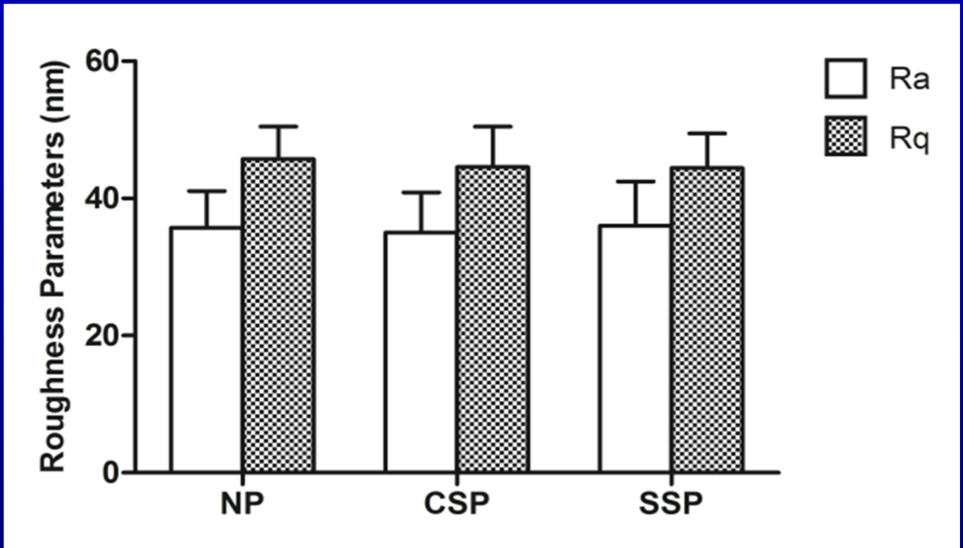
SSP

Stainless Steel: Separating Surface Roughness from Grain Size

At this point, half of the samples (both treated and as-received) were ground and polished **to obtain identical surface roughness for all samples**.

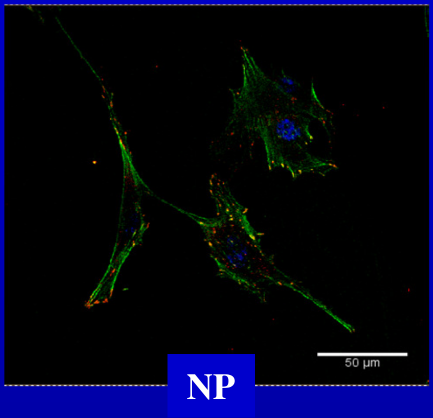


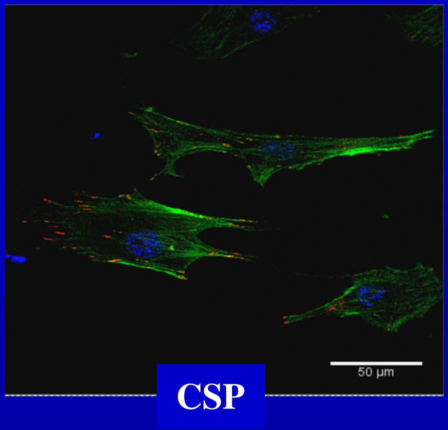
As-treated

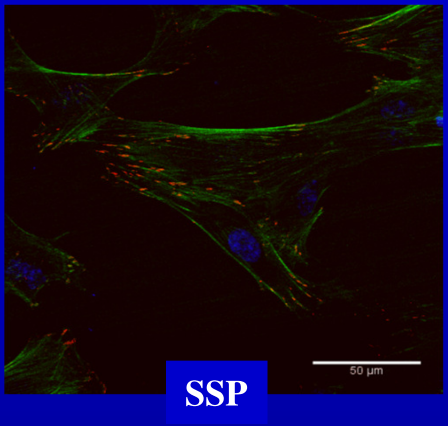


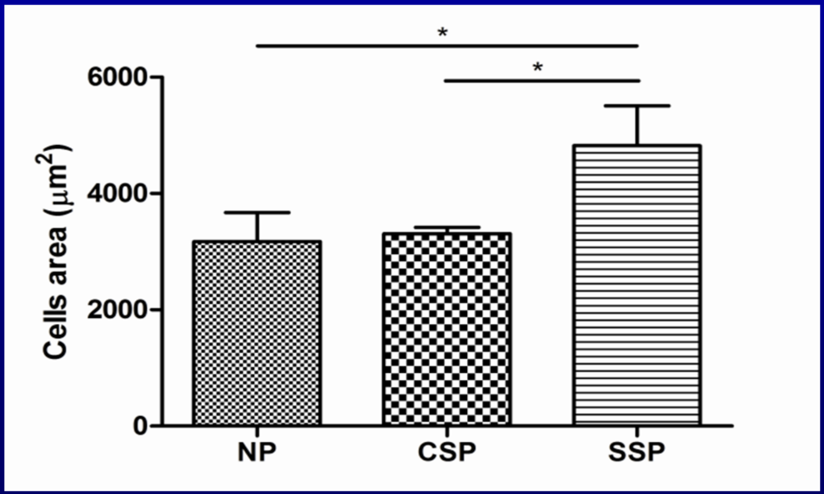
Polished

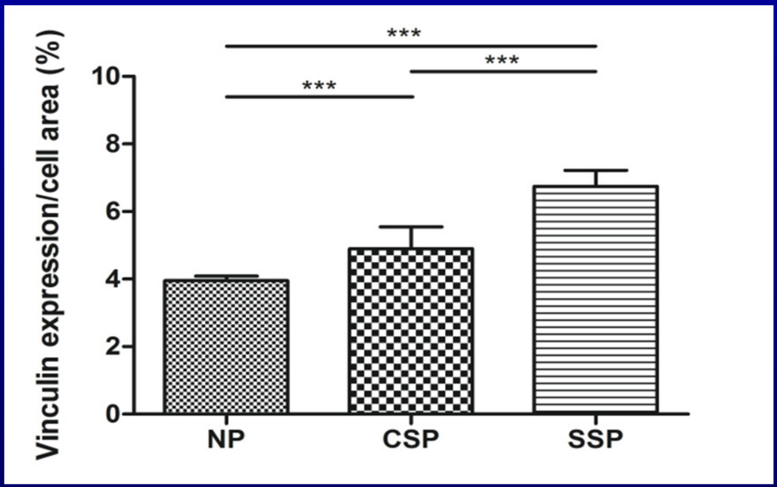
Osteoblast Morphology and Spreading on Polished Samples (1 Day)









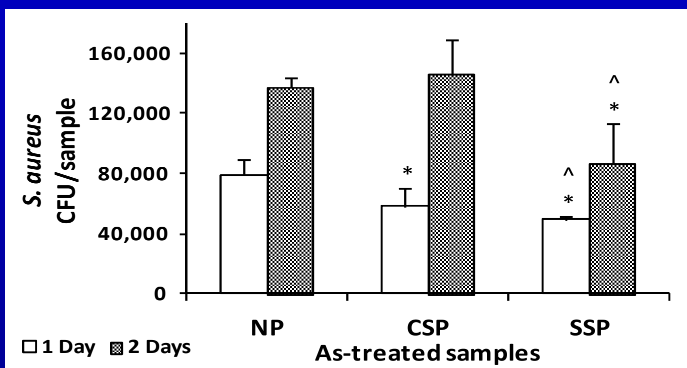


Data is mean ± St. Dev.; N=3,
*p<0.05
***p<0.001

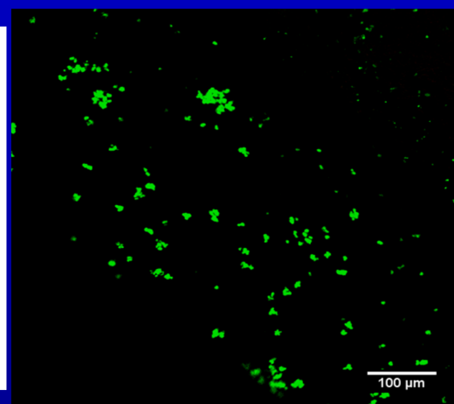
So both nanoscale surface features and nanoscale grain sizes increase osteoblast functions, but what about bacteria ?

No, only nanoscale surface features.
An example, *Staphylococcus aureus*

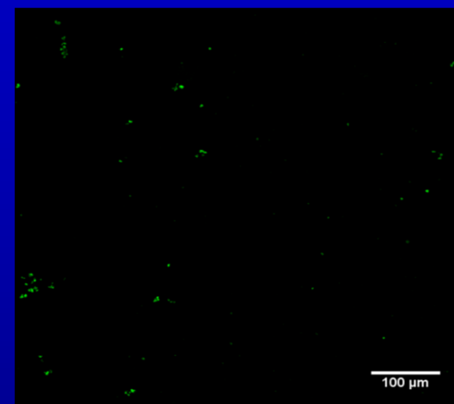
As-treated



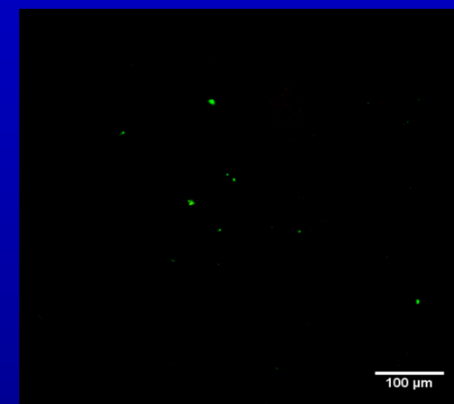
NP



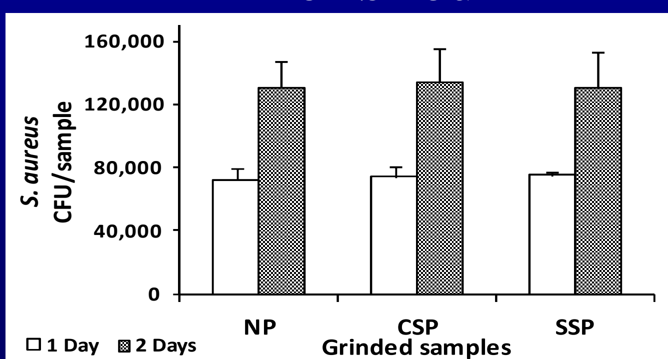
CSP

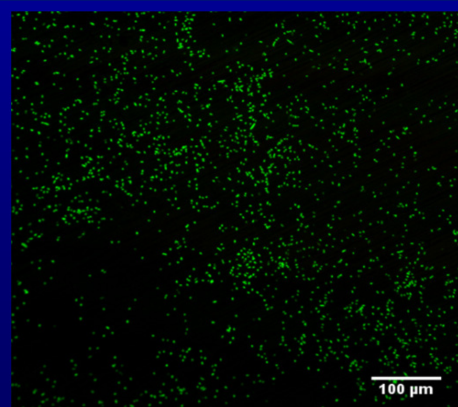


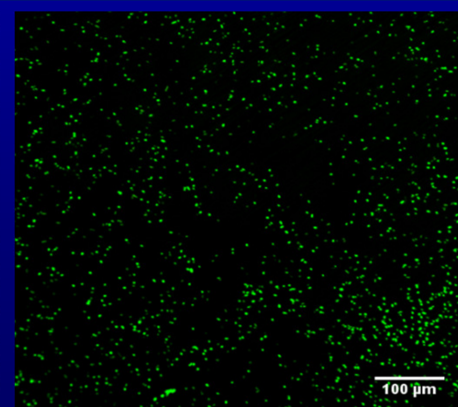
SSP

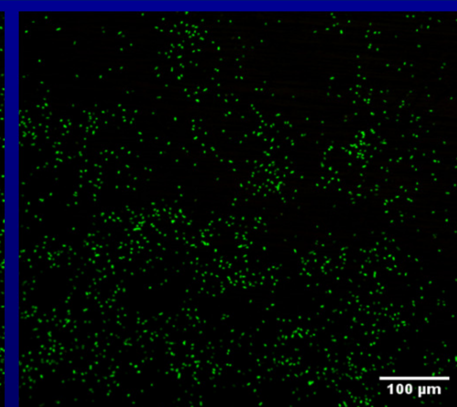


Polished







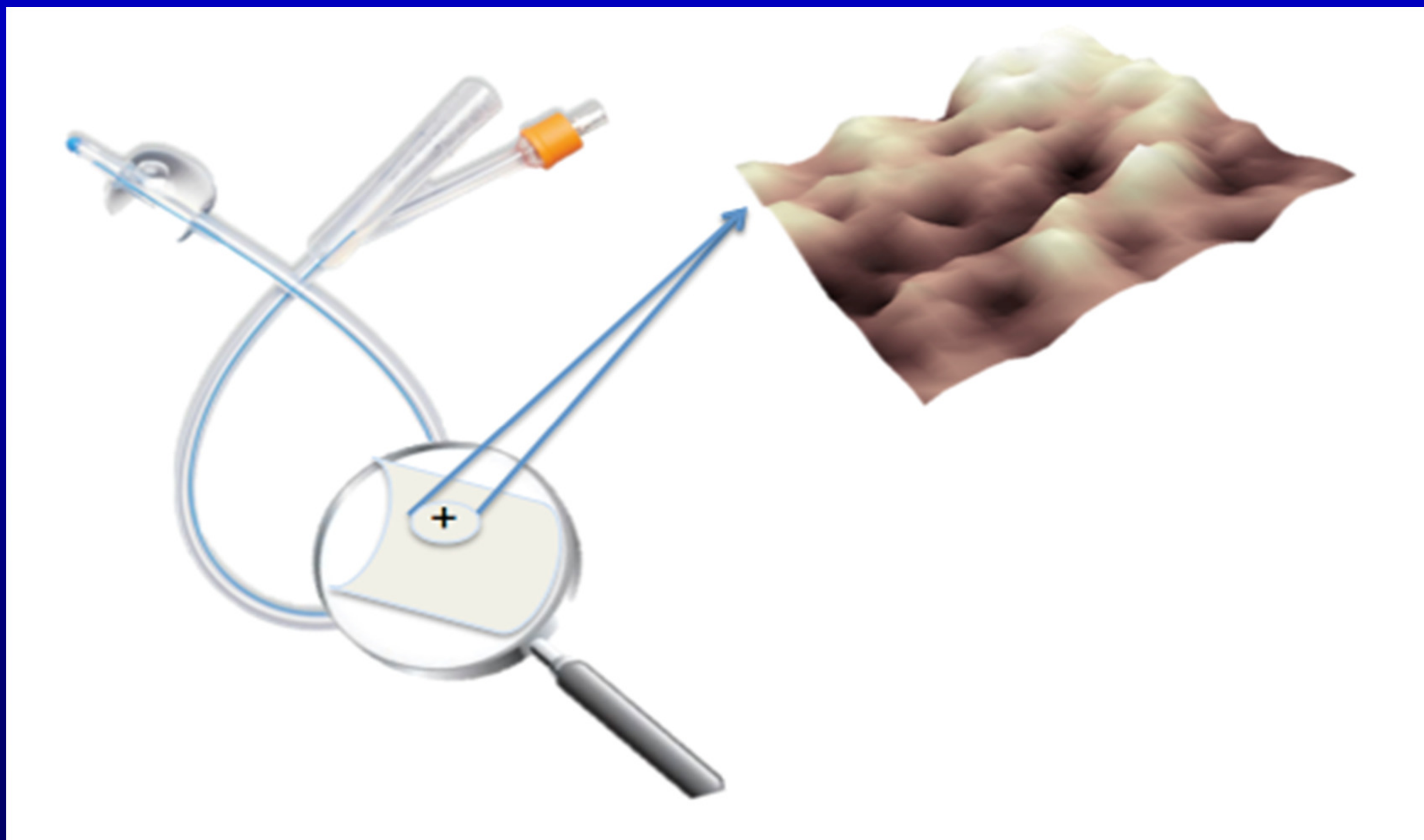


N=3; Data is mean +/- St. Dev,;
*p<0.01 compared to NP at the same time point;
^p<0.01 compared to CSP at the same time point

<u>Challenge #3</u>: We need a better understanding of the mechanism by which fundamental material properties decrease bacteria response.

Example: Catheters and Endotracheal Tubes

→ Develop a catheter that inhibits bacteria growth through fabricating antibacterial nano-patterns on the surface of catheter materials.



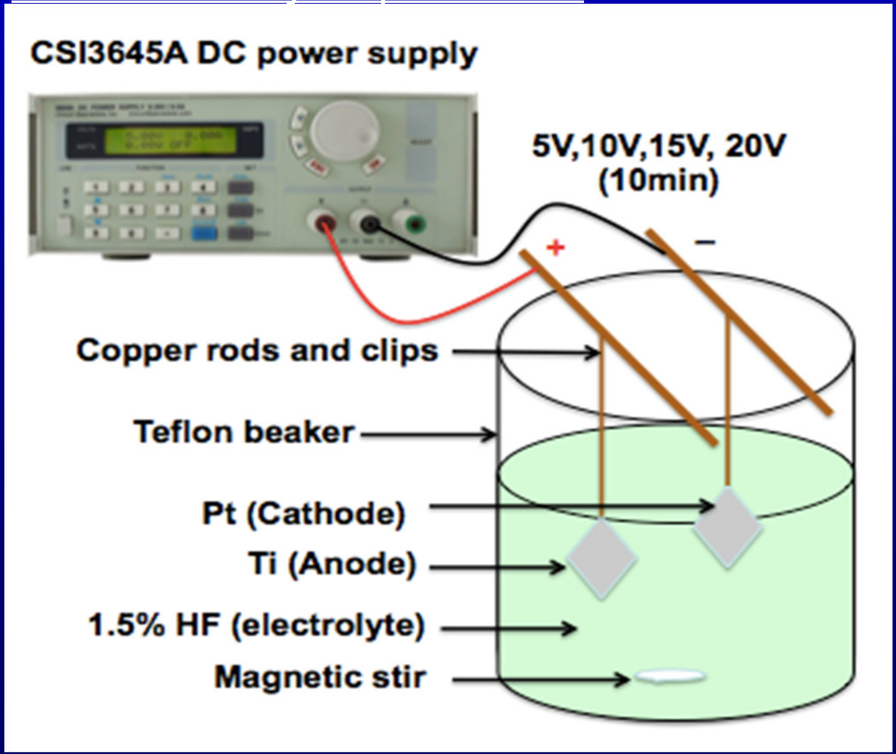
Noimark et al. The role of surface in catheter-associated infections. *Chem. Soc. Rev*. 2009, 38, 3277-3512.

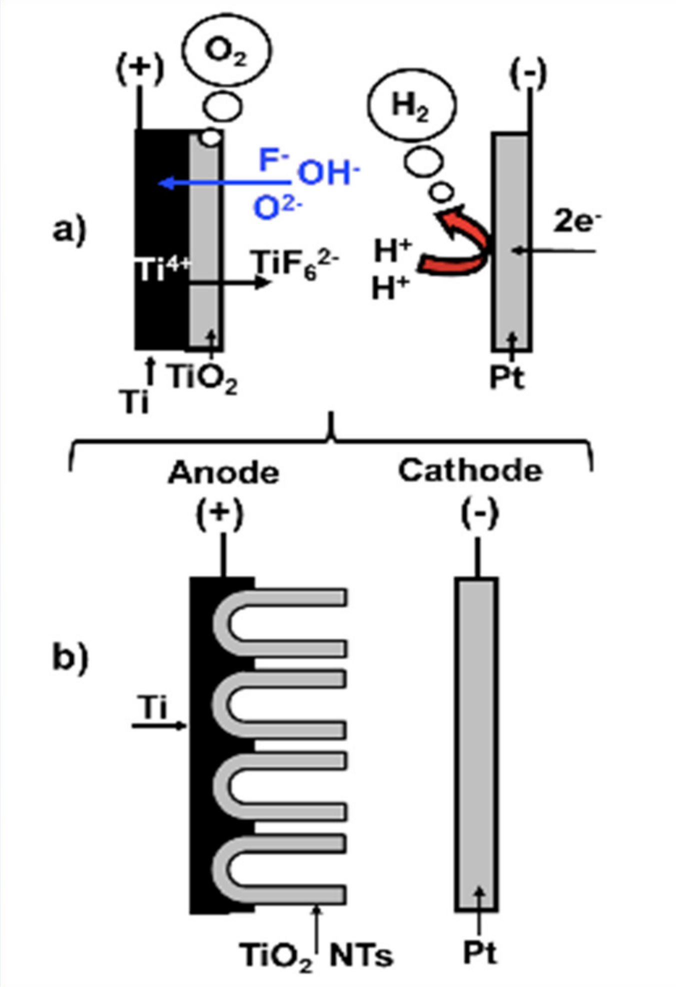
Methods

Template method: a material with a special structure was used as a template to imprint its structure onto another material

Step 1: Preparation a nano-patterned template

- Simple fabrication procedure;
- Low cost;
- Limited facility requirement.



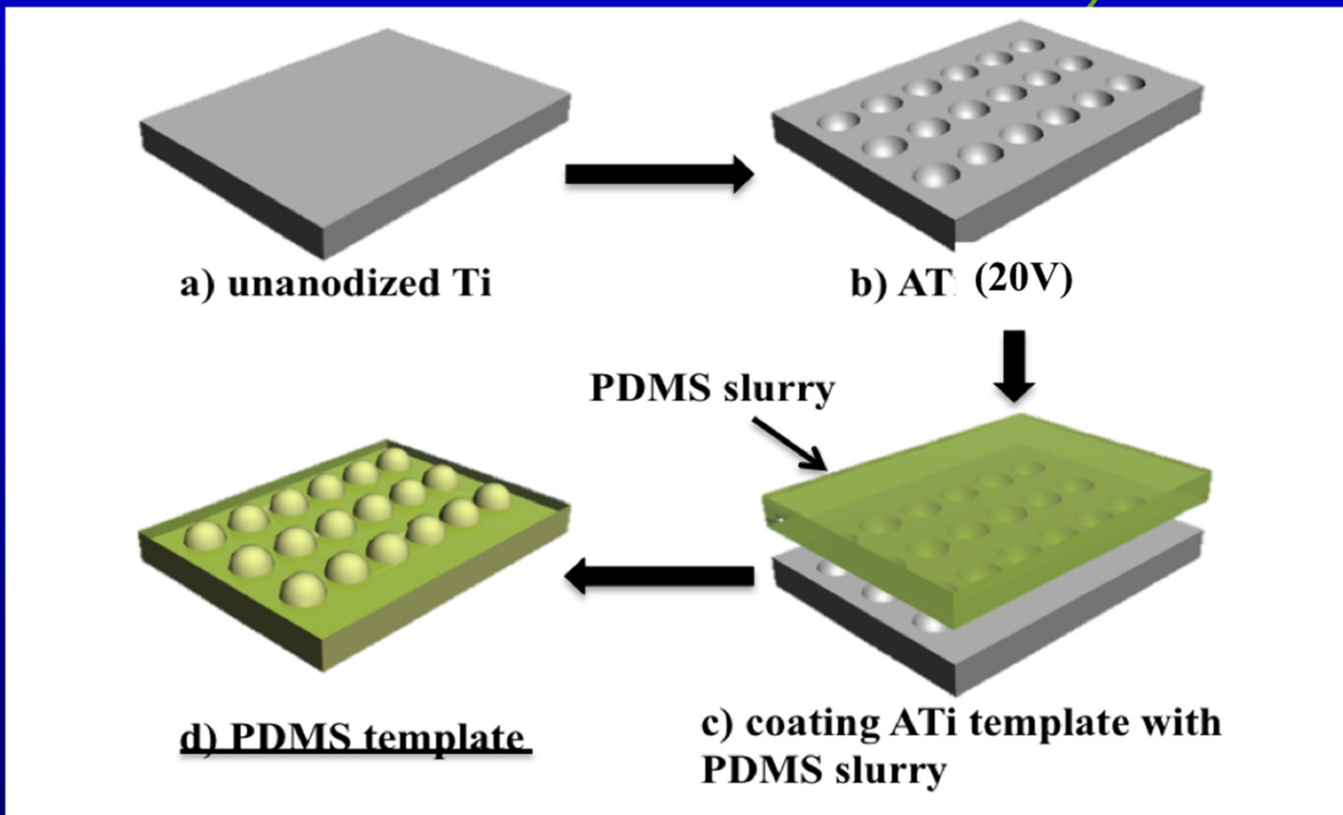


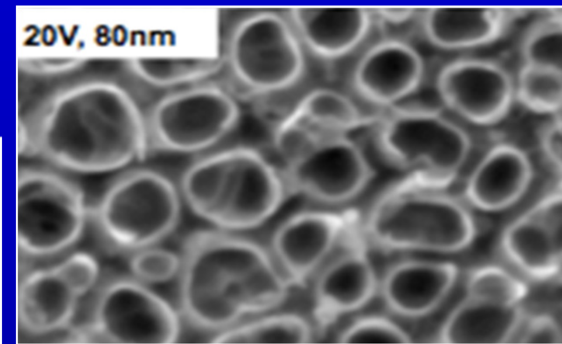
Anodization system to create nanotubular structure.

Rajyalakshmi et al. Reduced adhesion of macrophages on anodized titanium with selected nanotube surface features. *Int. J. Nanomedicine*. 2011, 6, 1765-1771.

Methods

Step 2: Preparation of PDMS replica

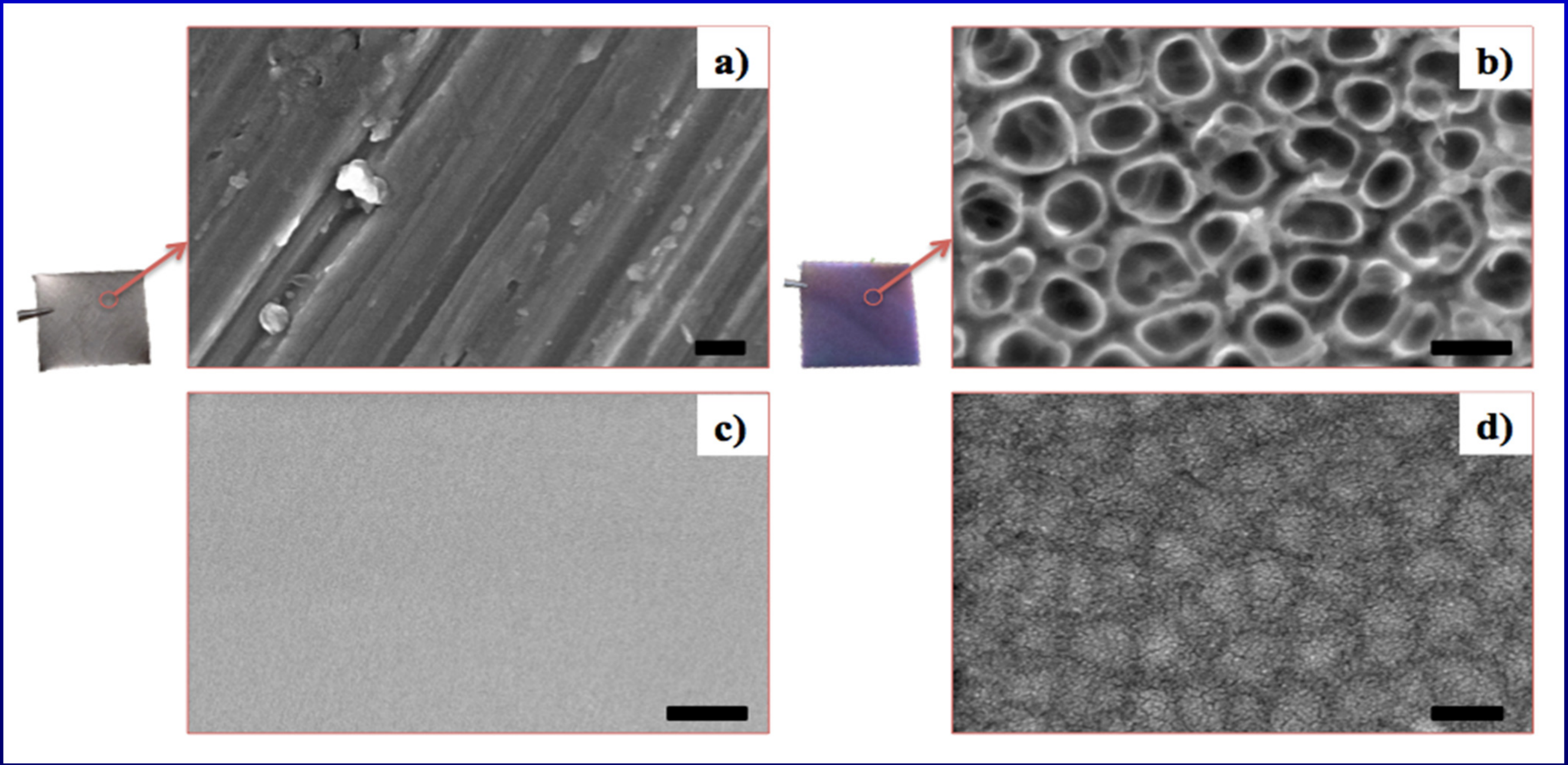




Process of fabricating the PDMS nanostructures. (ATi: anodized titanium)

Results

→Successful fabrication of nanostructures on PDMS surface

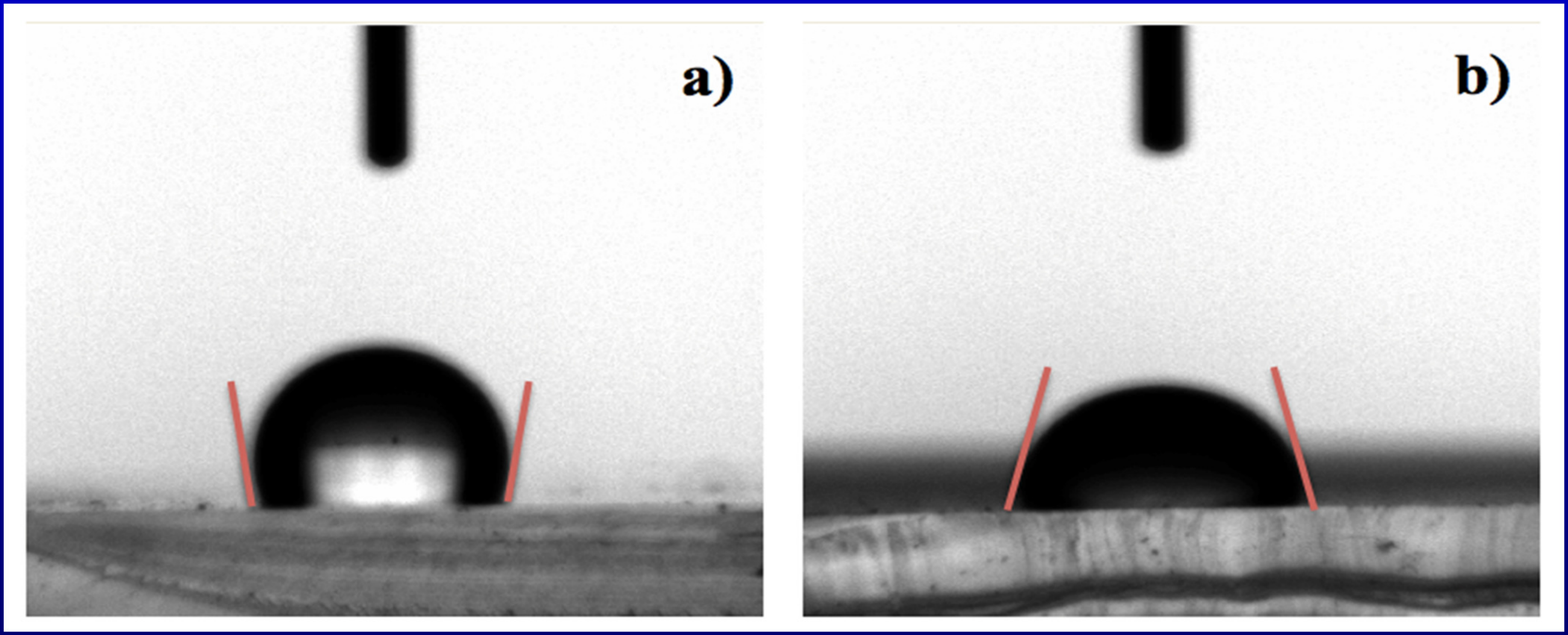


SEM images of a) unanodized Ti, b) anodized Ti, c) p-PDMS and d) nano-PDMS. Scale bars are 100 nm.

Abbreviations: plain-PDMS (p-PDMS); nano-patterned PDMS (nano-PDMS)

Results

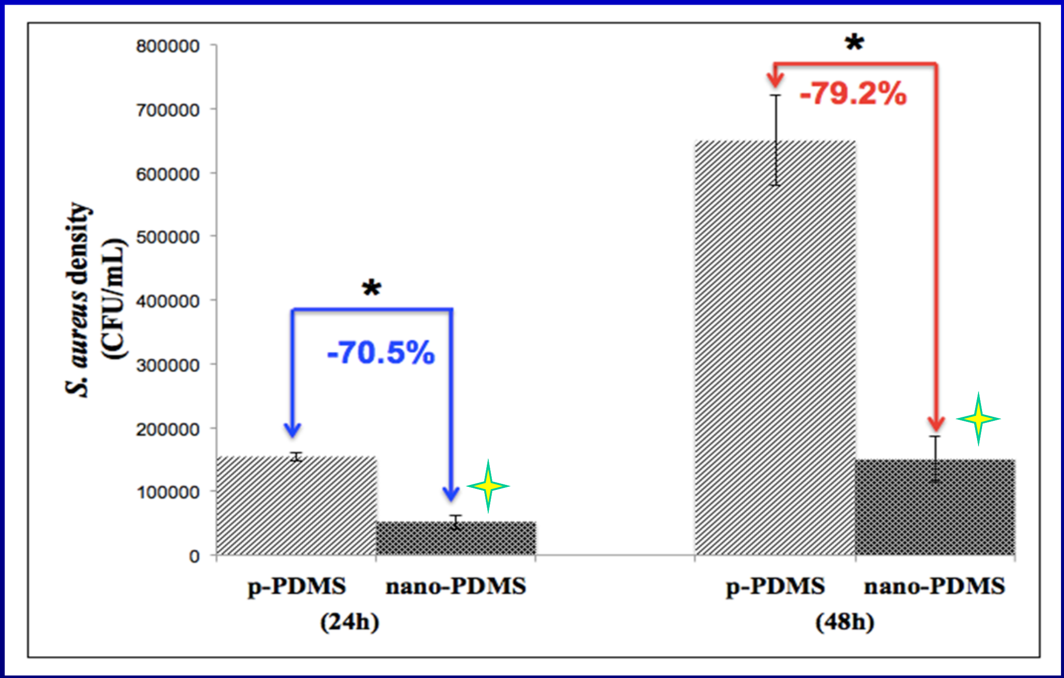
→Increased surface wettability upon nanostructuring



Water contact angle images of a) p-PDMS (99.2 °) and b) nano-PDMS (66.6 °).

Bacterial Assays (CFU)

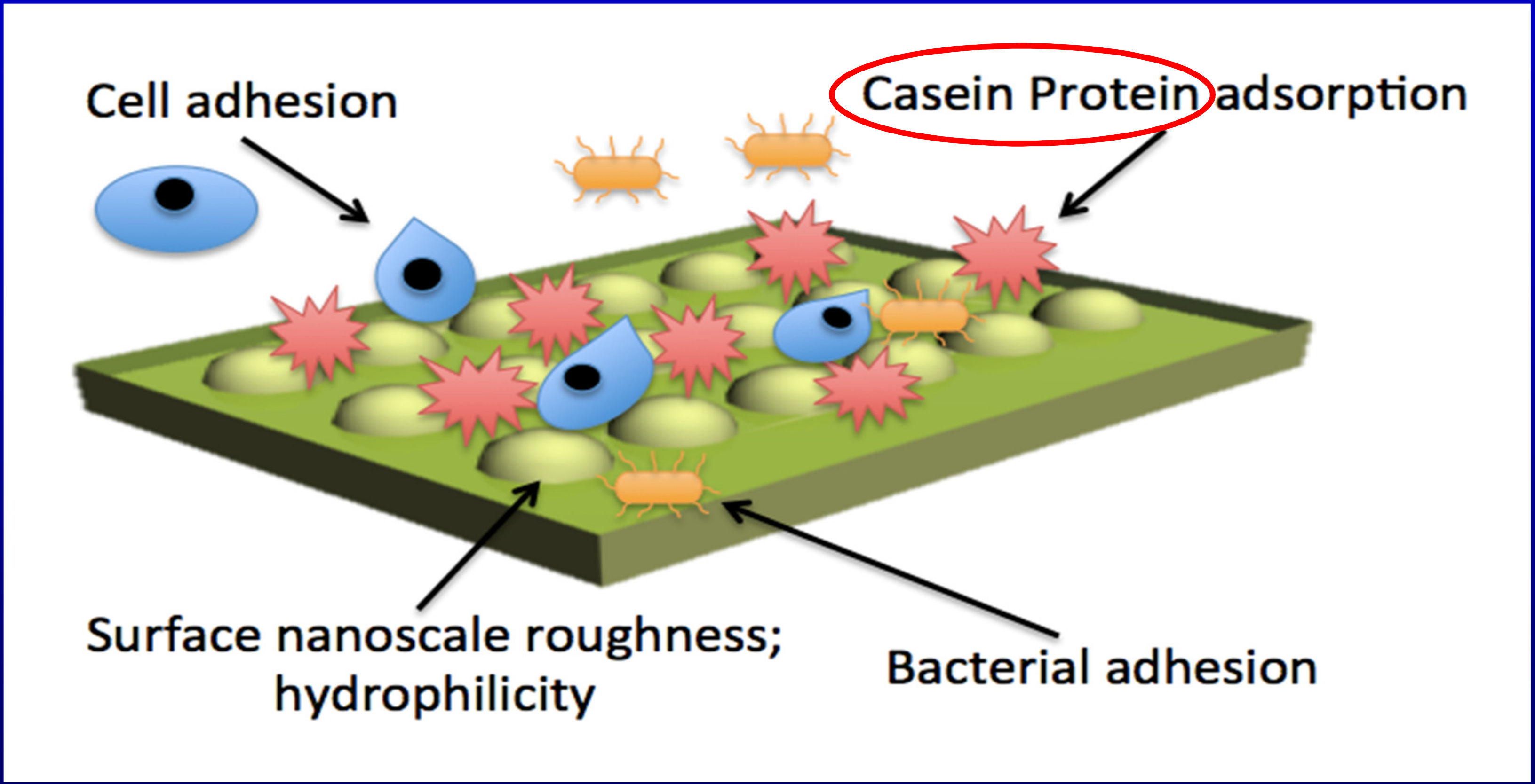
→Decreased bacterial adhesion and growth on nano-PDMS



S. aureus growth on the surface of nano-PDMS and p-PDMS.
Data represents mean ± SD, n=3. *p < 0.05 compared with p-PDMS at the same time period, *p<0.05 compared with nano-PDMS (24 h).

Mechanism

Nanoscale roughness, unique wettability → protein adsorption →cell/bacteria activities

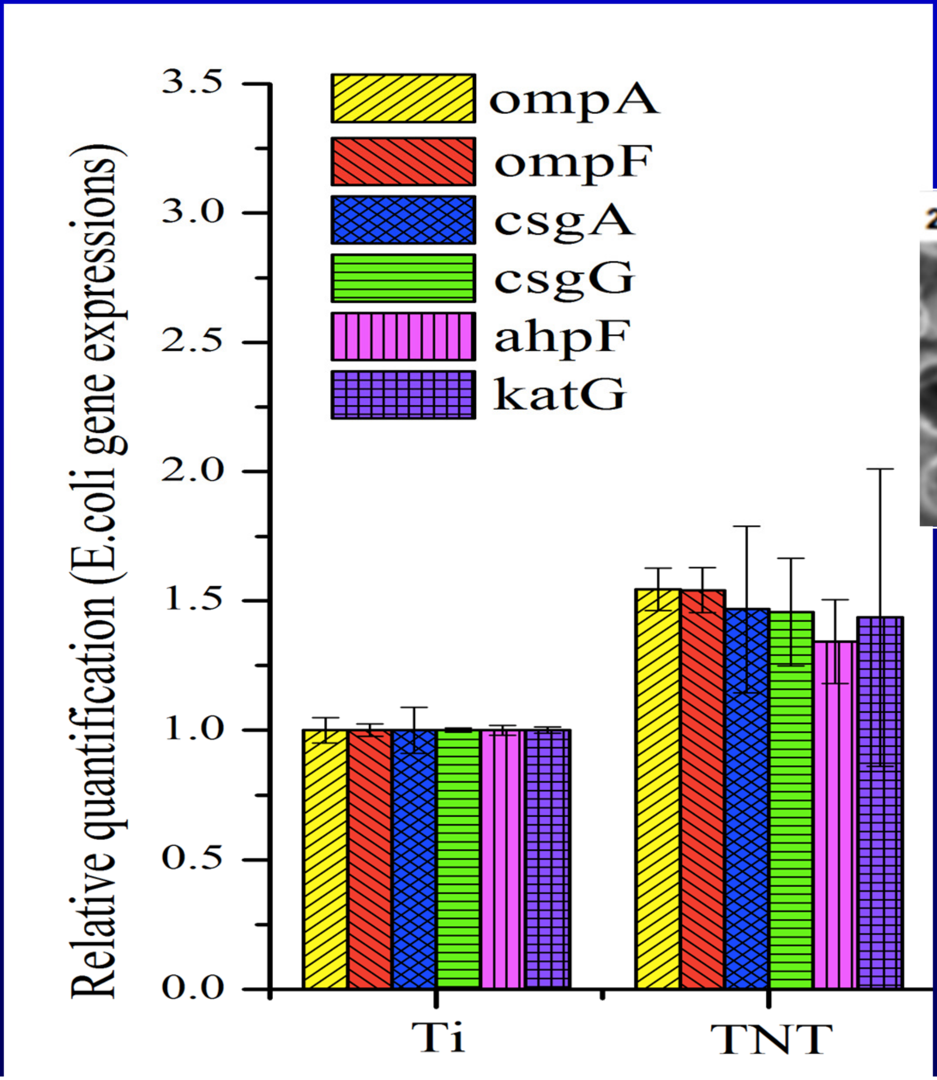


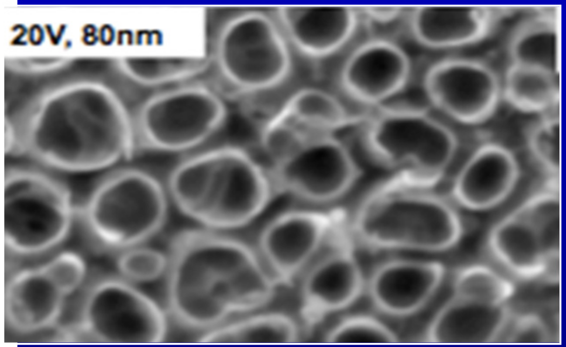
- Key protein in TSB (bacterial culture medium);
- Intrinsic anti-fouling property

Schematic diagram shows how this nanofabricated catheter surface design works for bacteria inhibition.

Anselme et al. The interaction of cells and bacteria with surfaces structured at the nanometre scale. *Acta. Biomater*. 2010, 6, 3824-3846.

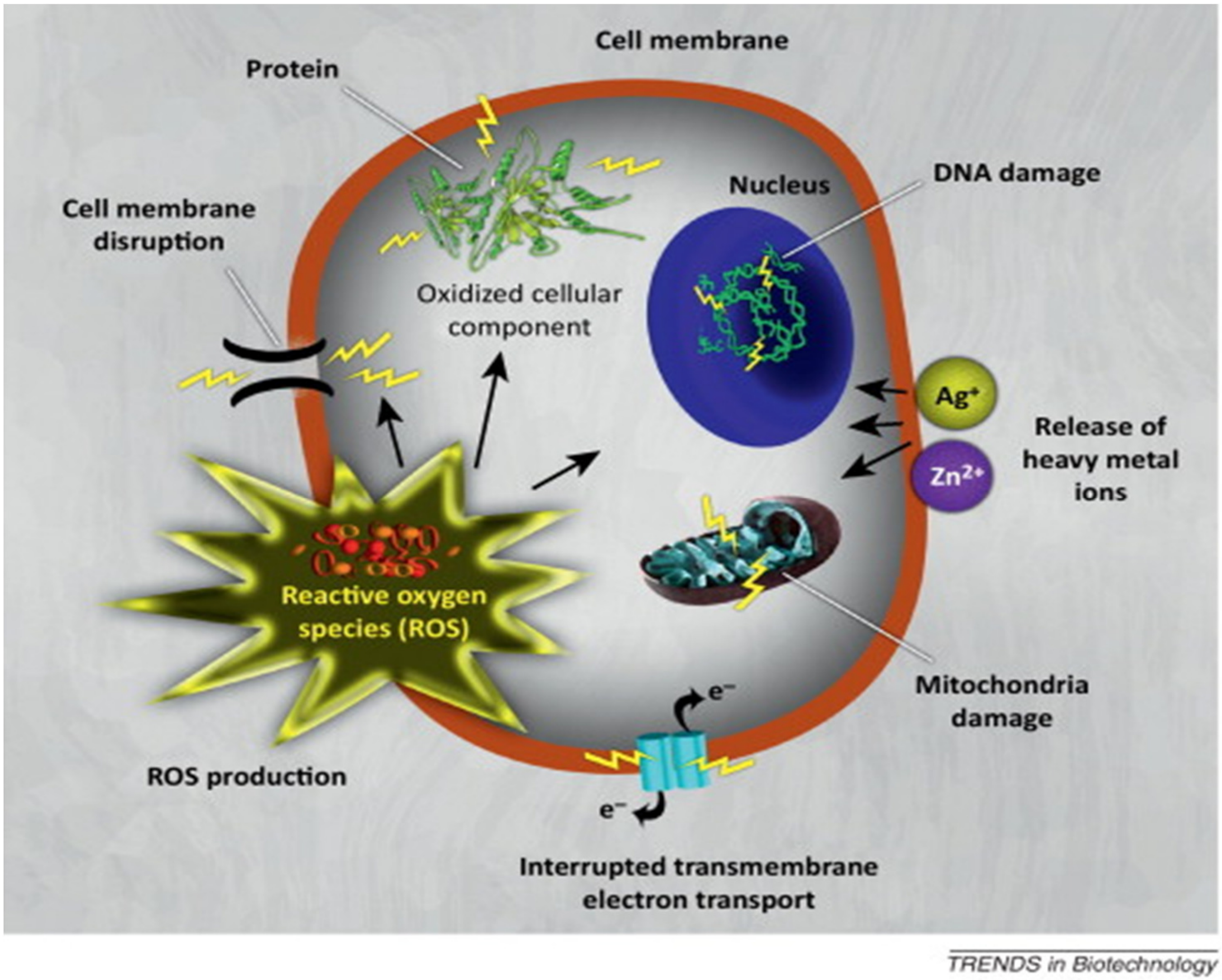
Another example: Genetic Changes in *E coli* on Anodized Ti





Part 2: Nanoparticles

Antibacterial Nanoparticles



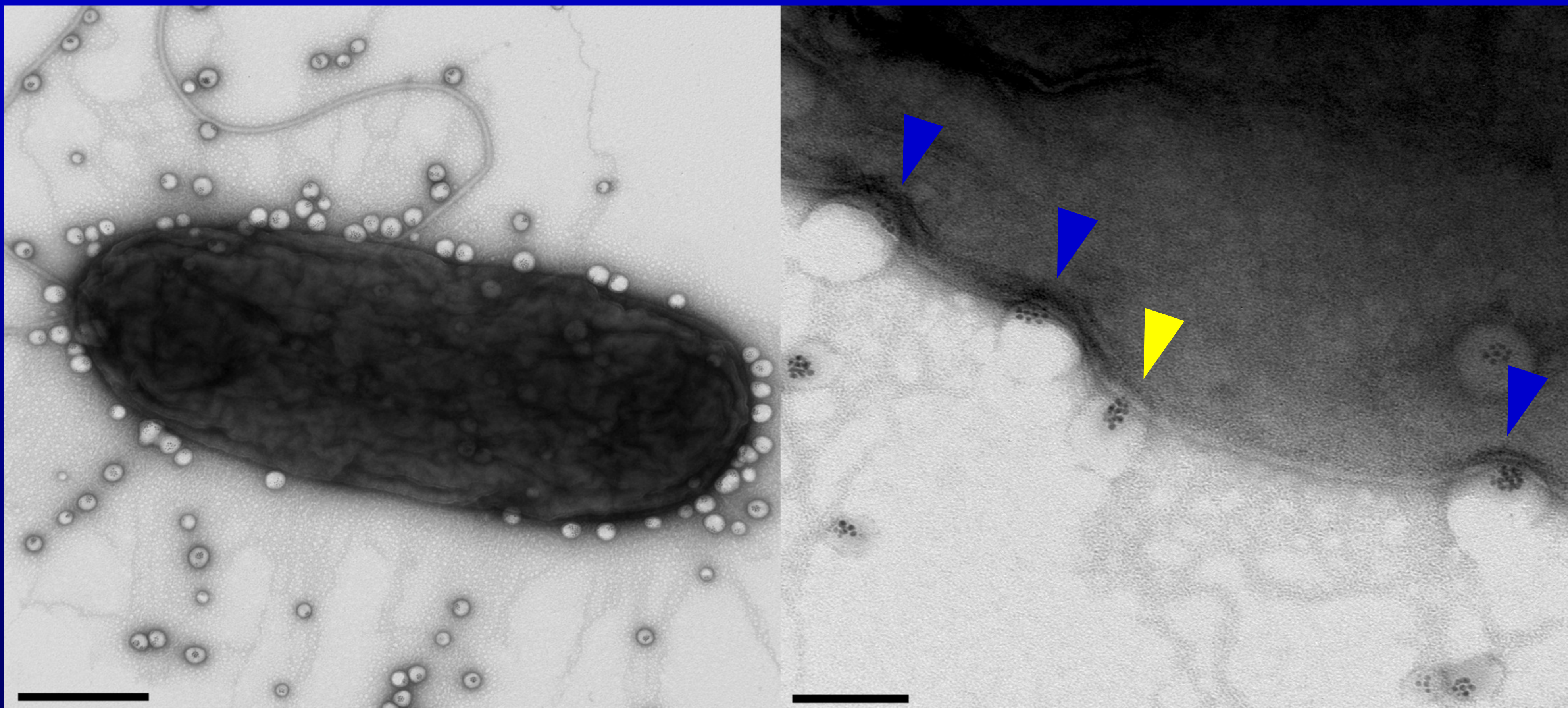
Nanoparticle-based drug delivery:

- Greater surface area to volume ratio
- Customization of nanoparticle materials
- Tissue-specific delivery by size, incorporation of targeting ligands

Healthy mammalian cells do not experience the negative effects of many nanomaterials at the same concentrations as diseased cells or pathogenic bacteria[1,2,3]

1. Phong A. Tran and Thomas J Webster 2013 *Nanotechnology* **24** 155101
2. Watson, Gregory S., et al. *Acta biomaterialia* (2015).
3. Stolzoff M. et al. *Biomacromolecules* 2015

Nanoparticles



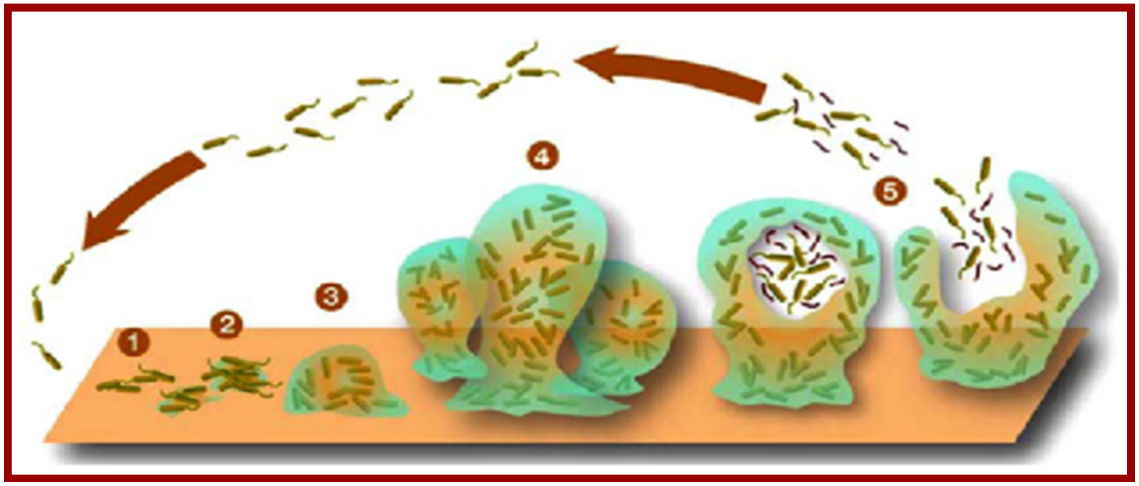
Scale Bars = 100nm

Geilich BM, et. al. *Nanoscale.* 7 (2015) 3511-3519

Post-Biofilm Treatment

- Biofilms are responsible for over 60% of infectious conditions in developed countries
  - Source of chronic infection and inflammation
  - Almost always necessitates device removal
- Bacteria adhere to surface through secreted exopolysaccharide matrix
  - Forms protective state
  - Impeneterable to antibiotics and host immune cells

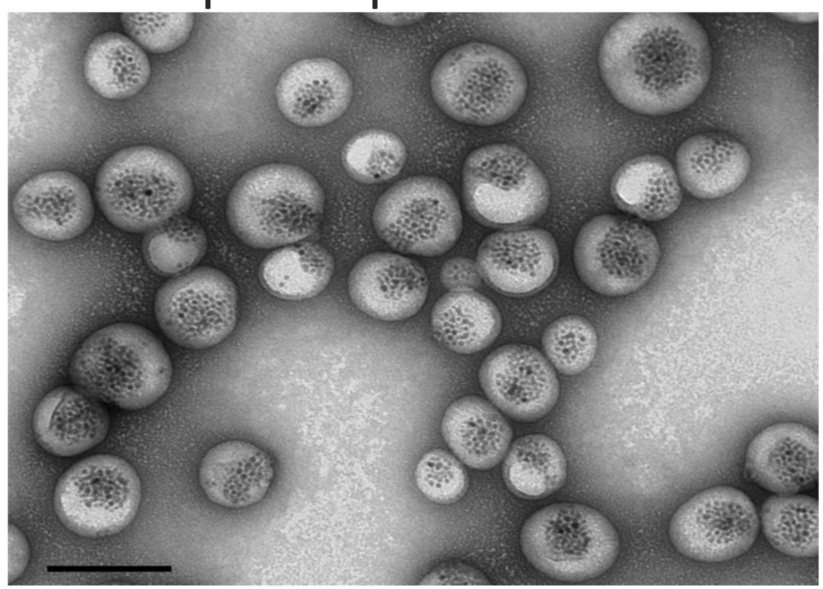
Can we modify nanoparticles to aid in the treatment of device-related infections?



Chen L, Wen Y. *Int J Oral Sci.* 2011;3(2):66-73.
Cunningham AB. Biofilm Hypertextbook, MSU Biofilm Engineering. 2005.

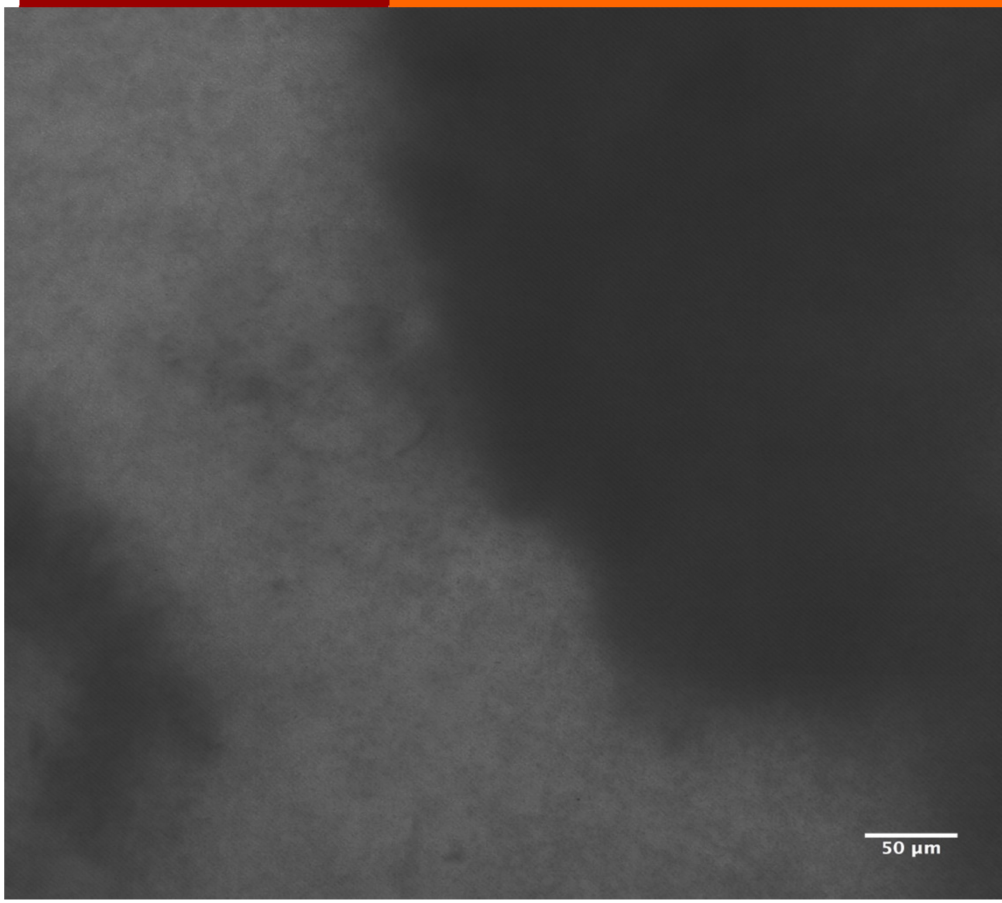
Iron Oxide Polymersomes

- Iron oxide nanoparticles have also been shown to display antibacterial action
- Synthesis technique slightly modified to allow embedding of 5nm hydrophobic SPIONs
- Exploit magnetic properties to help encapsulated antibiotic penetrate biofilm
- Same nanoparticle and antibiotic concentrations as AgPs

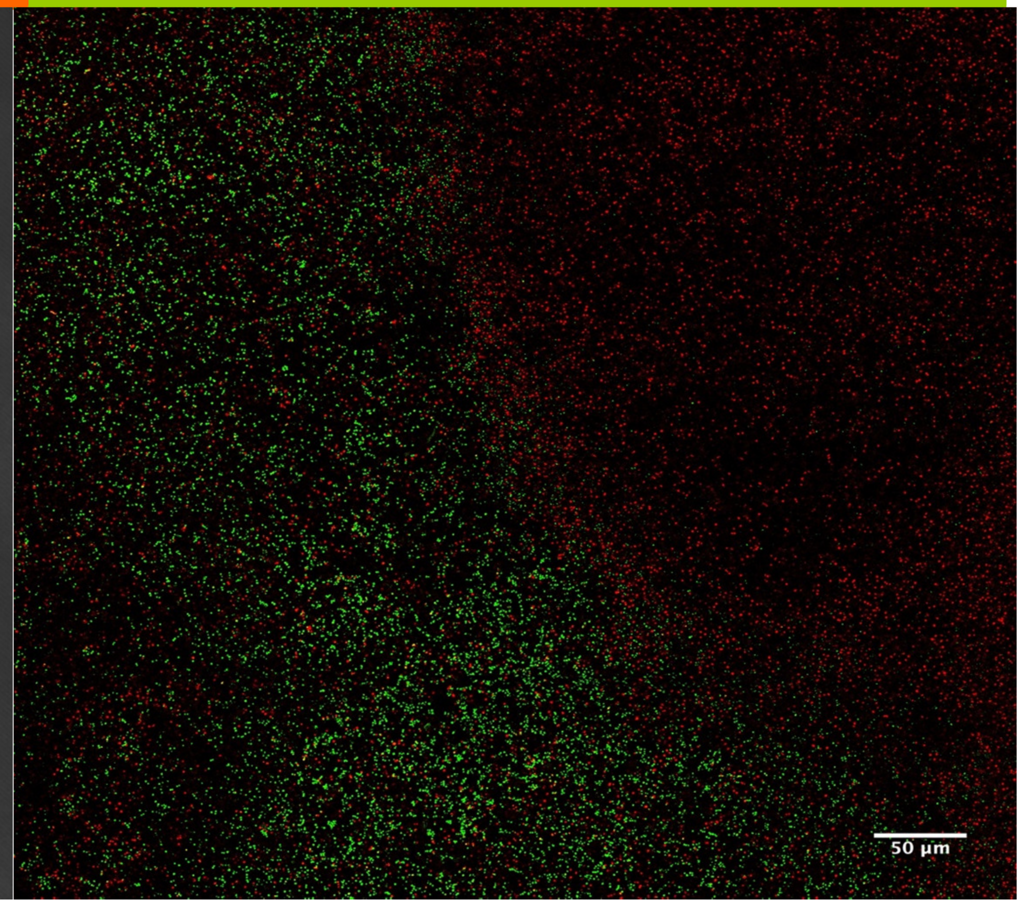


Geilich BM, et. al. Unpublished Data

Treatment +Magnet



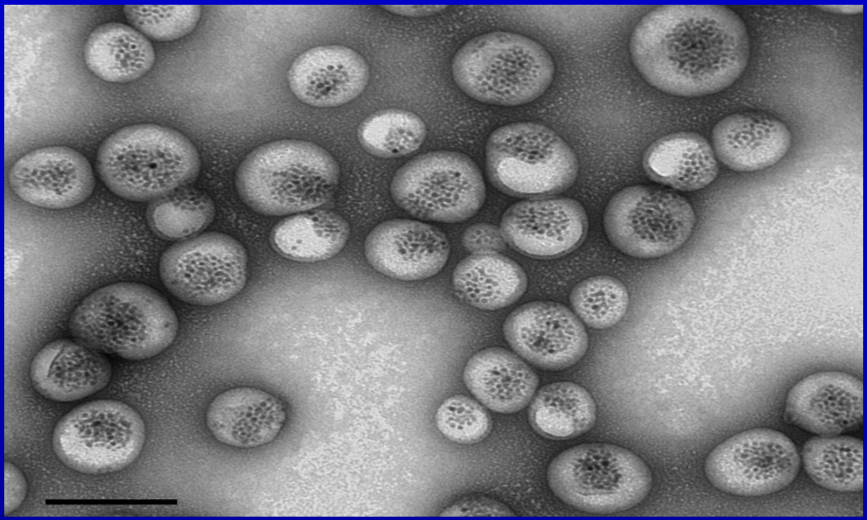
Brightfield

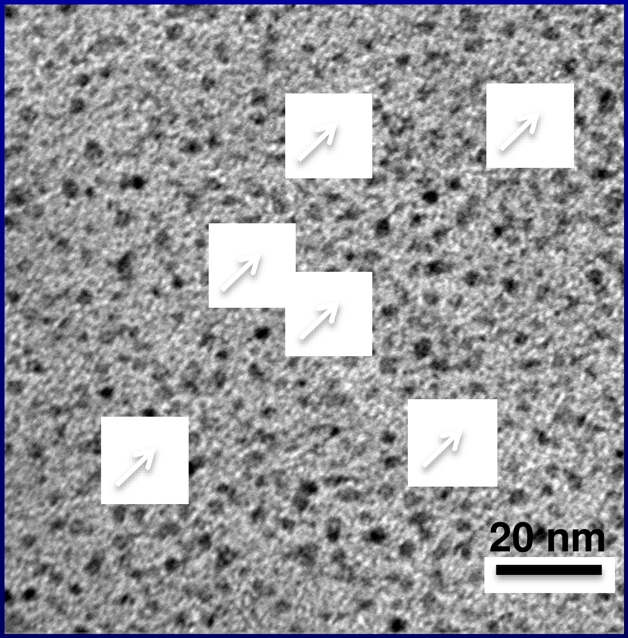


Live/Dead

Geilich BM, et. al. Unpublished Data

“Hot” Nanoparticles





- **Nanoparticles can penetrate cells and tissues before freezing down so that when thawed, they can decrease reactive oxygen species.**

- **Nanoparticles can quickly degrade to not create adverse cellular/organ function later.**

- **<u>Examples include</u>:**
  Selenium, silver, ceria, iron oxide, magnesium oxide, zinc oxide, self-assembled materials, liposomes, polymersomes, and others.

But what about green nanoparticles ????

Harmful chemicals are often used to make nanoparticles…

PROJECT 1

Synthesis of metallic nanoparticles by bacteria

What if bacteria can generate the “definitive weapon” against antimicrobial resistance?

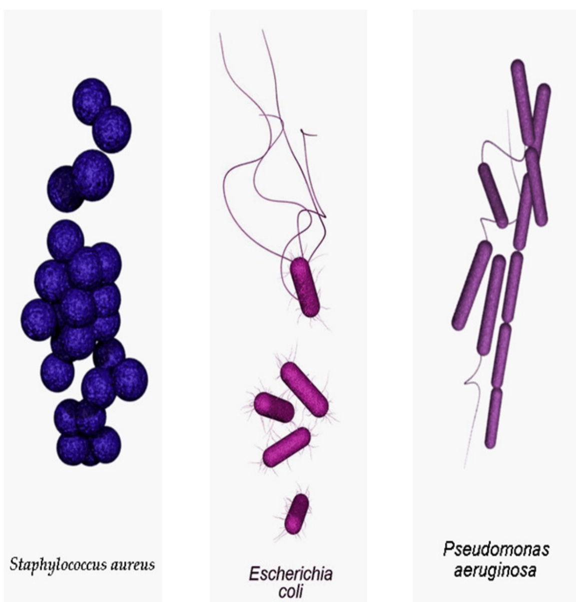
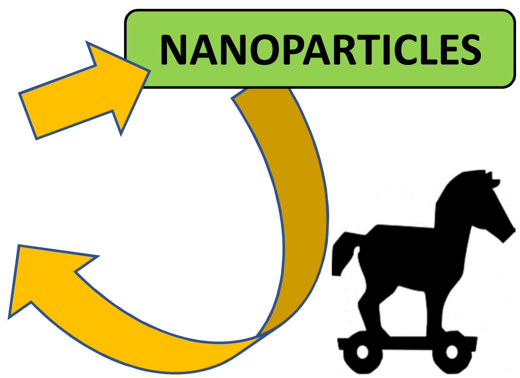
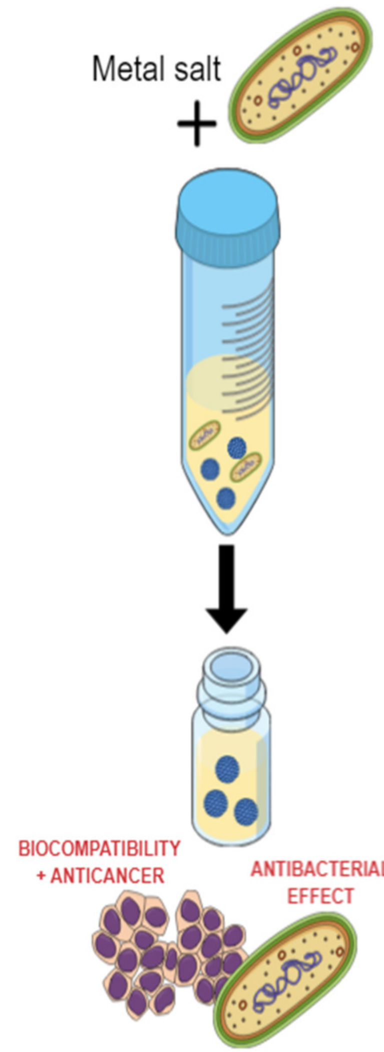
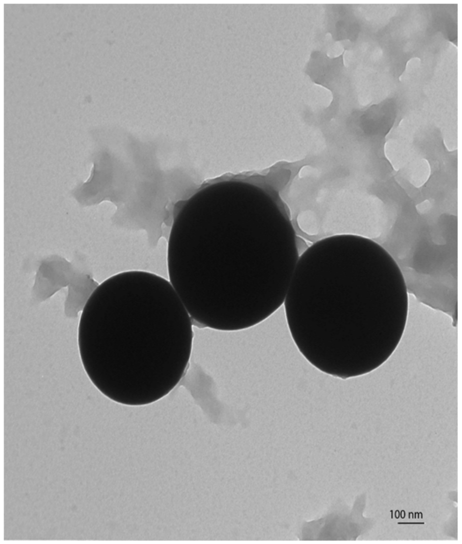


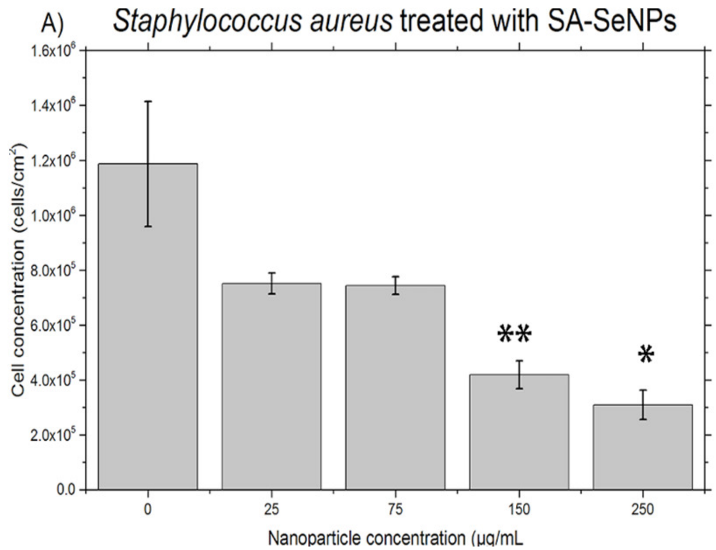
Image from research data



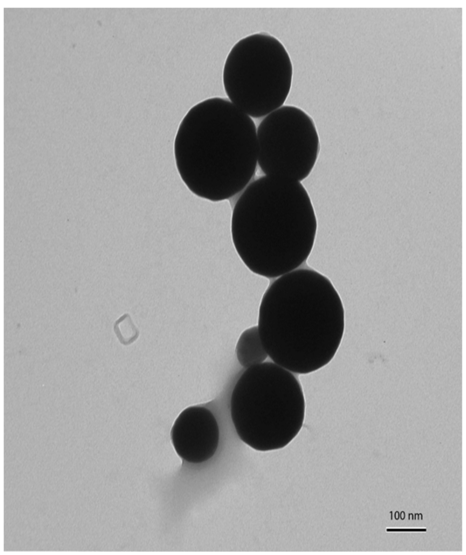
OBJECTIVE

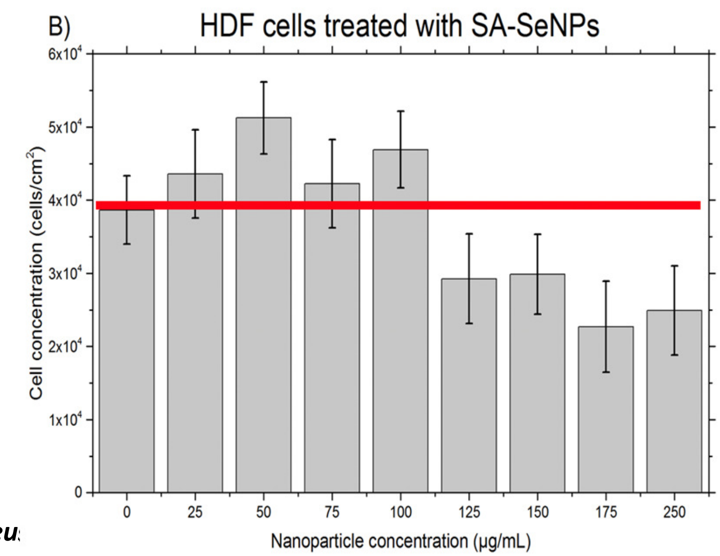






Staphylococcus aureus treated with SA-SeNPs. *Values represent the mean ± standard deviation, N=3.* Colony counting assay of bacteria after being treated for 8 hours with different selenium nanoparticle concentrations. N=3. *p<0.01 versus control, **p<0.005 versus control..





NPs synthesized by _Staphylococcus aureu_

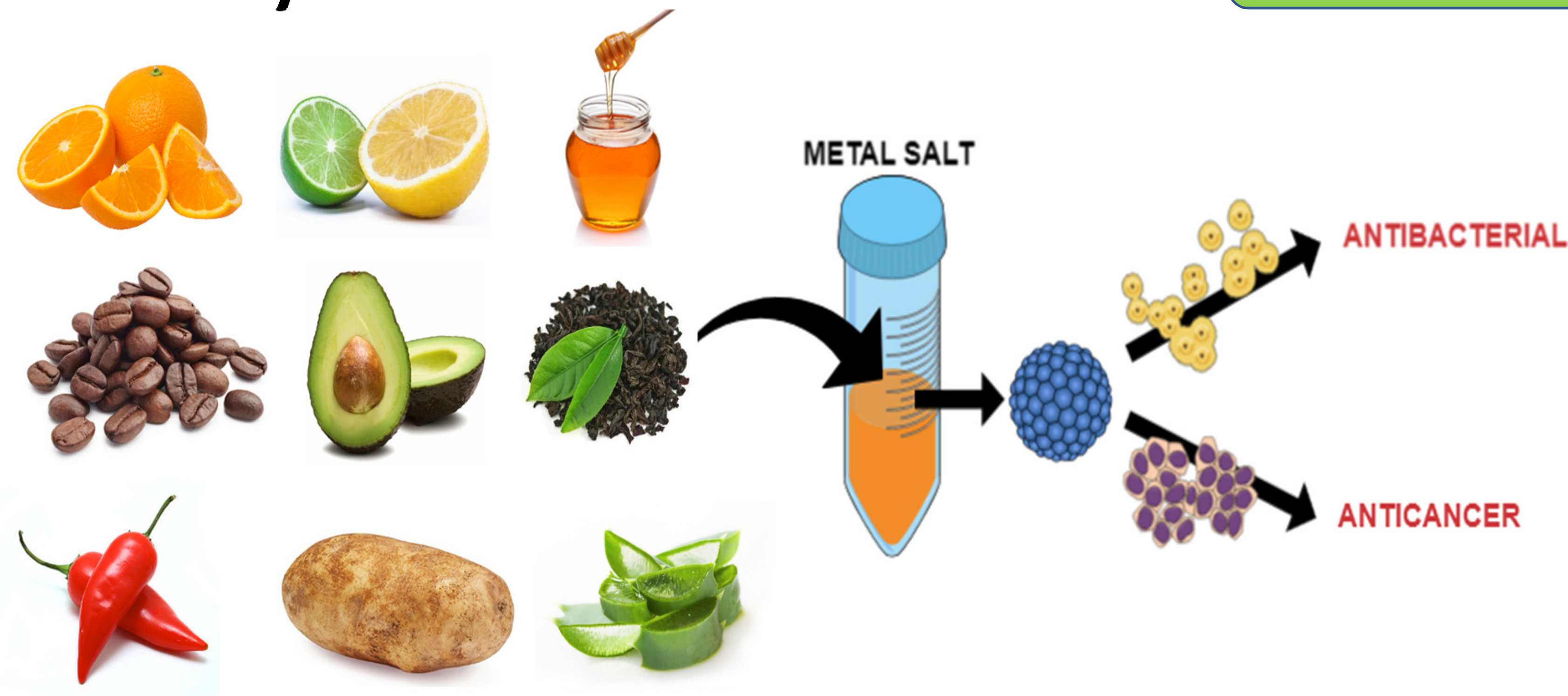
Particle cytotoxicity to human dermal fibroblasts (HDF). Values represent the mean ± standard deviation, N=3. p<0.05 compared to controls for all the samples which showed no statistical difference.

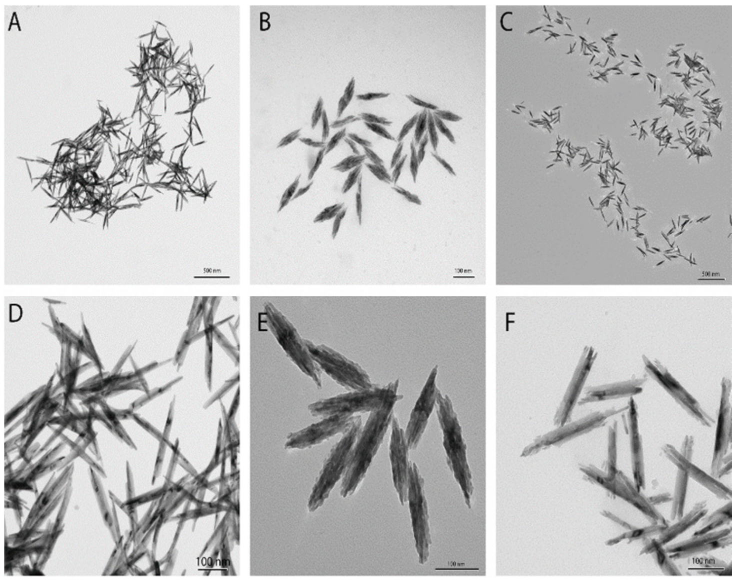
PROJECT 2

Synthesis of metallic nanoparticles by dietary compounds

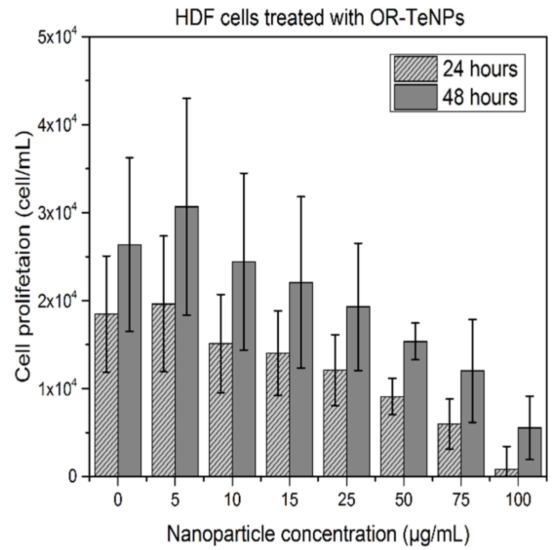
What if your food could cure the disease you have?

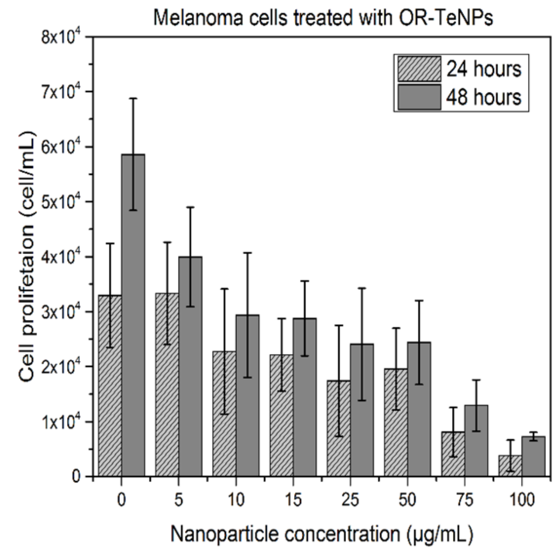
OBJECTIVE

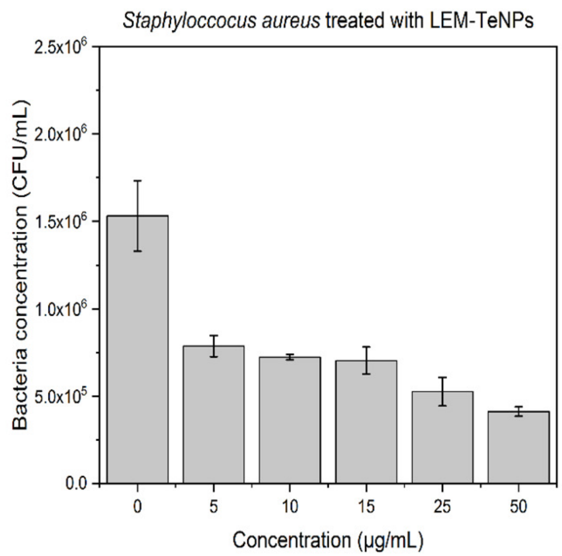


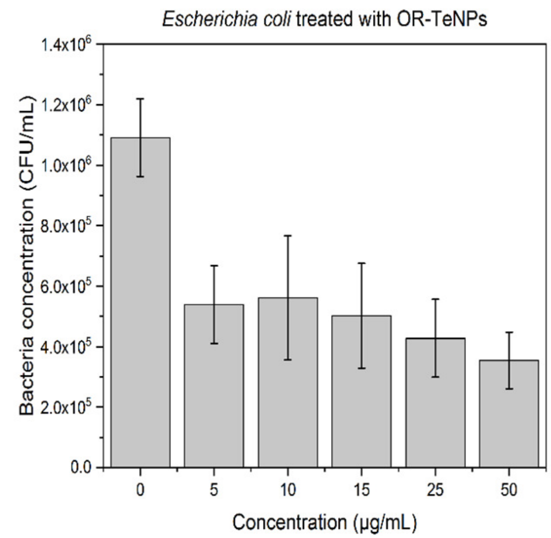


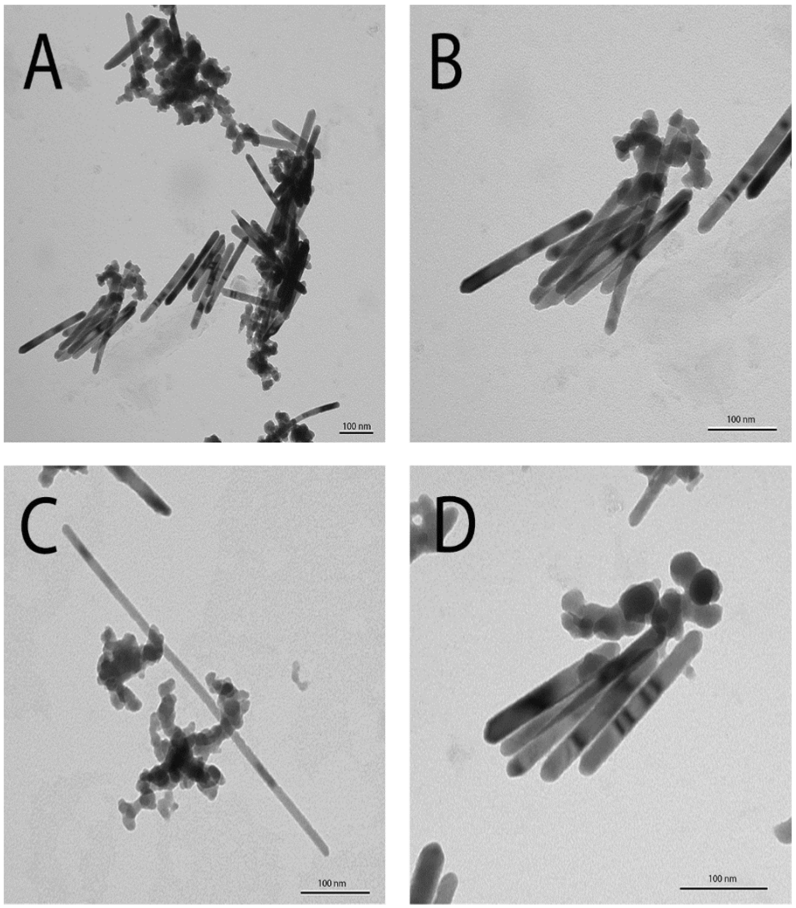
Tellurium nanoparticles made with orange (A,D), lemon (B, E) and lime (C, F) juices. Different shapes were observed.

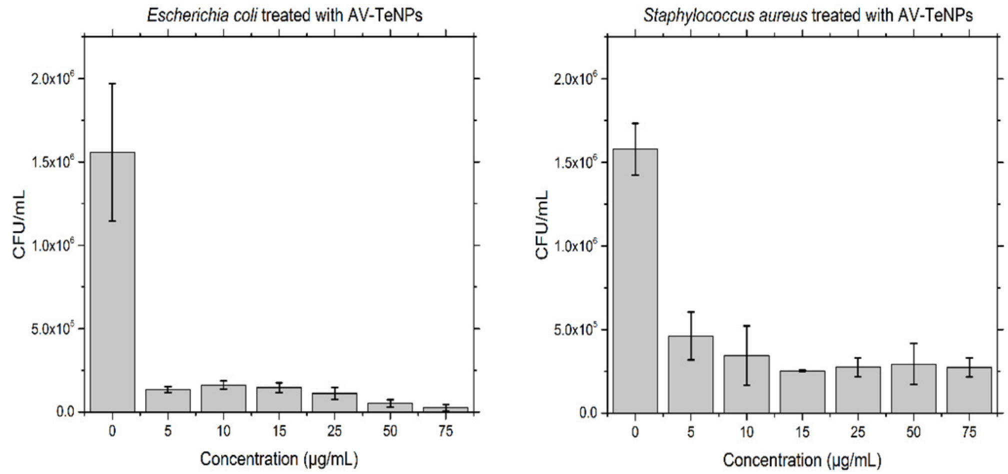


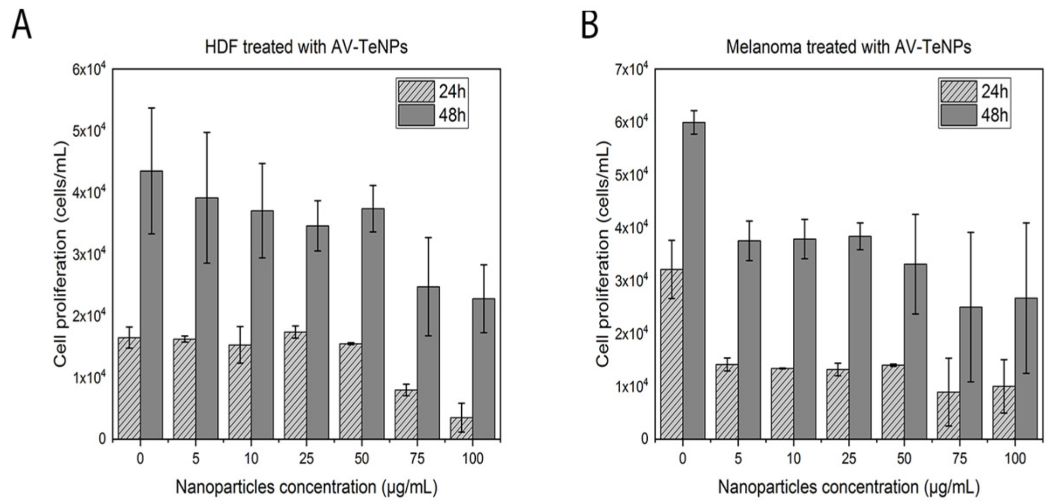










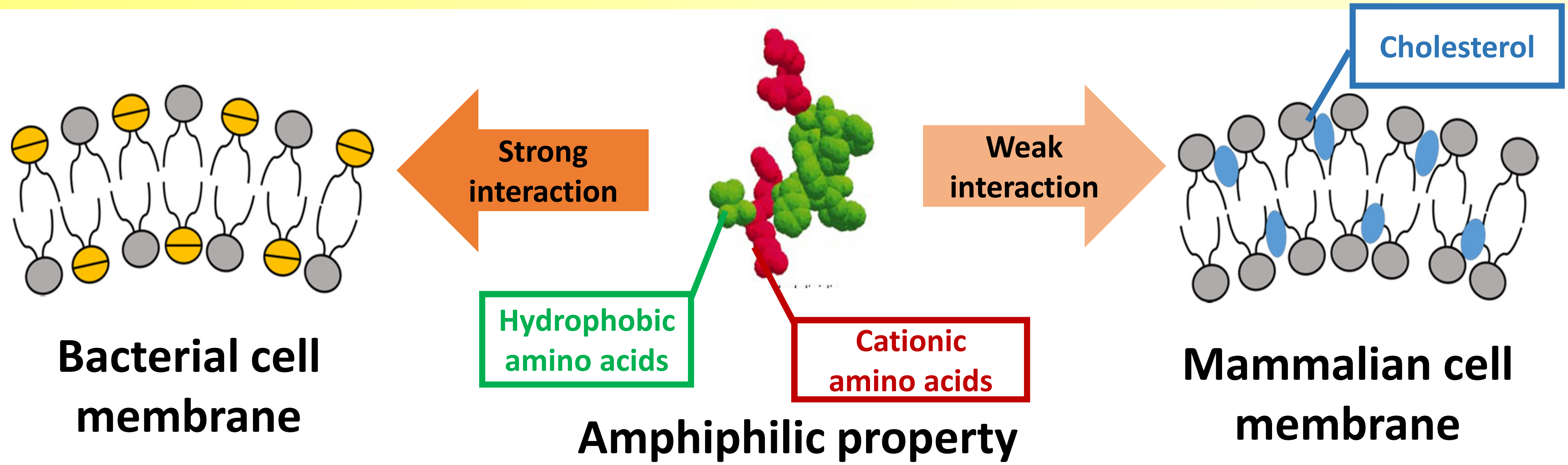


Tellurium nanoparticles made with aloe vera

Challenge #4: While using less toxic materials to make nanoparticles -
we can also discover new exciting nanoparticle properties.

Part 3: Self-Assembled Nanomaterials

Antimicrobial Peptides (AMP)



Bacterial membrane disrupting activities

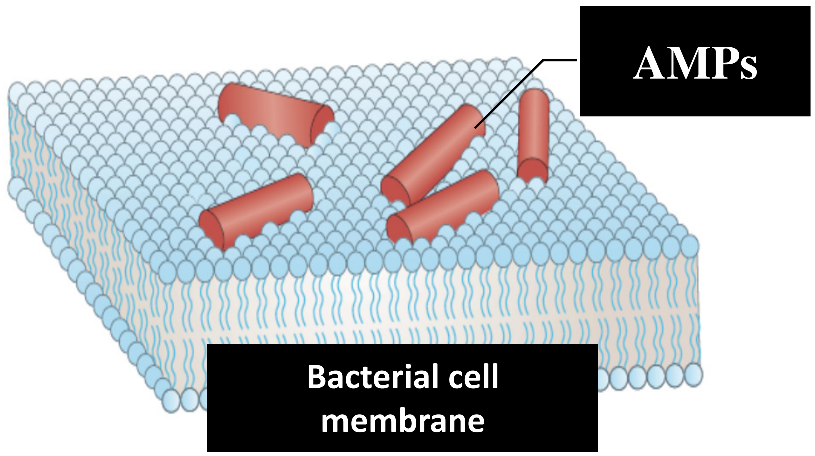
- Electrostatic attachment on negatively charged bacterial membranes
- Membrane insertion via the hydrophobic interactions with the lipid core region of the membrane bilayer
- Limited likelihood for bacteria to develop resistance

Selectivity towards bacterial cells

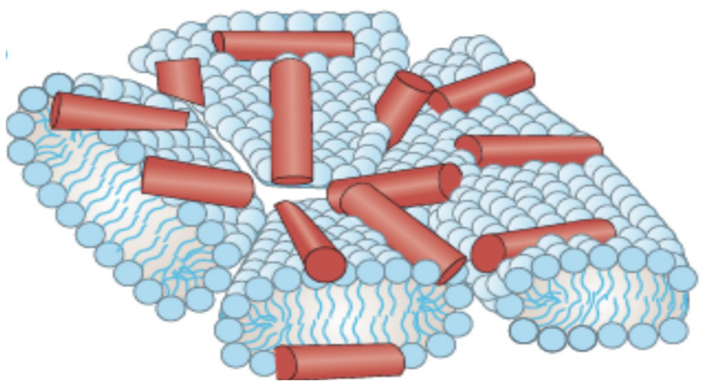
- Higher proportion of zwitterionic lipids in mammalian cell membranes
- Cholesterol that rigidifies the mammalian cell membranes

Zasloff, M., Nature, Vol.415, 389-395, 2002
Teixeira, V., Progress in Lipid Research, Vol. 51, 149–177, 2012.

Partitioning pathways of AMPs

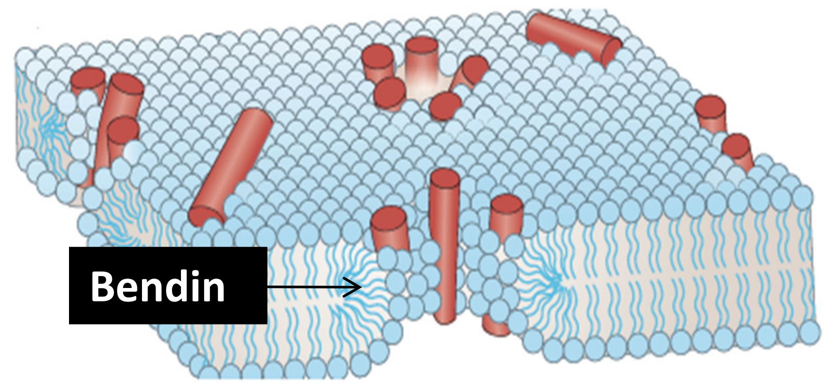


Peptides attach and accumulate on membrane



Peptides disrupt cell membrane by micellization

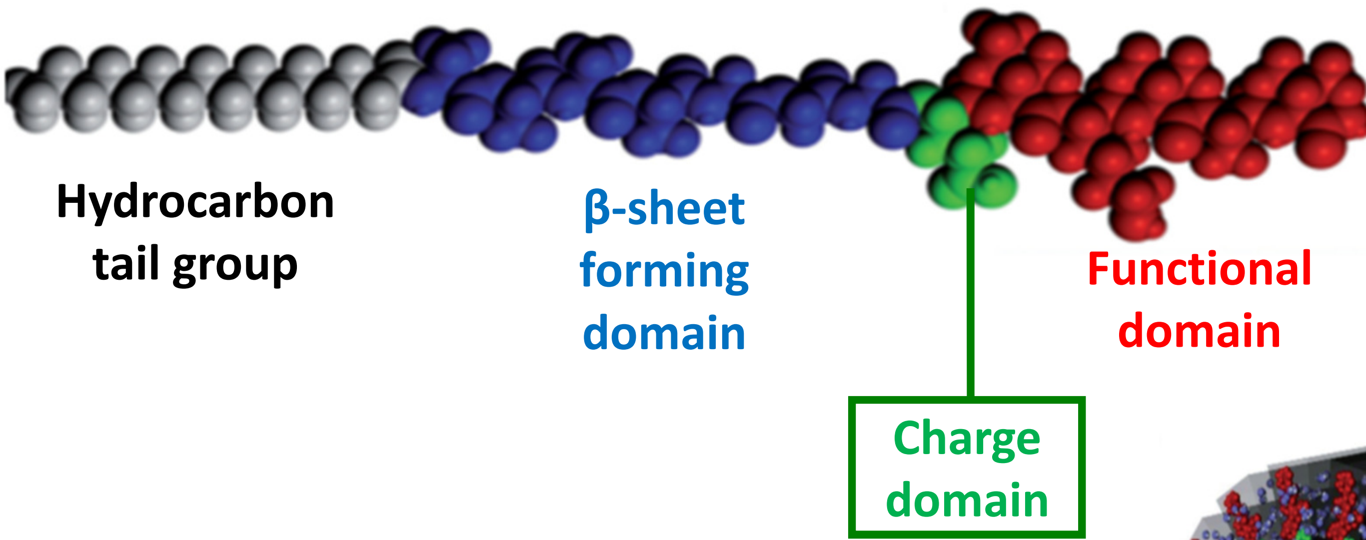
Secondary structures of AMPs can facilitate membrane insertion



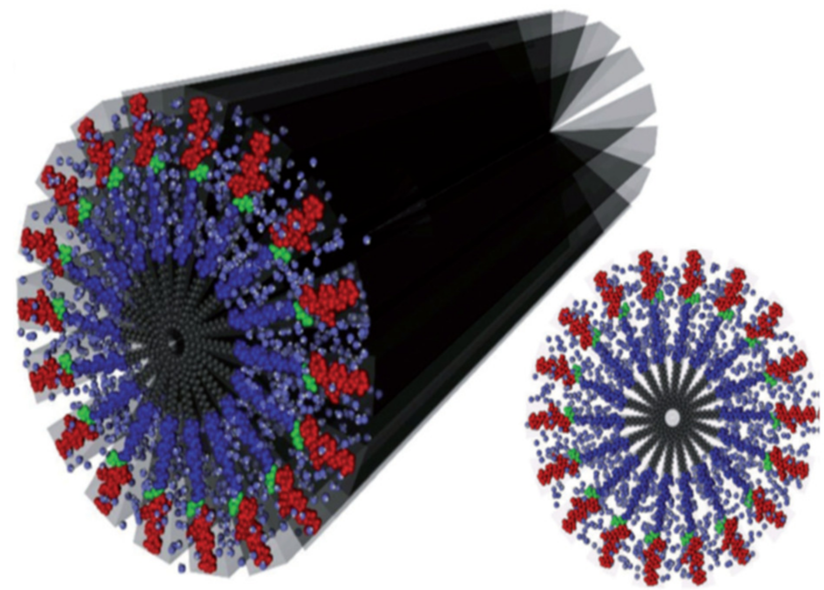
Peptides create bending on the membrane and cause membrane disruption

Melo, M. N et al., Nature Microbiology, Vol. 7, 2009

Self-assembling peptide amphiphiles (PA)



The functional domains of PAs can be functionalized with cationic antimicrobial sequence



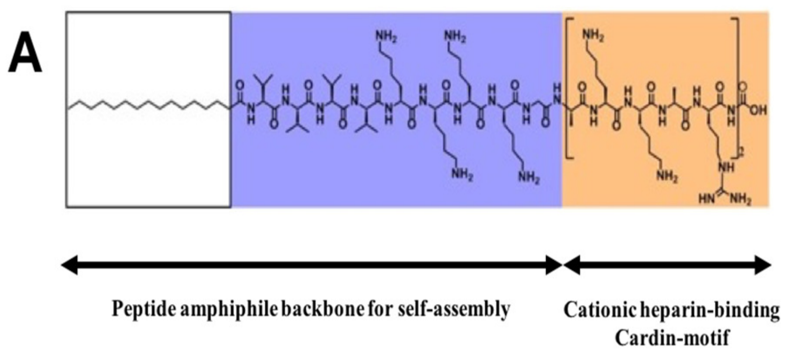
Advantages of self-assembling peptides:

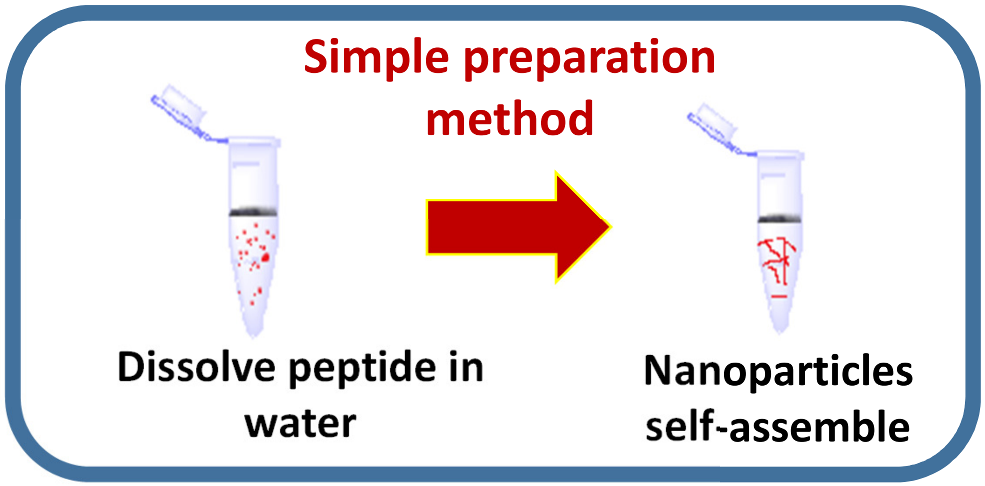
- High biocompatibility
- Promising versatility for a variety of morphologies
- Ability to form complex supramolecular

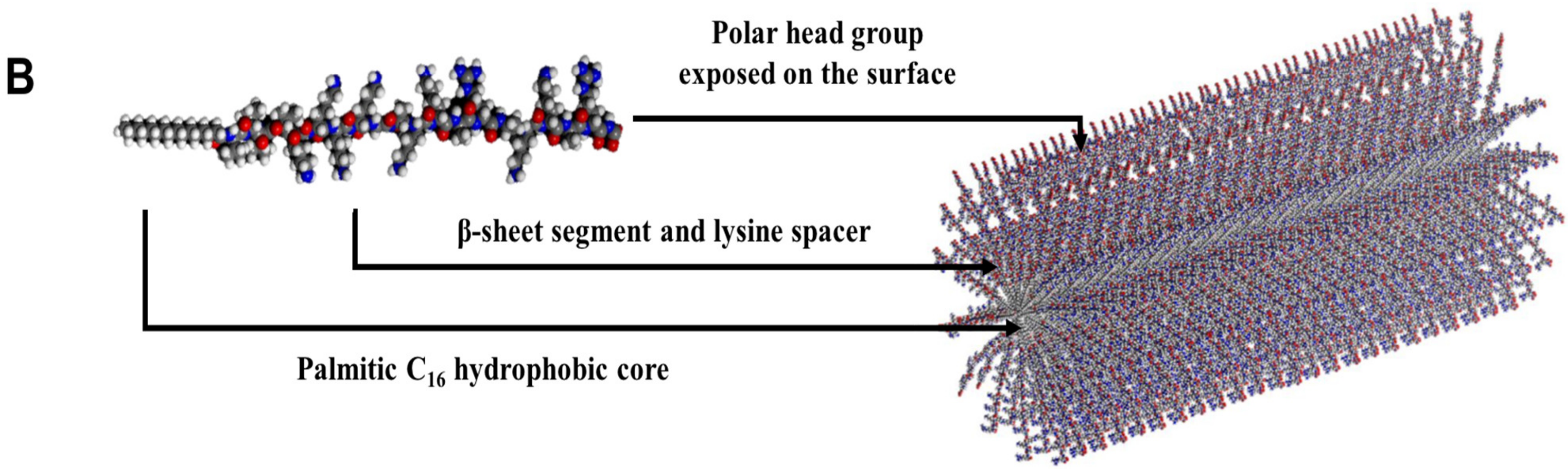
Stupp, S.I., et al., Isr. J. Chem. Vol. 53, 530 – 554, 2013

Self-assembling antibacterial cationic peptide amphiphiles (ACA-PA)

Self-assembling antibacterial cationic amphiphilic peptide (ACA-PA)



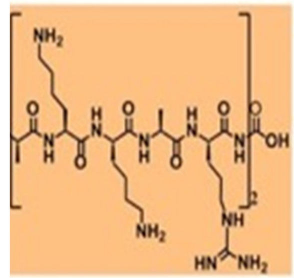




Other peptide molecules as comparison

Bi-Cardin peptide

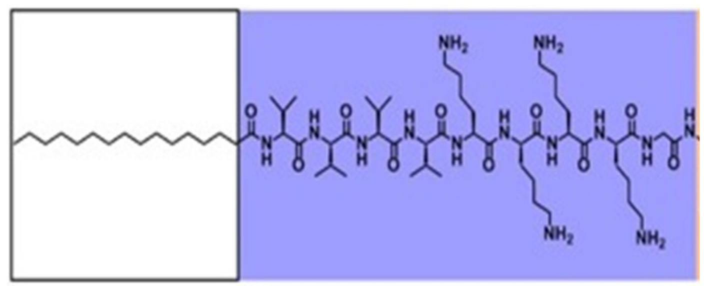
- ✓ **Sequence: (AKKARK)$_2$**
- ✓ **Contains the cationic heparin-binding group only with no self-assembly property**



CVK-PA

✓**Sequence: C$_{16}$-V$_4$K$_4$**

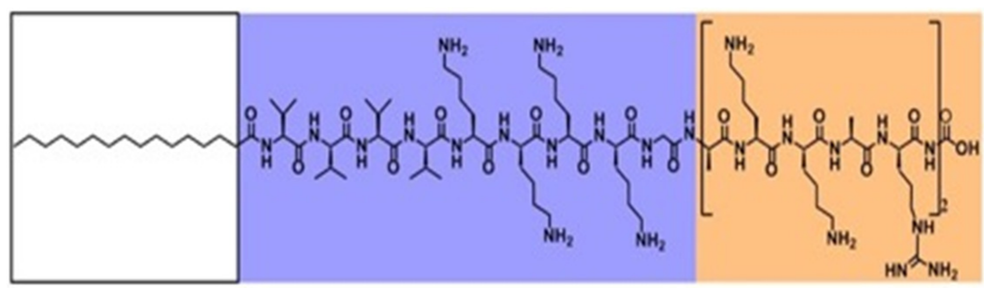
✓**Contains the β-sheet self-assembly backbone only**



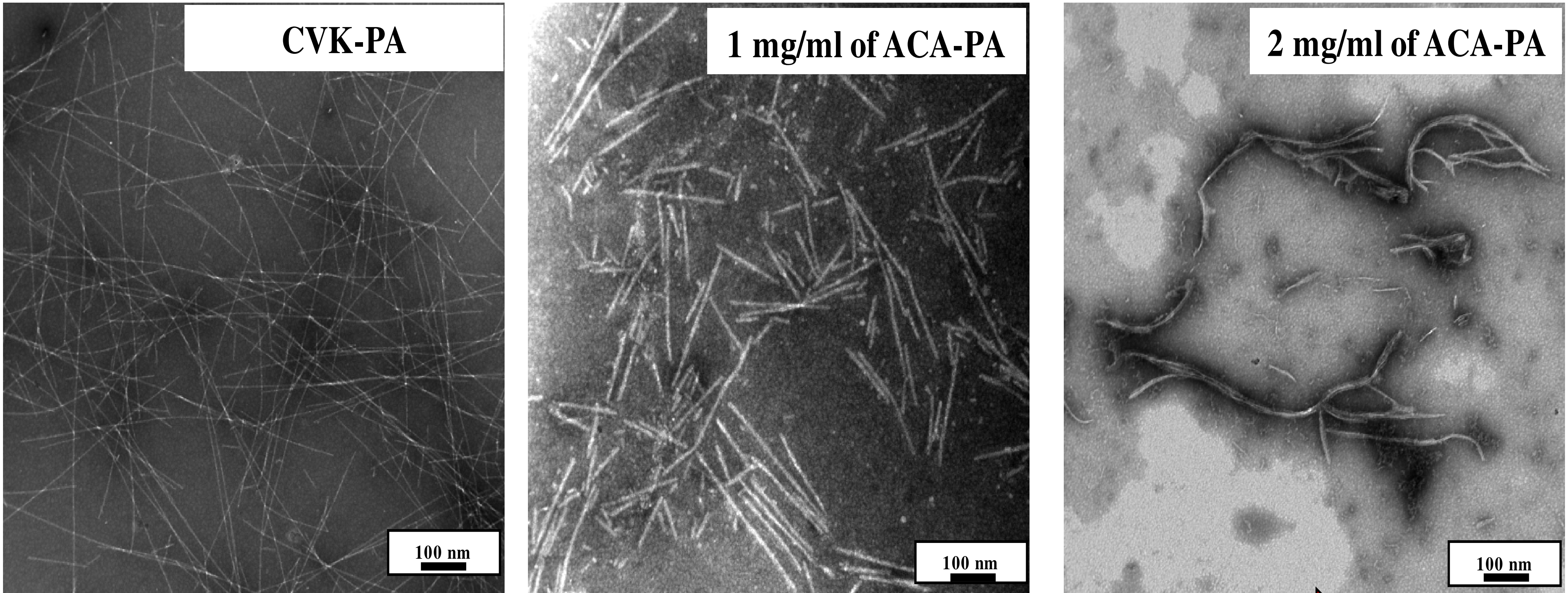
ACA-PA

✓**Sequence: C$_{16}$-V$_4$K$_4$G(AKKARK)$_2$**

✓**Contains the cationic heparin-binding group and the β-sheet self-assembly backbone**

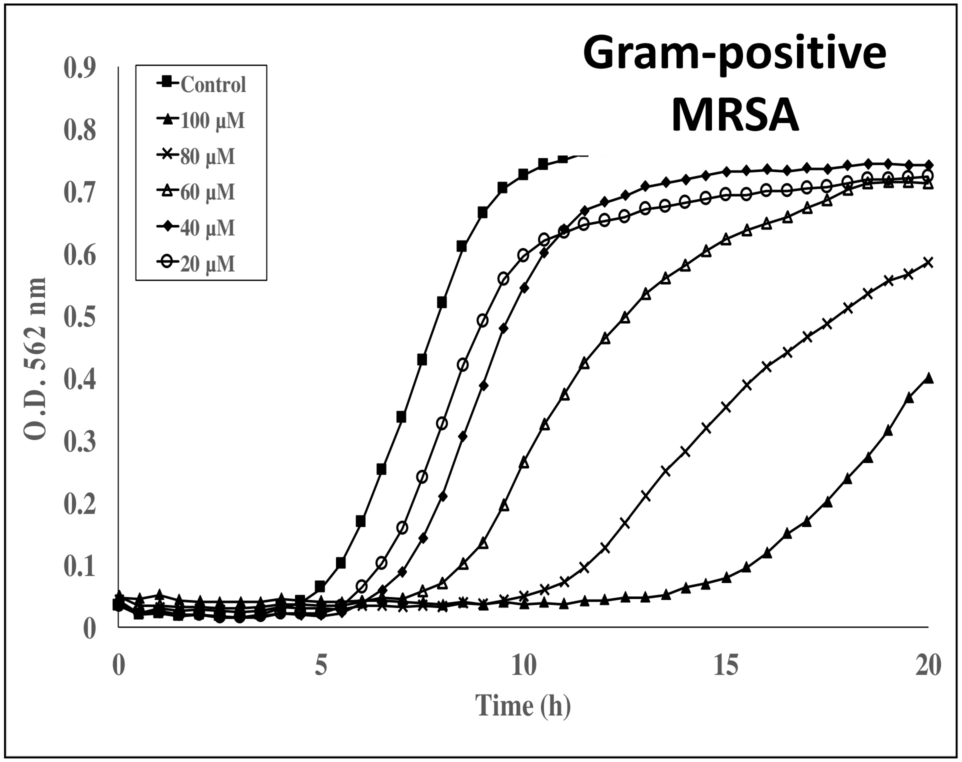


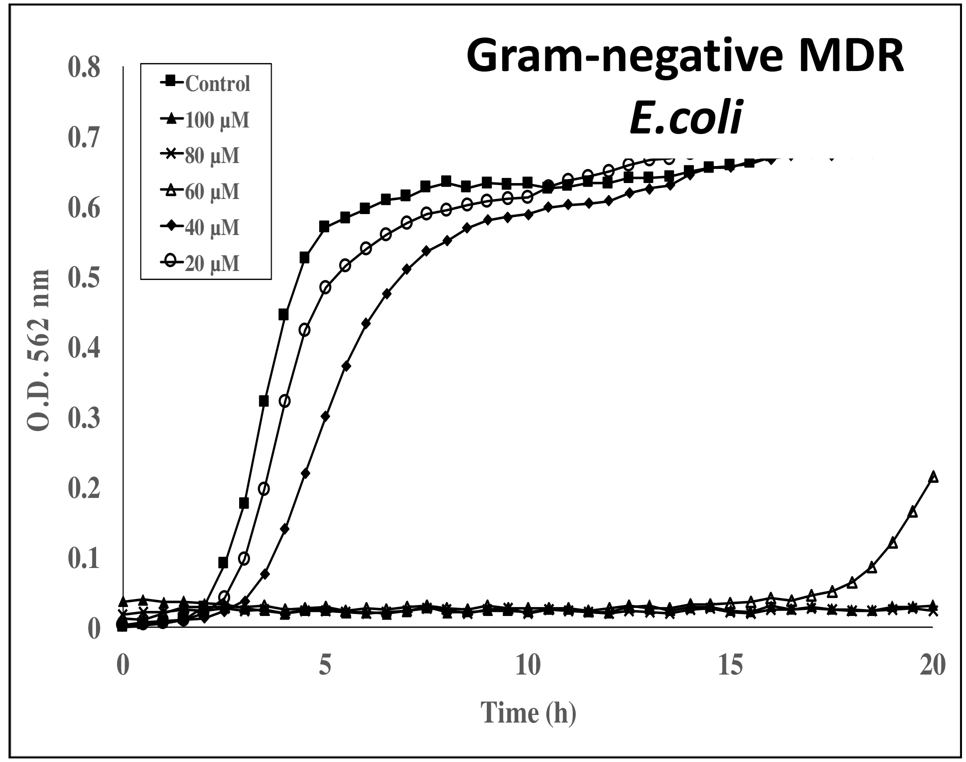
Morphological characterization of self-assembled structure



Morphological transition as concentration increased

Bacterial growth inhibition of self-assembling ACA-PAs





For Gram-positive MRSA:

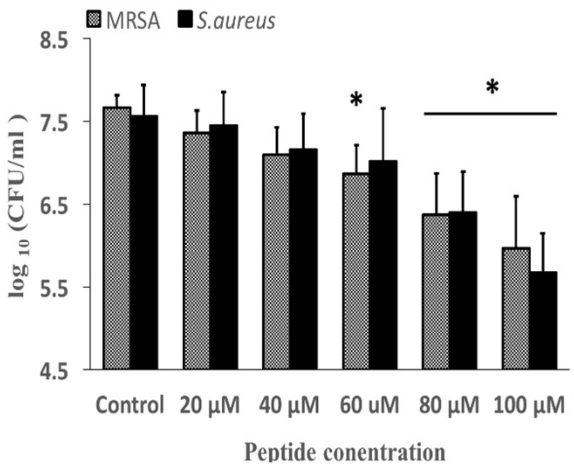
✓ The ACA-PAs exhibited a concentration-dependent inhibitory effect regardless of peptide self-assembly

For Gram-negative multidrug-resistant *E.coli* (MDR *E.coli*):

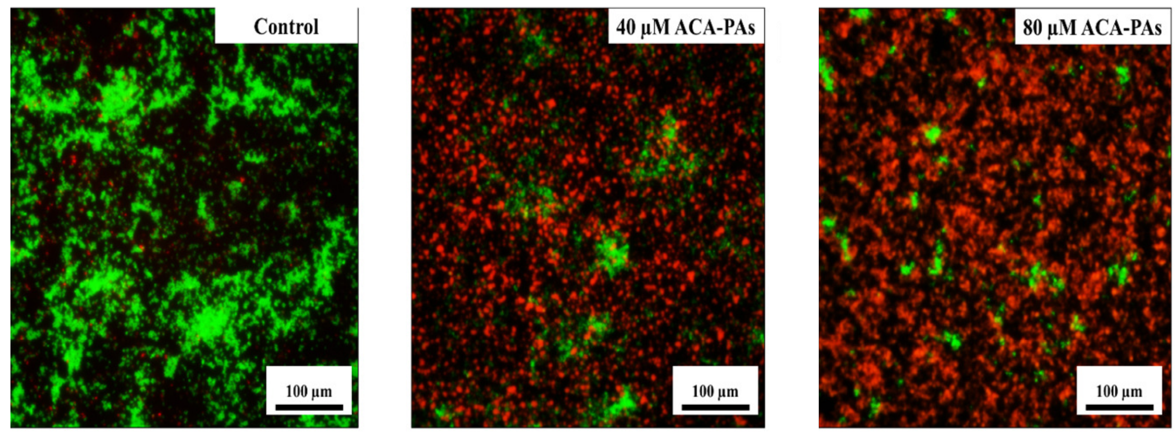
✓ Self-assembly the ACA nanorods significantly enhanced the antibacterial property, and remarkably inhibited the growth of the bacteria upon self-assembly

Bactericidal effects of ACA-PAs against bacteria

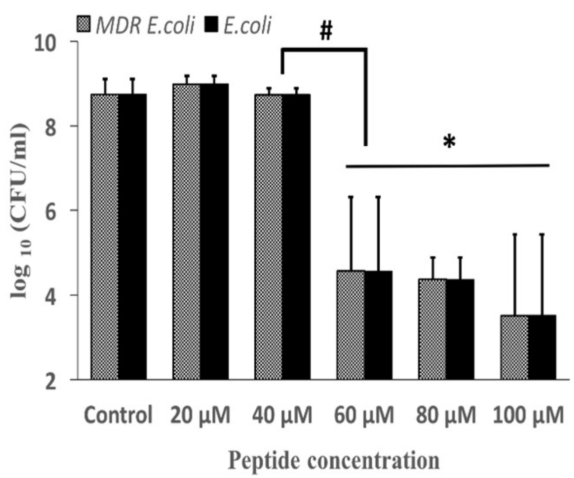
Gram-positive bacteria



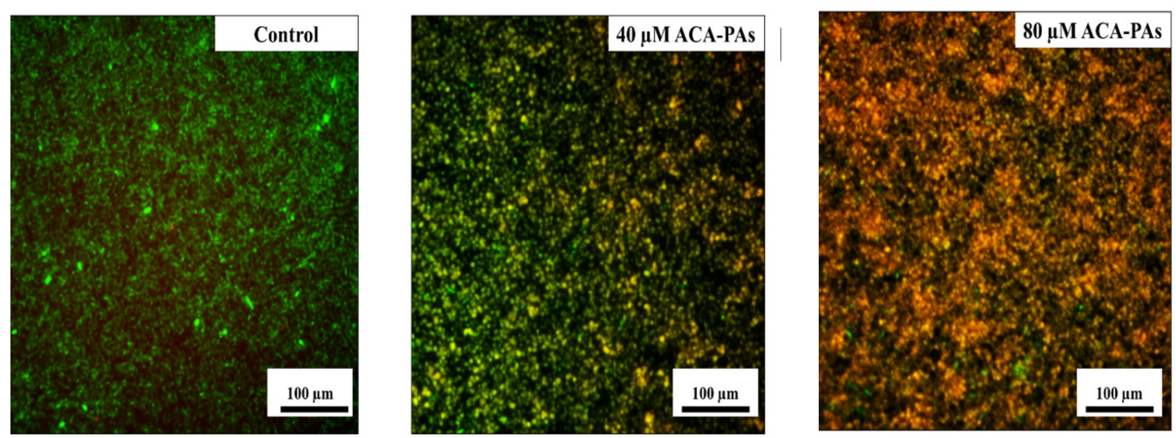
MRSA



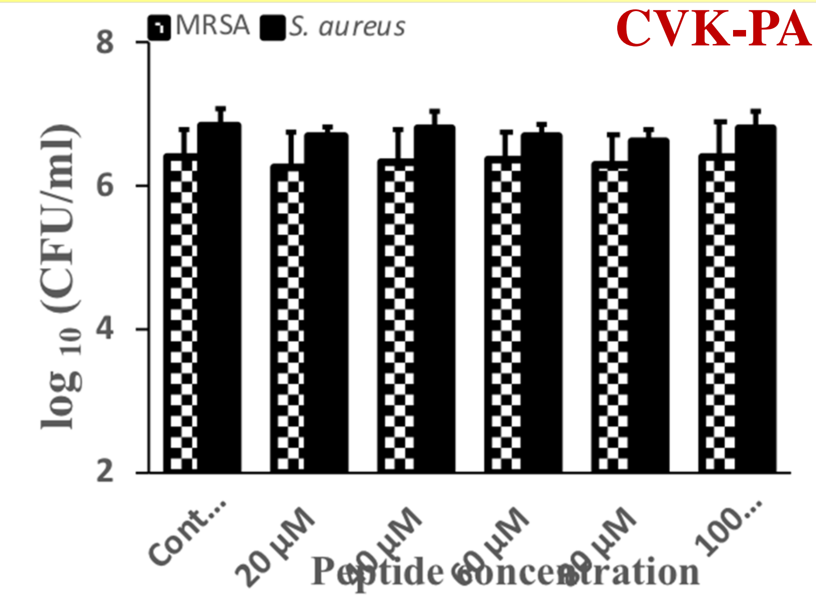
Gram-negative bacteria

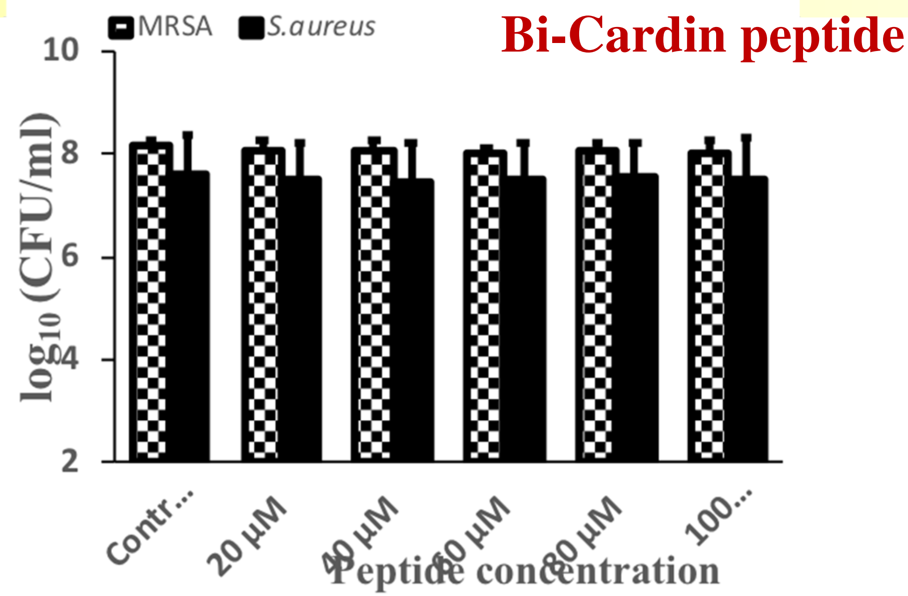


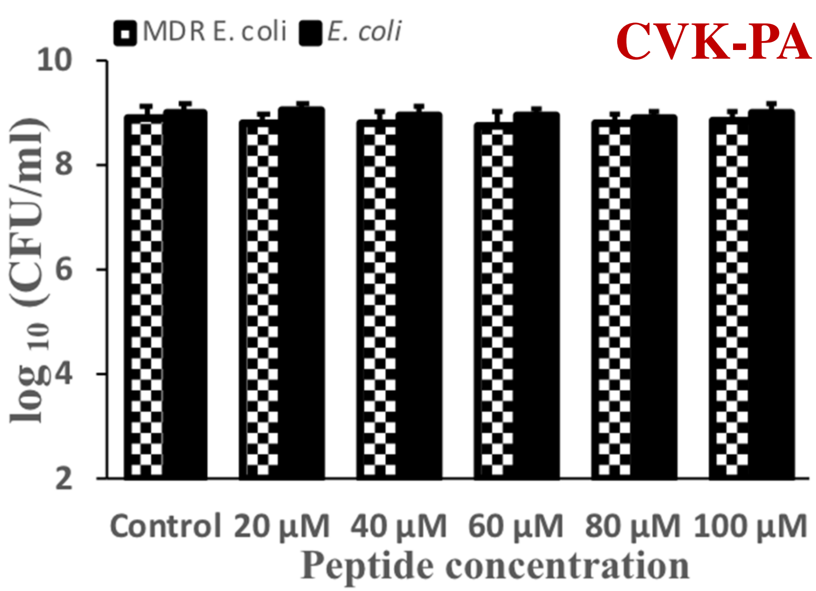
Multidrug resistant *E. coli*

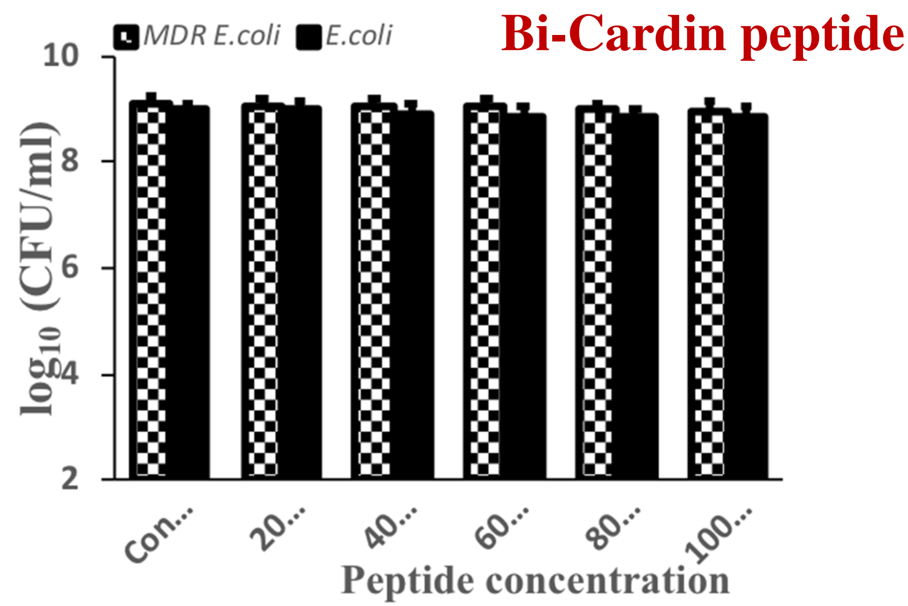


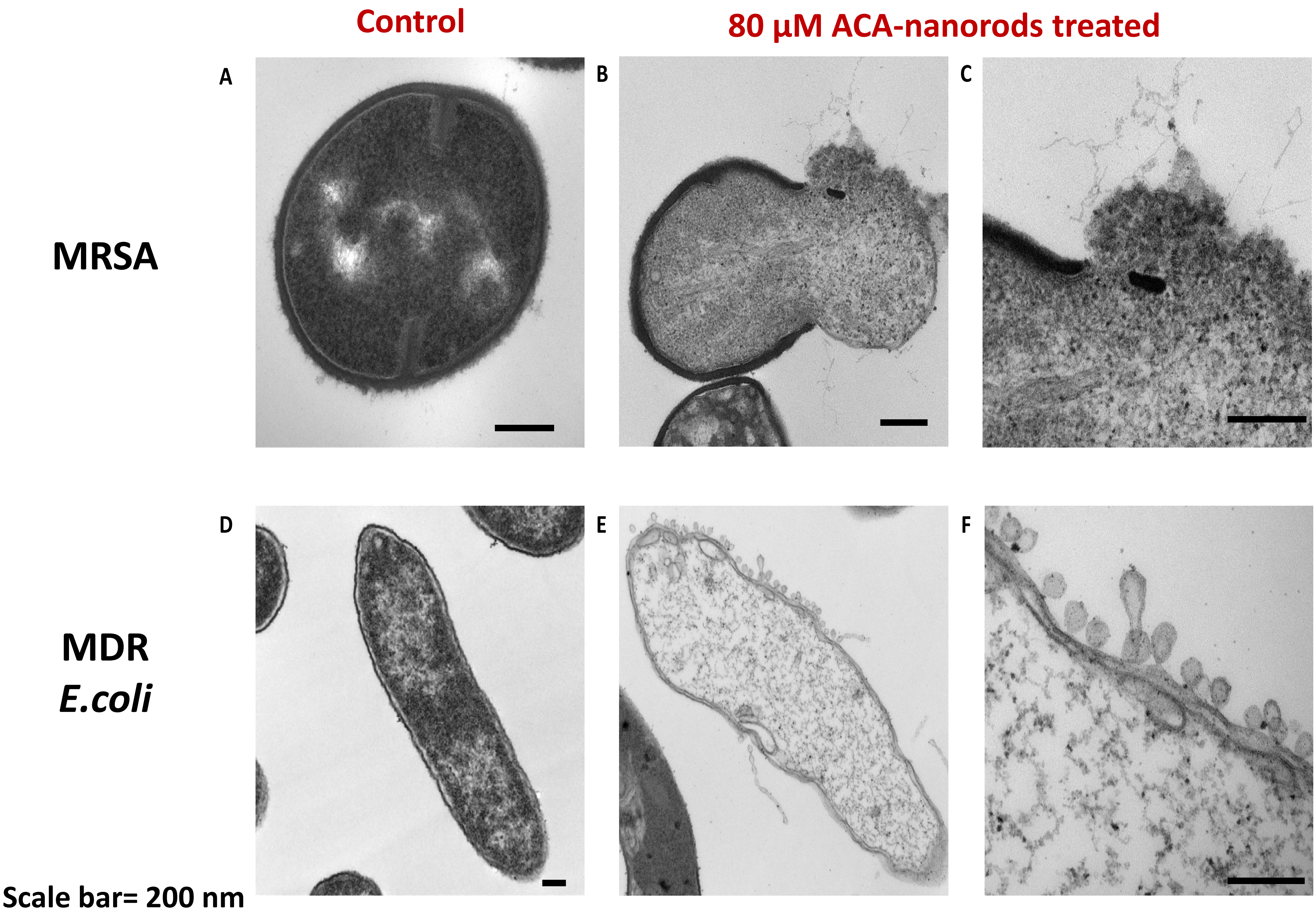
The CVK-PA and Bi-Cardin peptide showed no antibacterial activity







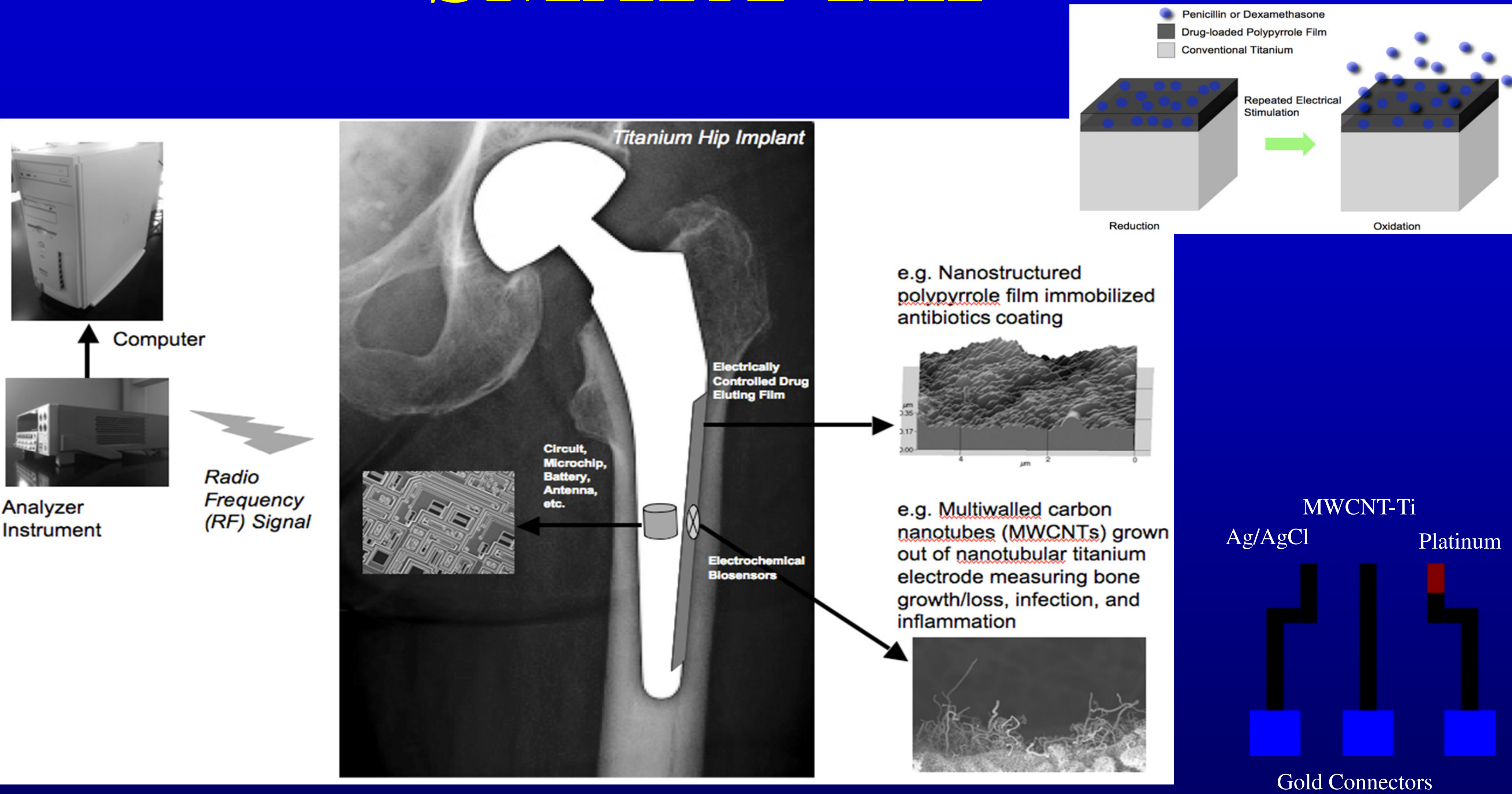




Control
80 µM ACA-nanorods treated
A
B
C
MRSA
D
E
F
MDR
E.coli
Scale bar= 200 nm

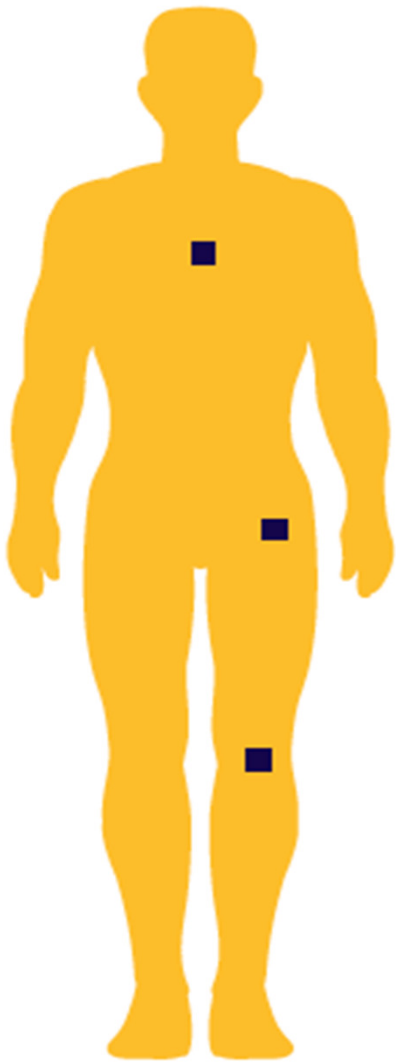
<u>Challenge #5</u>: We need to be more proactive (and not always reactive) in medicine.

SMART HIP™



Real-time Detection of Proteins/Cells/Tissue using Sensors and Releasing Drugs from a PLGA/Polypyrrole Coating

Ortho-tag



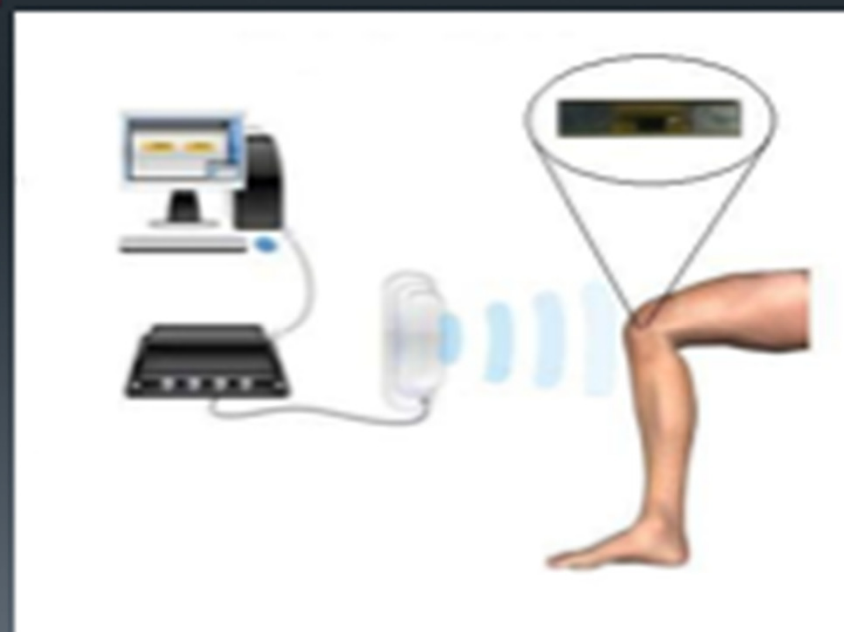
On the Forefront of In-Body Communication and Biosensing on the Nanoscale

Ortho-tag's technologies enable and enhance wireless in-body communication, data exchange and storage, and the nanodiagnostic functionality of smart medical implants, providing a versatile, in vivo platform that connects digital health applications and sensors with the human body.

www.ortho-tag.com

Ortho-Tag System Overview

- The Ortho-tag system incorporates proprietary RFID systems
  - Touch probe replaces traditional RFID antenna for transcutaneous energy transfer and communication
  - RFID reader and software facilitates communication with implanted tag
- Instantaneous data retrieval without the need to rely on medical records or device removal

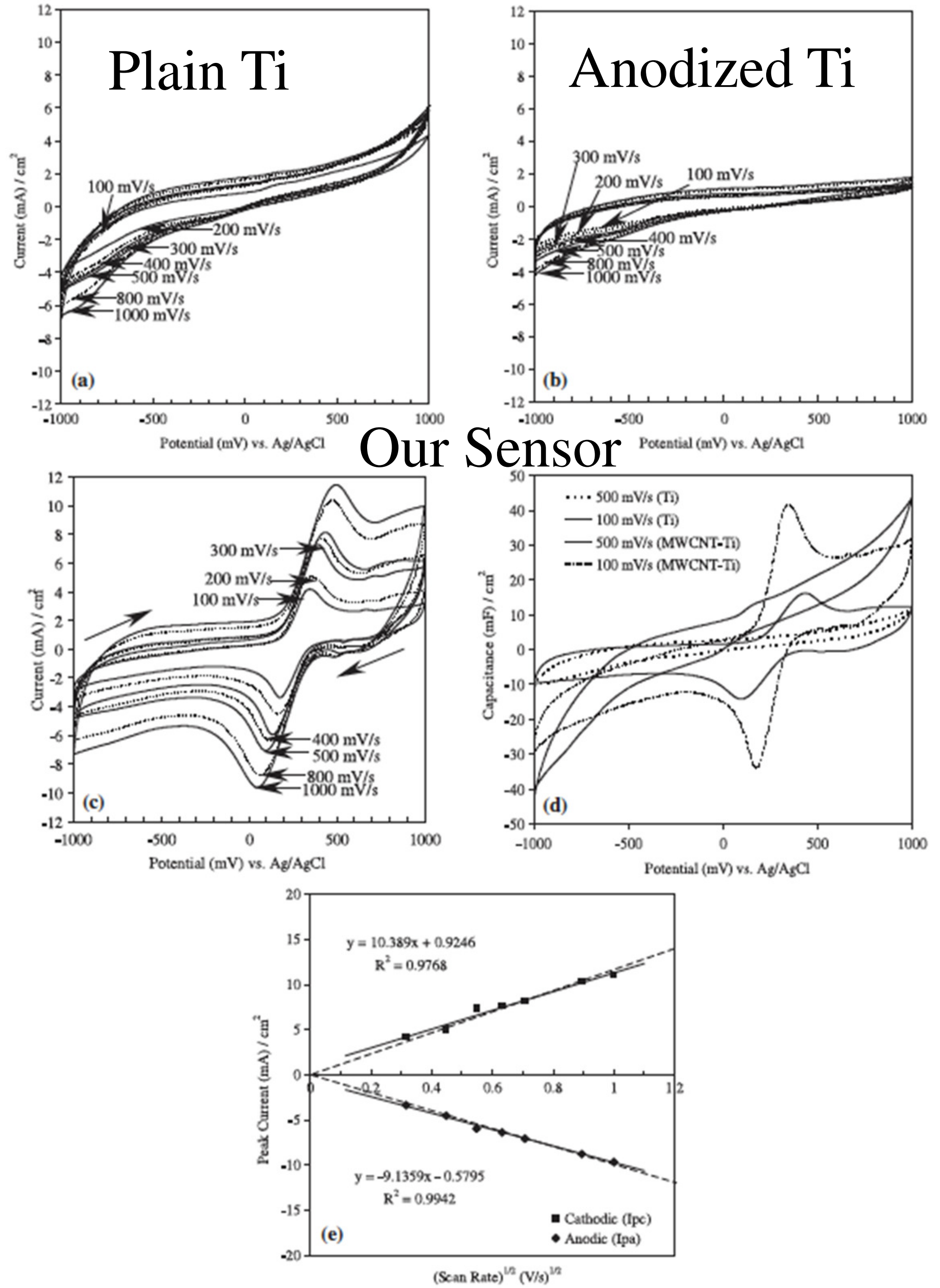


But does this translate in vivo ??

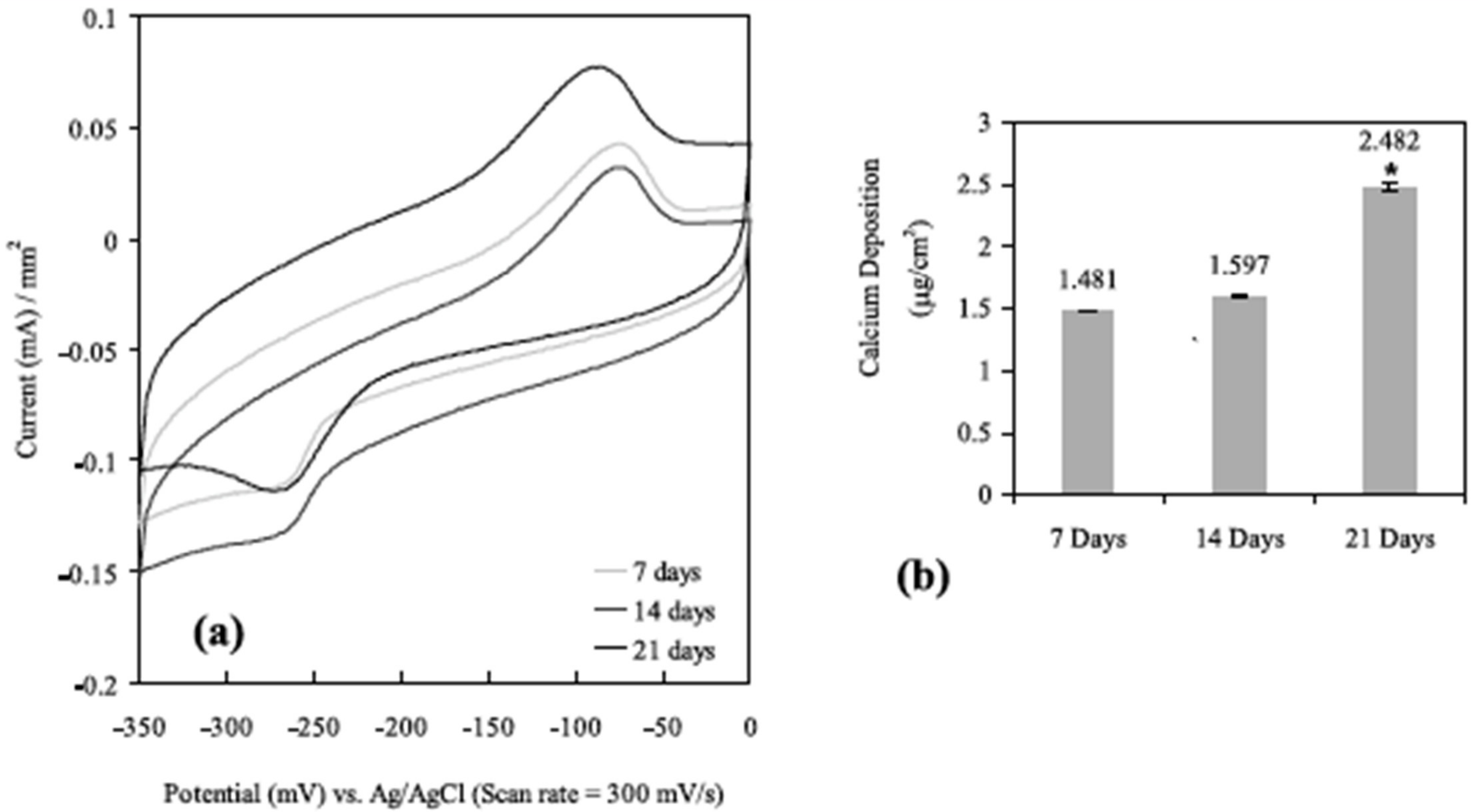
- Implanted square titanium-based sensors into rat calvaria
- Some samples, forced an infection via pre-seeding 10^5 *Staph. epi* (and other bacteria in separate experiments) CFU per implant
- Determine bacteria presence, macrophage presence, and bone growth via characteristic cyclic voltammograms
- Assessed tissue growth up to 3 months

Characteristic CVs:

Proving We Transitioned Ti into a Sensor



Characteristic CVs: Showing Increased Bone Growth With Time



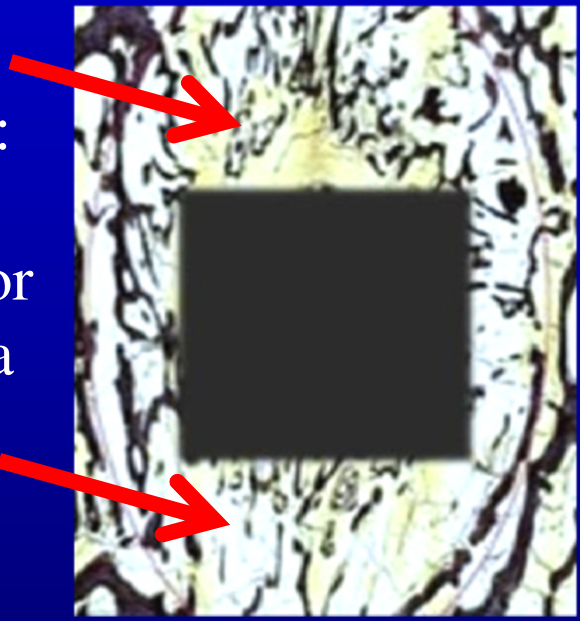
Reversal of Infection to Increased Bone Growth: 7 Days Post Implantation

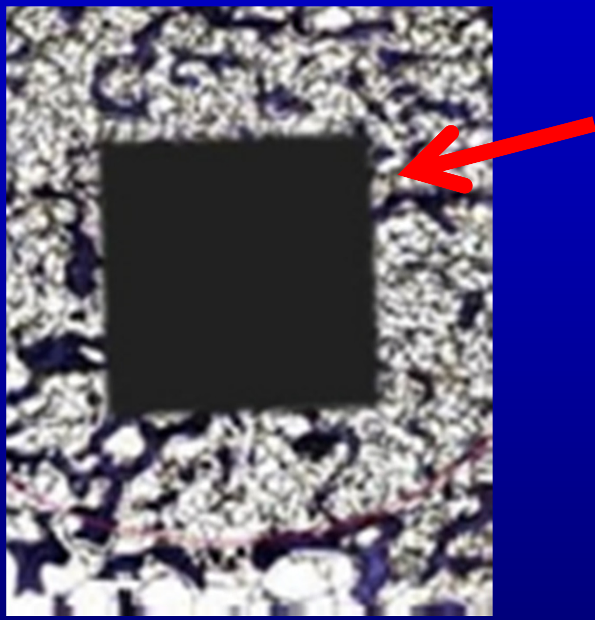
Push-Out Strength: 0.11MPa

0.71 MPa

Yellow:

Stain for bacteria





Purple:

Stain for bone growth

Pre-seeded with *Staph epi*
Plain Ti

Pre-seeded with *Staph epi*
Release of gentimicin and BMP-7 after 1 day
Our sensor

Similar results were achieved for *Pseudomonas*, MRSA, and *E. coli*

And remember,
what is wrong with this ??

vs.

How many sensors do we have in both ?

My Dream for the Future of Healthcare

- Our version of medicine must fight bacteria without drugs.
- Our version of medicine must transition to predictive not reactionary.
- Our version of medicine must treat individuals not generalized for the whole population or age groups.
- Our version of medicine must be dynamic not static.
- Unless we change, our life expectancy in the U.S. will continue to decline, unlike the rest of the world.

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Thank You!

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