Goodbye Hospitals and Hello Nanosensors

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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.?
Pop Quiz:

Is life expectancy **increasing** or **decreasing** 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

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

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

Methicillin-resistant *Staphylococcus aureus* (MRSA)

https://www.cdc.gov/drugresistance/
https://amr-review.org/Publications.html
Problems with Infection


>2 million resistant infections/yr
>23,000 deaths/yr

$20 billion in excess direct healthcare costs

Immediate public health threat requiring urgent and aggressive action

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

Number of Antibacterial New Drug Application Approvals per Year

Antibiotic deployment

Sulfonamides
Penicillin
Streptomycin

Tetracycline
Chloramphenicol

Vancomycin
Ampicillin

Methicillin
Erythromycin

Cephalosporins

Daptomycin
Linezolid


Antibiotic resistance observed

>2 million resistant infections/yr
Longer treatment durations
Undesirable side-effects


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
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- 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

Vascular Endothelialization

Antibacterial

Many endothelial cells

Few Staph Epi

Increased bone growth

Bone Growth

Orthopedic Soft Tissue

Perpendicular collagen fibers
Why Use Nanotechnology To Fight Bacteria ????
Part 1: Nanostructured Materials
**Possible Reason: Biophysical model**

**Mechanism:** 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.

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*.

![Cicada Wing](image)

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.

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\textsuperscript{1-6}

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
Silicon Nitride: 3 Months (bacteria inoculation)

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

Rat calvaria model
Example: Commercialized by Nanovis, LLC
Anodized Titanium

PROCEDURES:
Pretreatment: chemical polishing using HF/HNO₃ mixture
Anodization: 0.5 or 1.5% HF
Voltage: 20V
Time: 20 min
Clean: acetone and ethanol
Sterilize

Sketch map of anodization system
Anodized Ti Nanotubular Screws

Low Magnification (10K)

Nanotubular Pin

Grooved Valley

Raised Peak

Anodization

High Magnification (100K)

Low Magnification (10K)

High Magnification (100K)
Closed Wound with No Infection Surrounding Nanotextured Screws Only

Nanovis, LLC is now commercializing this as a pedicle screw.

Over 2,200 implanted with no infections.
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
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.
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

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
Challenge #3: We need a better understanding of the mechanism by which fundamental material properties decrease bacteria response.
→ Develop a catheter that inhibits bacteria growth through fabricating antibacterial nano-patterns on the surface of catheter materials.

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.](image-url)
Step 2: Preparation of PDMS replica

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

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

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

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

![Graph showing gene expressions](image-url)
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
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
  - Impenetrable to antibiotics and host immune cells

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

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
“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.

**Examples include:** 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
**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.

**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.
What if your food could cure the disease you have?

**OBJECTIVE**

1. Relatively cheap
2. Easy access
3. Versatility
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

Partitioning pathways of AMPs

Bacterial cell membrane

AMPs

Peptides attach and accumulate on membrane

Peptides disrupt cell membrane by micellization

Secondary structures of AMPs can facilitate membrane insertion

Bendin

Peptides create bending on the membrane and cause membrane disruption

Advantages of self-assembling peptides:

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

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

A

Peptide amphiphile backbone for self-assembly
Cationic heparin-binding Cardin-motif

B

Polar head group exposed on the surface
β-sheet segment and lysine spacer
Palmitic C₁₆ hydrophobic core

Simple preparation method
Dissolve peptide in water
Nanoparticles self-assemble
Other peptide molecules as comparison

**Bi-Cardin peptide**
- Sequence: \((\text{AKKARK})_2\)
- Contains the cationic heparin-binding group only with no self-assembly property

**CVK-PA**
- Sequence: \(\text{C}_{16}\text{V}_4\text{K}_4\)
- Contains the β-sheet self-assembly backbone only

**ACA-PA**
- Sequence: \(\text{C}_{16}\text{V}_4\text{K}_4\text{G}(\text{AKKARK})_2\)
- Contains the cationic heparin-binding group and the β-sheet self-assembly backbone
Morphological characterization of self-assembled structure

- CVK-PA
- 1 mg/ml of ACA-PA
- 2 mg/ml of ACA-PA

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**

<table>
<thead>
<tr>
<th>Peptide concentration</th>
<th>Control</th>
<th>20 μM</th>
<th>40 μM</th>
<th>60 μM</th>
<th>80 μM</th>
<th>100 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>log_{10} (CFU/ml)</td>
<td><img src="image1.png" alt="Bar Graph" /></td>
<td></td>
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</tr>
</tbody>
</table>

**MRSA**

![Images of MRSA with peptide concentrations](image2.png)

**Gram-negative bacteria**

<table>
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<tr>
<th>Peptide concentration</th>
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**Multidrug resistant E. coli**

![Images of MRSA with peptide concentrations](image4.png)
The CVK-PA and Bi-Cardin peptide showed no antibacterial activity.
Control

80 µM ACA-nanorods treated

MRSA

A

B

C

Scale bar= 200 nm

MDR

E.coli

D

E

F

Scale bar= 200 nm
Challenge #5: We need to be more proactive (and not always reactive) in medicine.
SMART HIPTM

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

Pre-seeded with *Staph epi*
Plain Ti

Release of gentimicin and BMP-7 after 1 day
Our sensor

Push-Out Strength: 0.11MPa
0.71 MPa

Yellow:
Stain for bacteria

Purple:
Stain for bone growth

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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Showalter Foundation

Whitaker Foundation
Thank You!

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