

Modified Chitosan Biomaterials for Biofilm-based Infection Prevention and Treatment



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Collaborative efforts and acknowledgments



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NSF CAREER

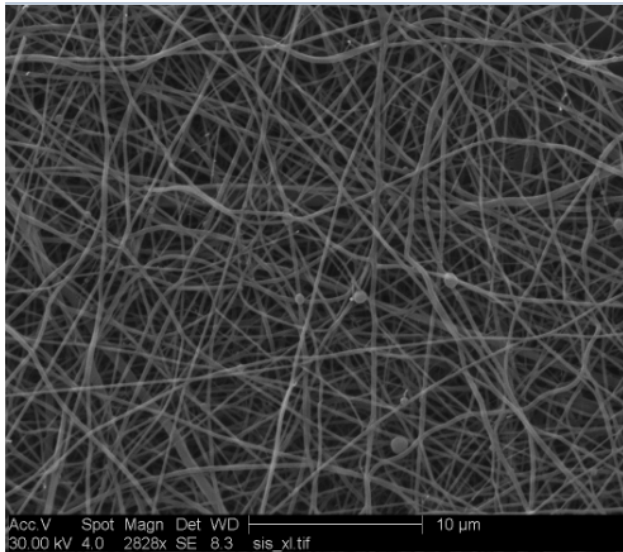


Military Burn Research Program
Peer Reviewed Medical
Research Program



UM Research
Development Award

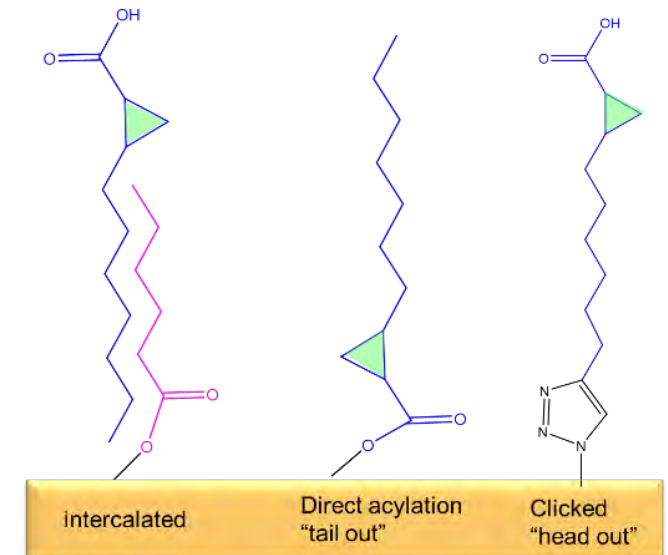
This talk will cover our efforts using chitosan as a local drug delivery system



Nanofibers



Injectable
paste



Material
functionalization

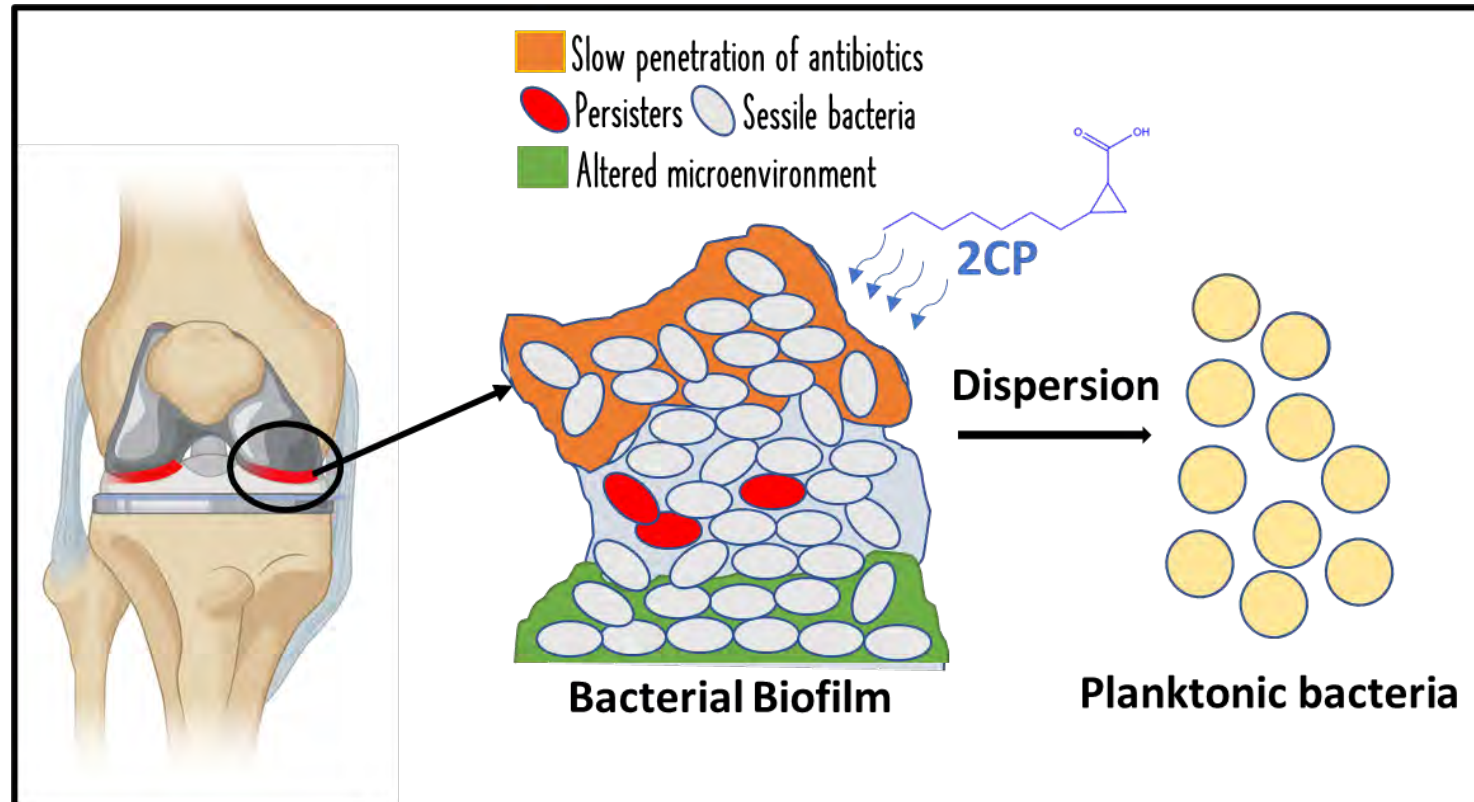


Bacterial biofilm

Biofilm is bacteria attached to a surface, which may also be encased in exopolymeric substance.

Up to 80% of all infections can be attributed to biofilm.

Implants can act as a substrate for bacteria to attach and form a biofilm

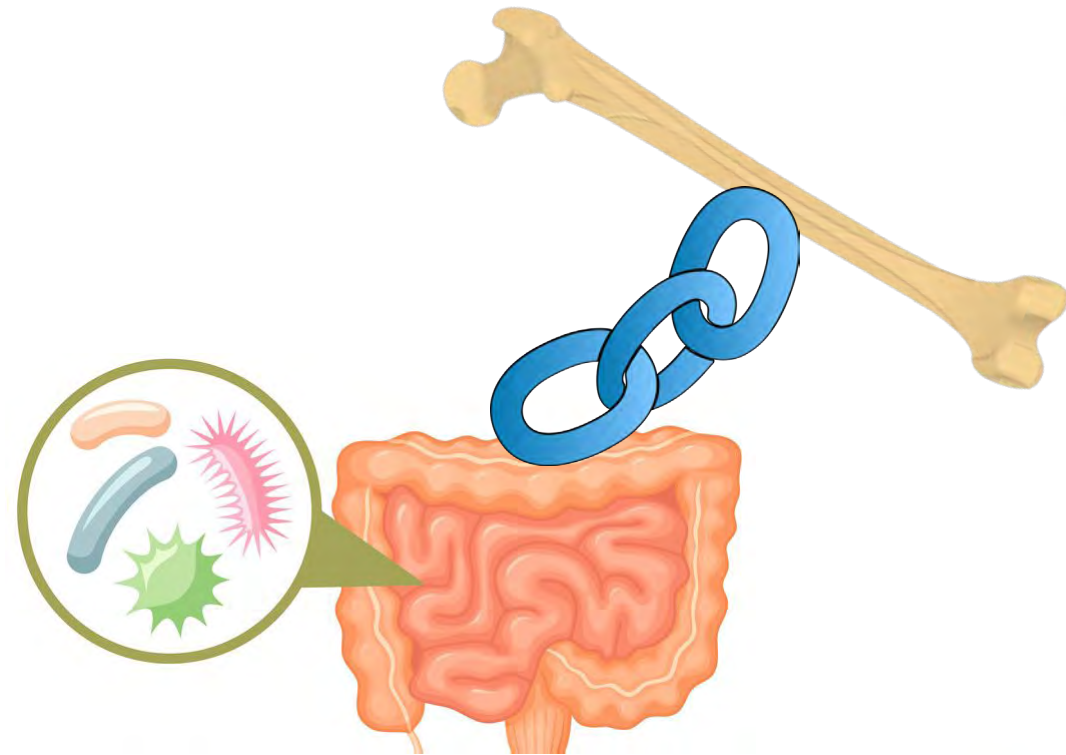
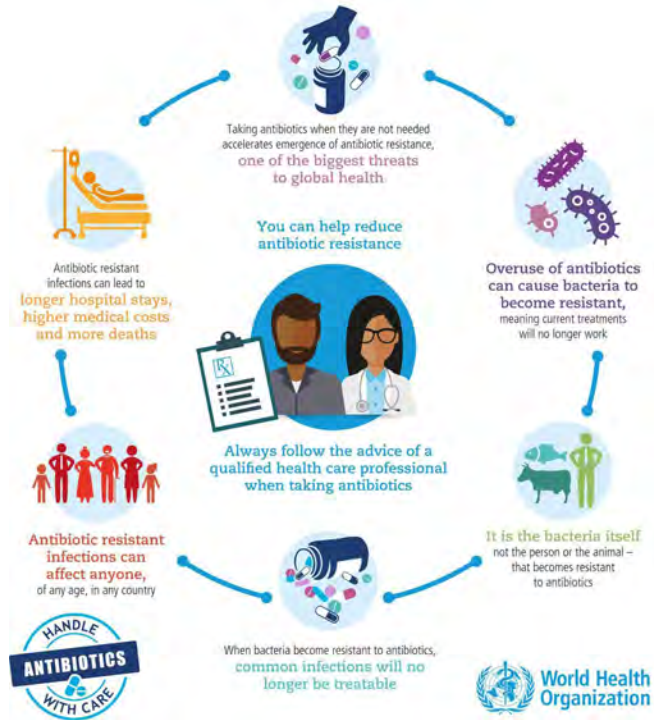


Pain management avoiding opioids is also advantageous



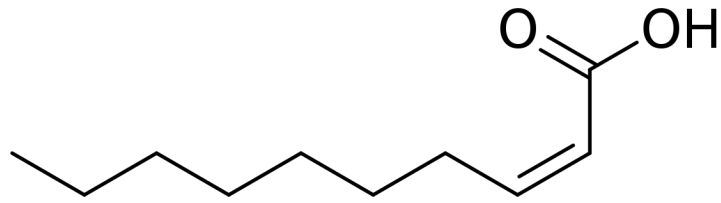
But—why not just load up on antibiotics and pain-relievers?

Routine use of antibiotics works against us

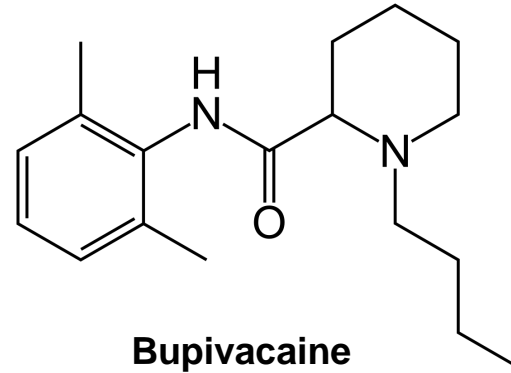


Resistance and microbiome effects

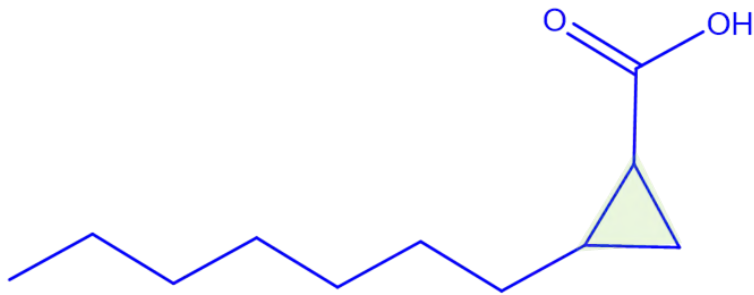
2-decenoic acid analogs and local anesthetics have been shown to have antimicrobial properties



Cis-2-decenoic acid

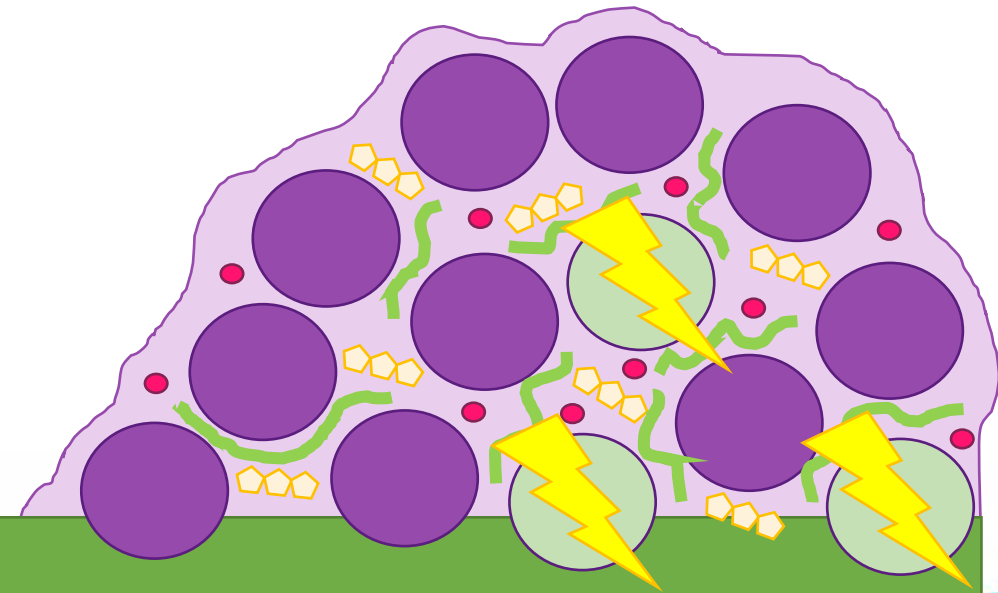


Bupivacaine

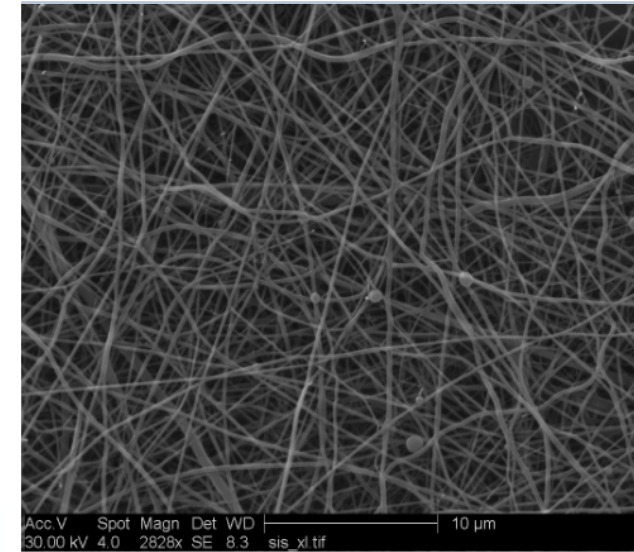
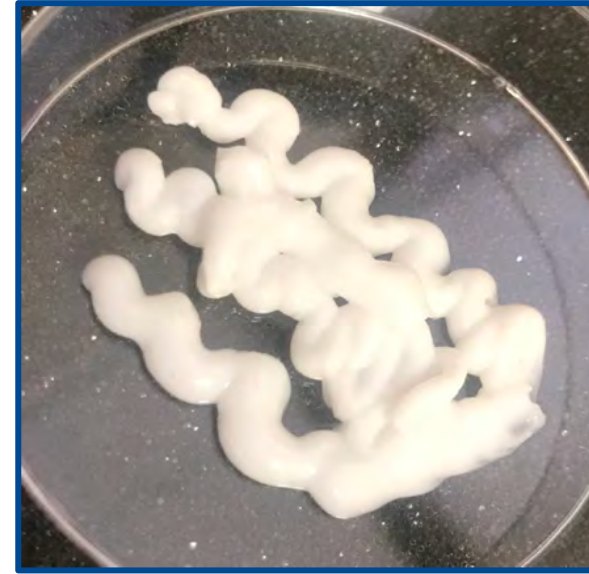
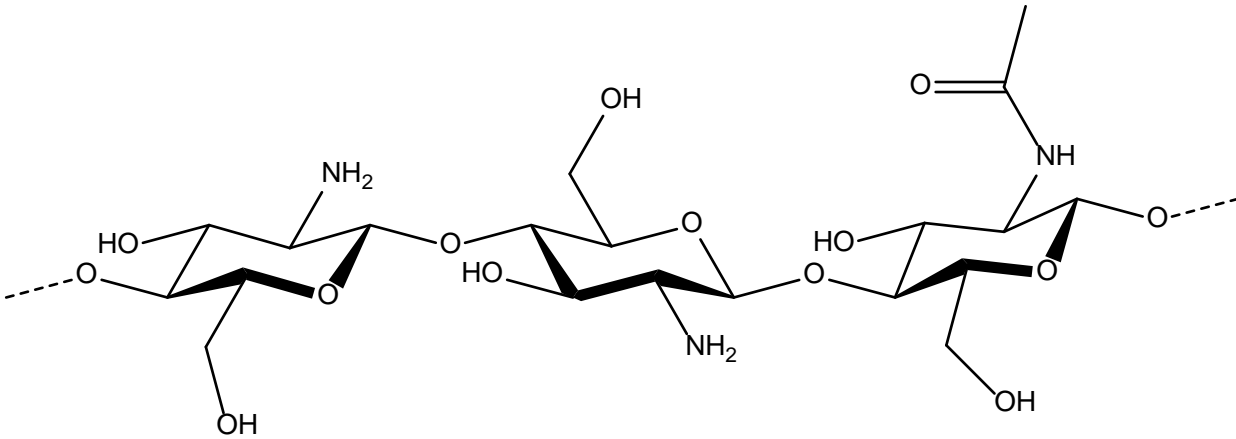


2-Heptylcyclopropane-1-carboxylic acid

Orthopedic Implant

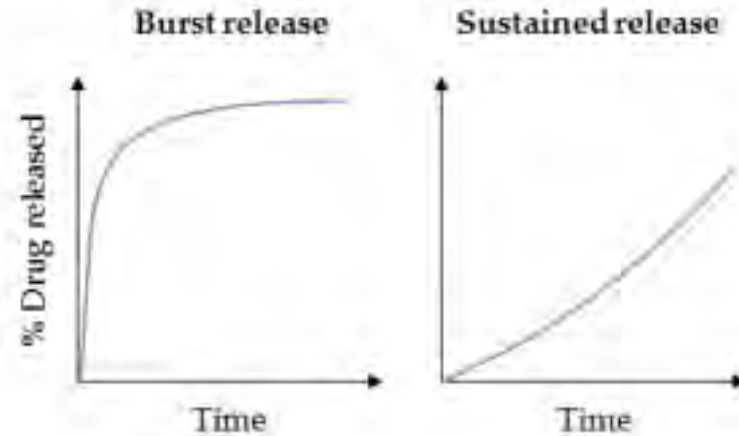


Chitosan is a versatile local drug delivery system



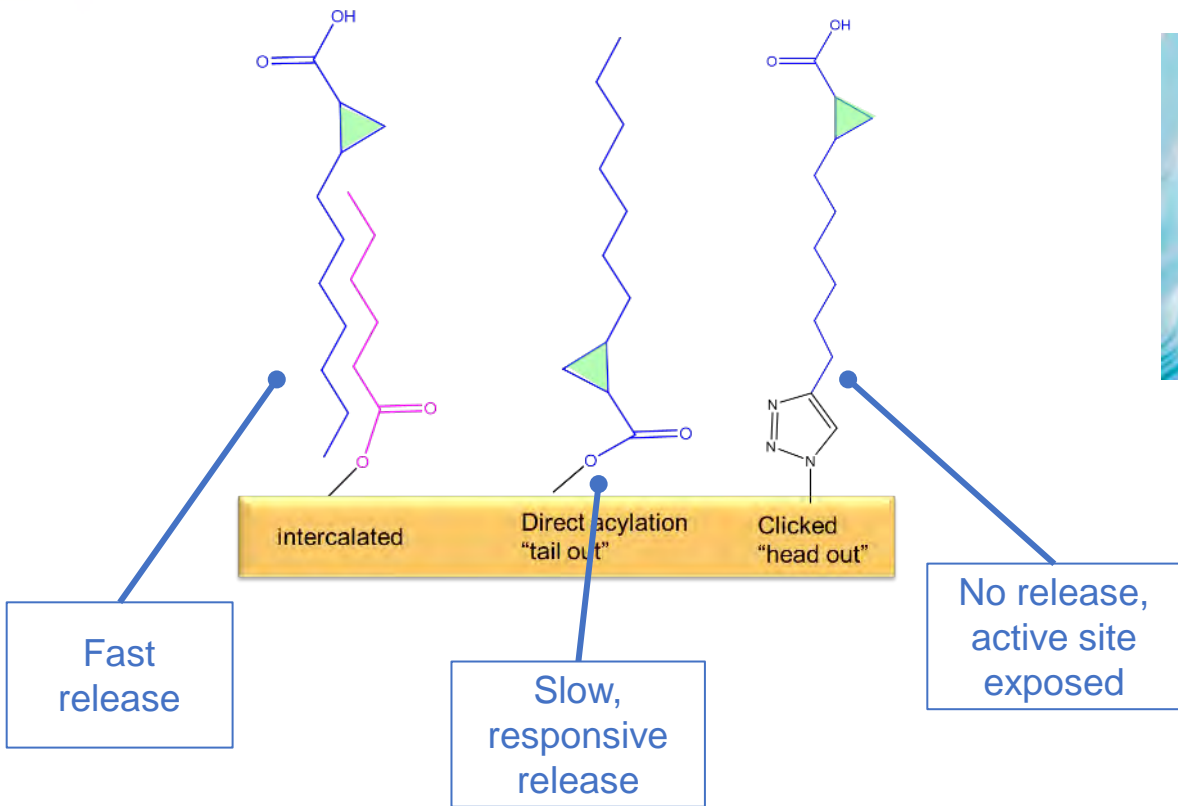
Shells of crustaceans, fungi

Hydrophobic therapeutics are challenging to load and release



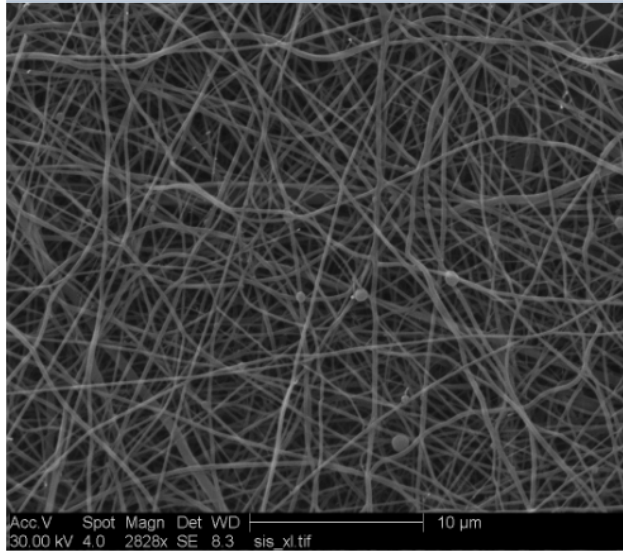
Solvent must also be biocompatible

Our goals: create functional delivery systems for these molecules



Functionalized chitosan

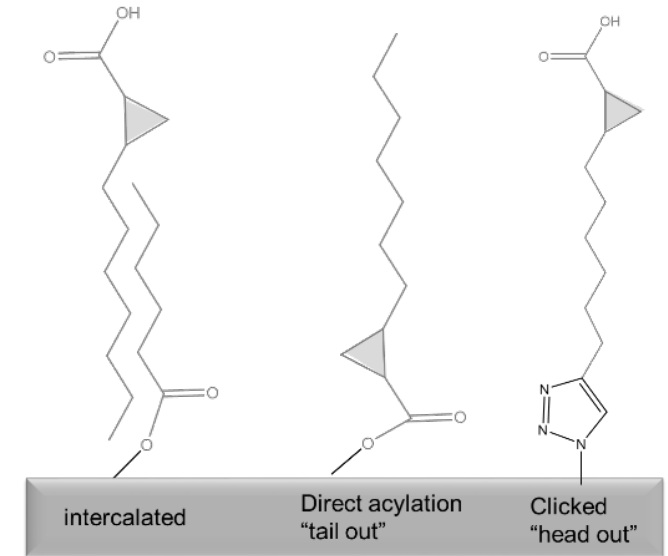
Outline



Nanofibers

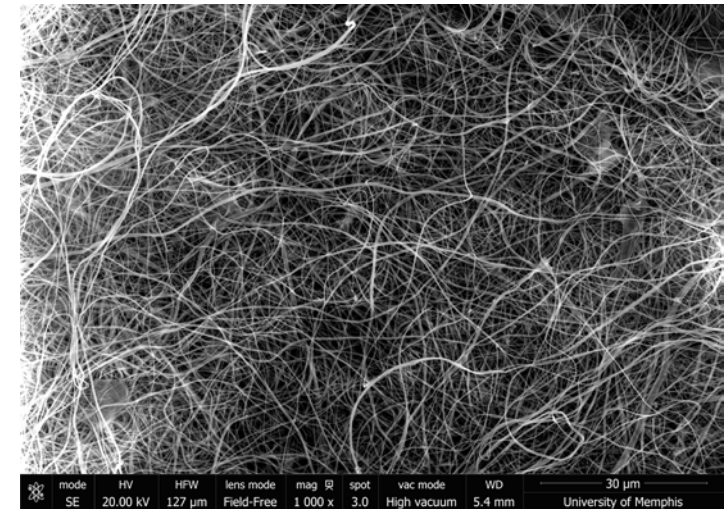
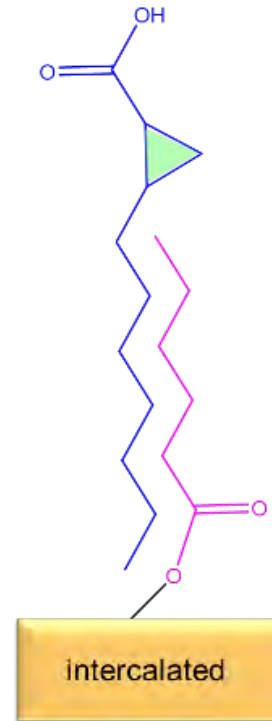
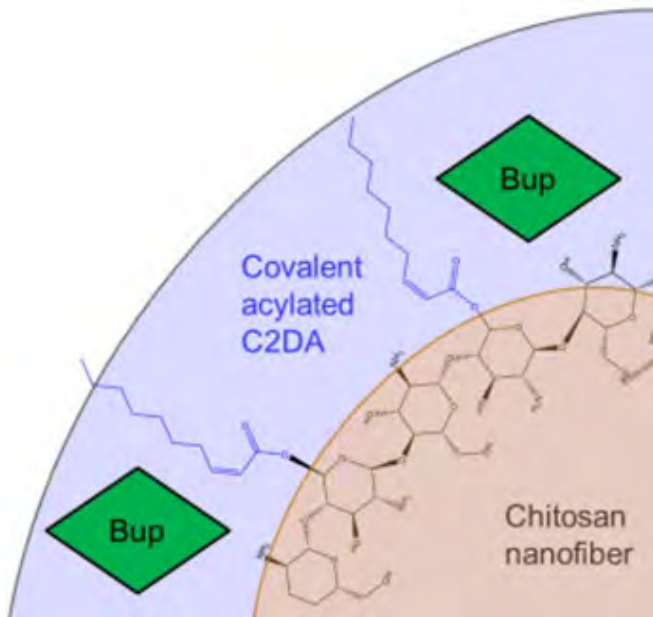


Injectable
paste



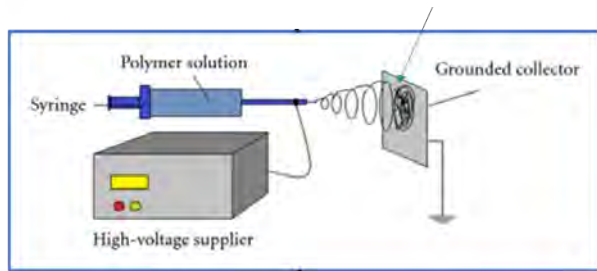
Material
functionalization

Acylated electrospun membranes for stabilization of nanofibers

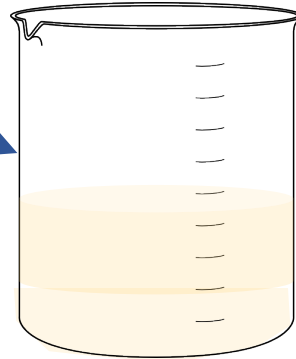


Varying acyl types may be used

Process for electrospinning, treatment and loading



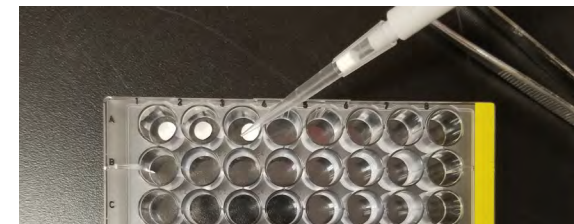
Electrospin



Add pyridine + acyl chlorides

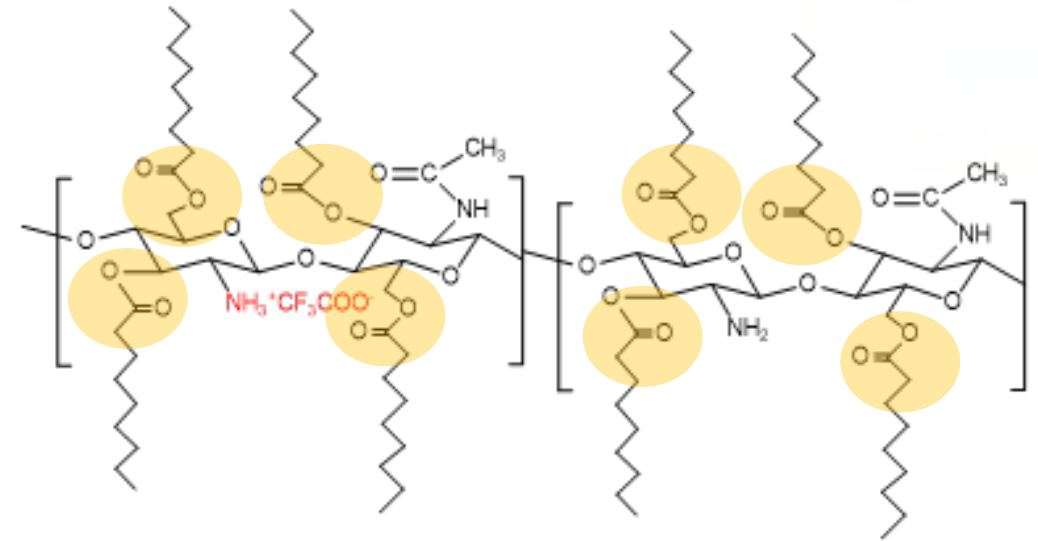
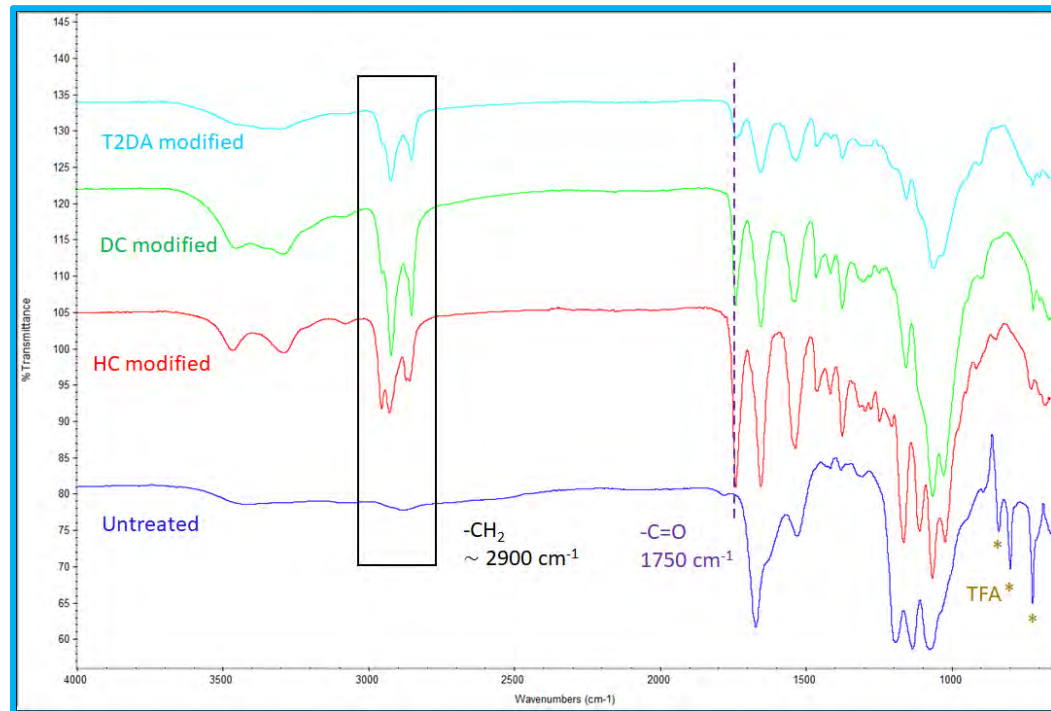


Wash



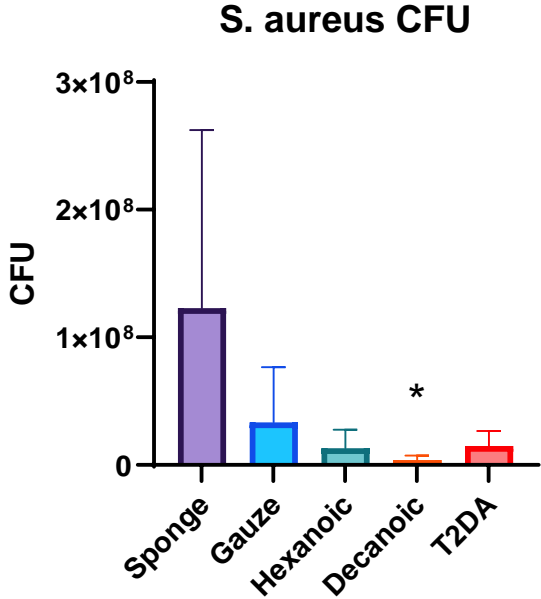
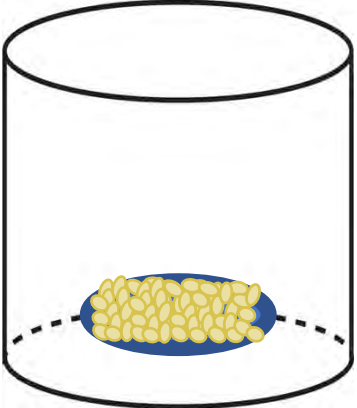
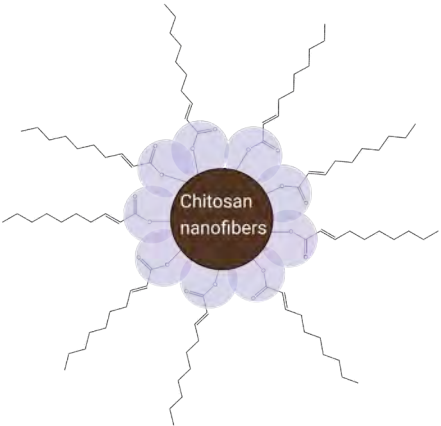
Load

Confirmation of reaction by FTIR



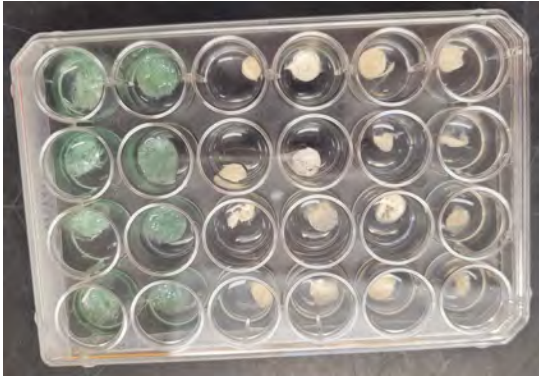
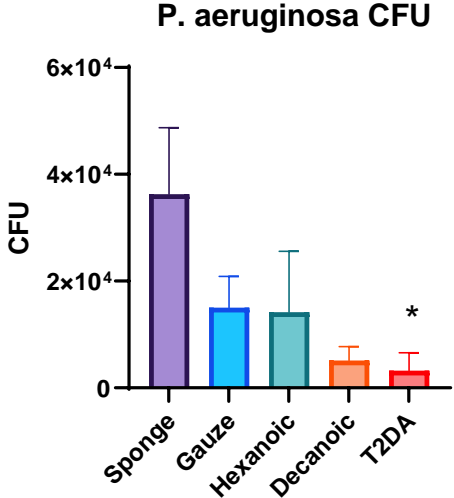
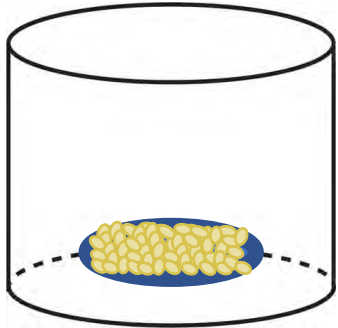
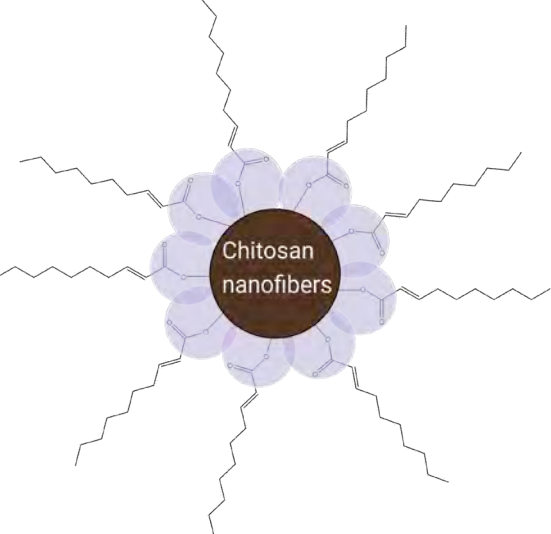
O-acylation confirmed

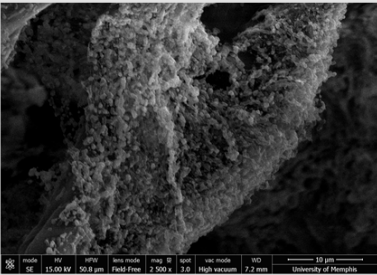
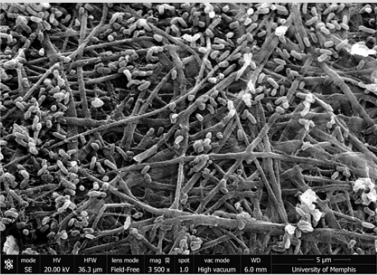
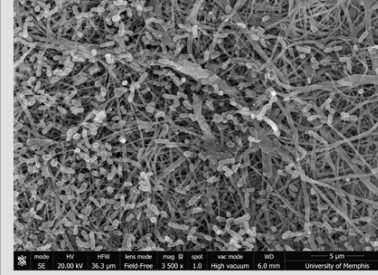
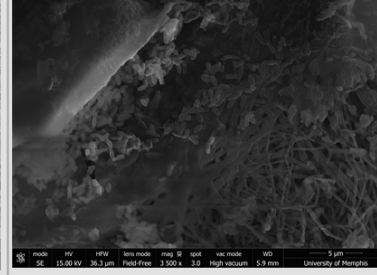
Acylated membranes resisted *S. aureus* biofilm formation



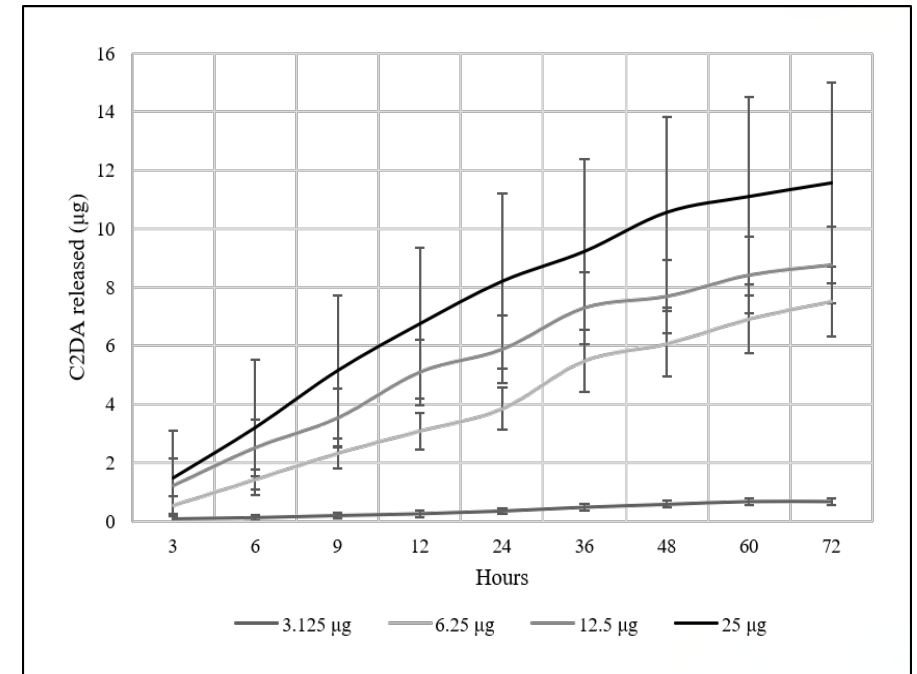
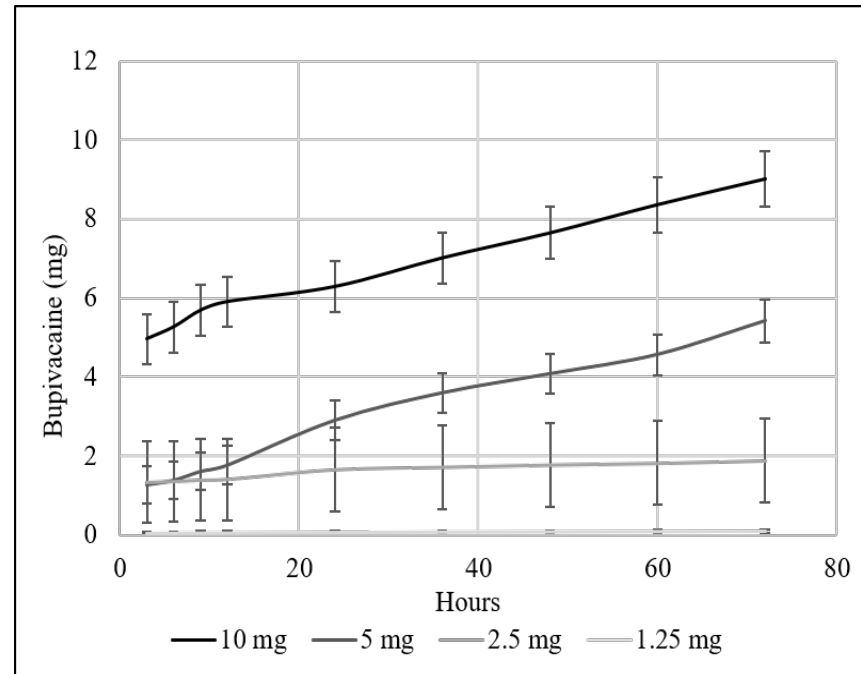
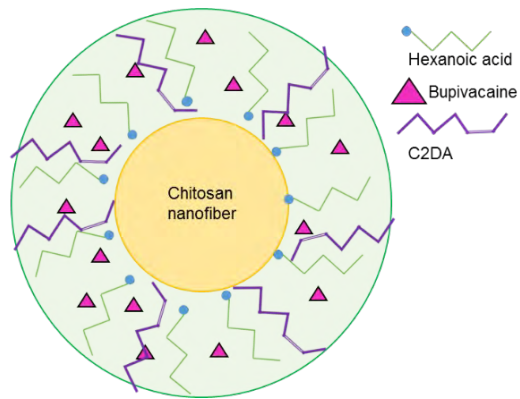
	Gauze	Hexanoic	Decanoic	2-decenoic
<i>S. aureus</i>				

Acylated membranes resisted *P. aeruginosa* biofilm formation

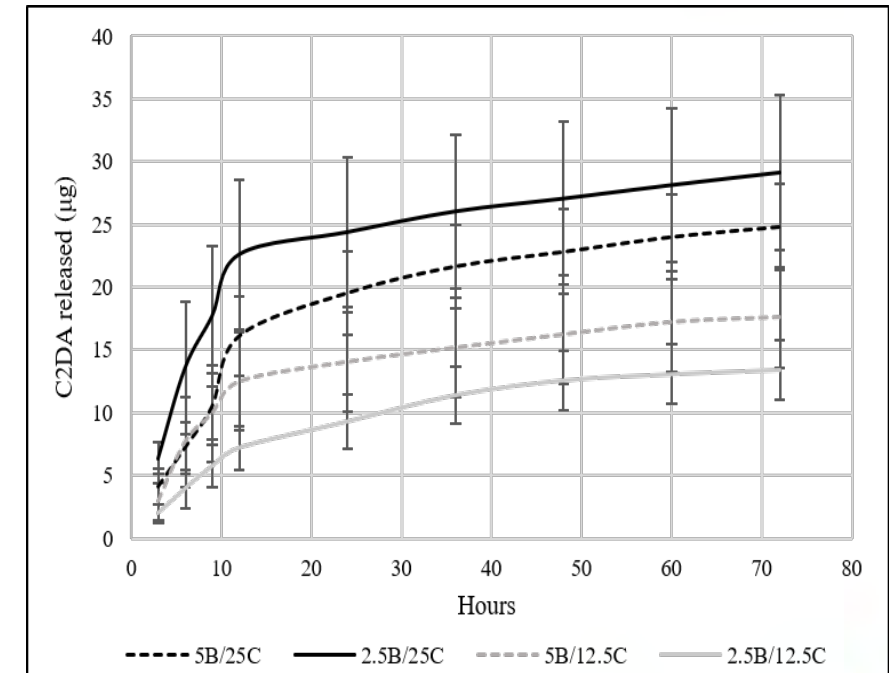
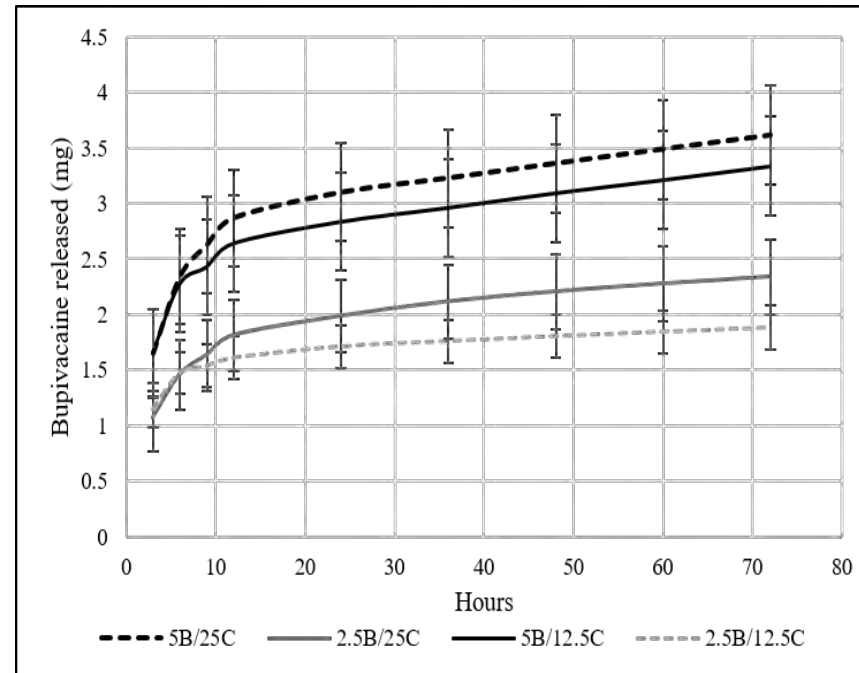
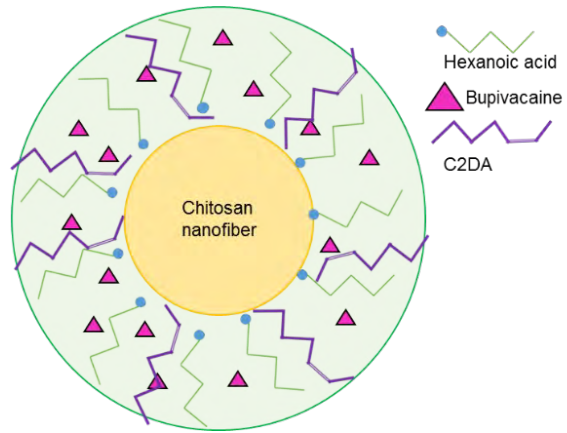


	Gauze	Hexanoic	Decanoic	2-decenoic
<i>S. aureus</i>				

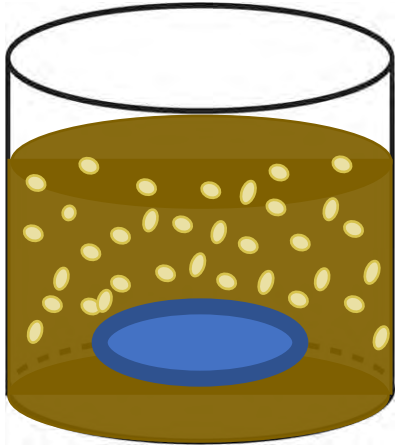
Release of hydrophobics from acylated membranes is controlled



Combining both C2DA and bupivacaine does not adversely affect release

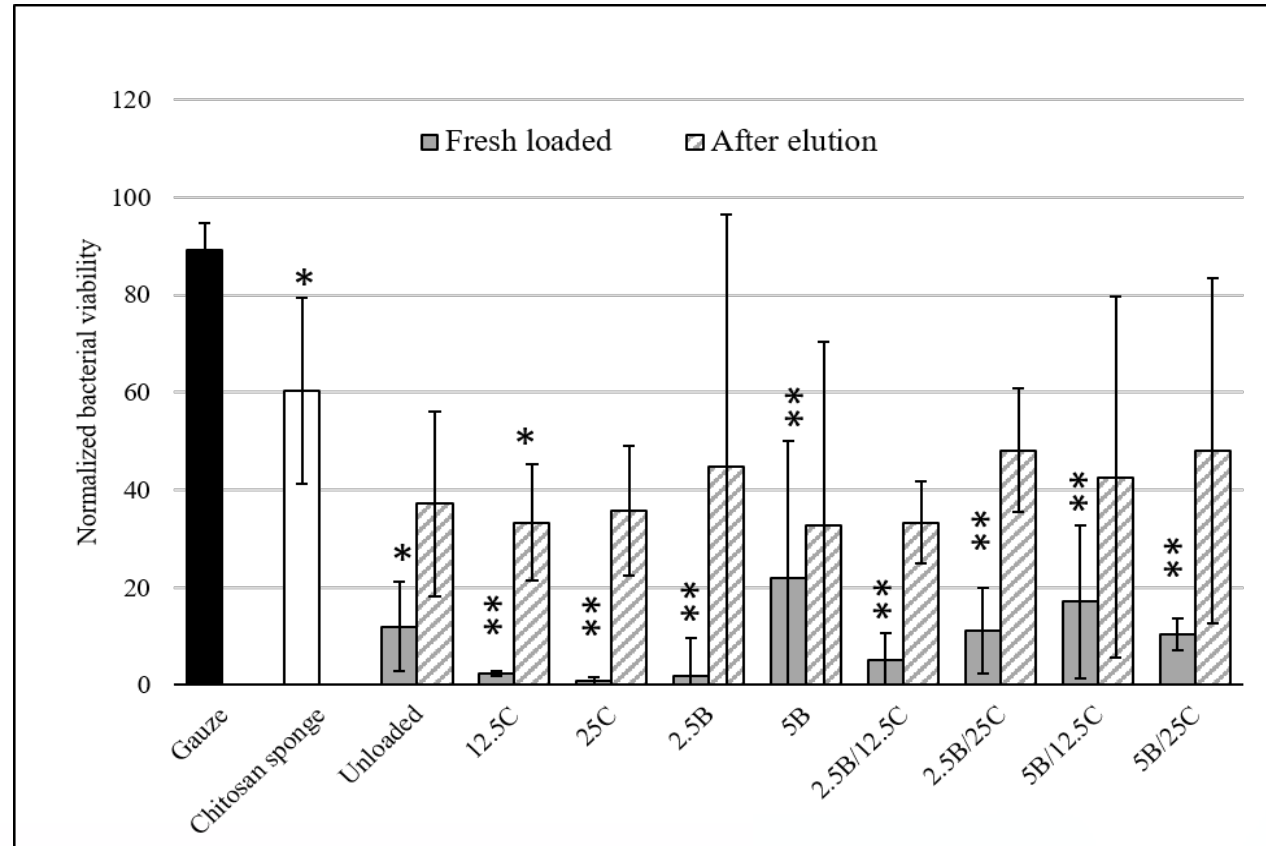


All fresh loaded membranes had significantly lower planktonic viability compared to controls

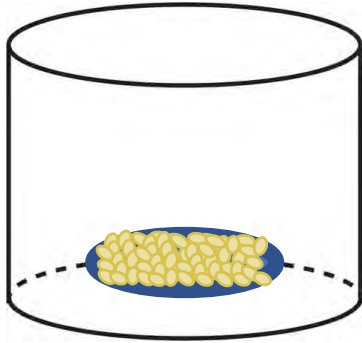


** = significantly lower bacterial viability than both the gauze and chitosan sponge

* = significantly lower bacterial viability than gauze only

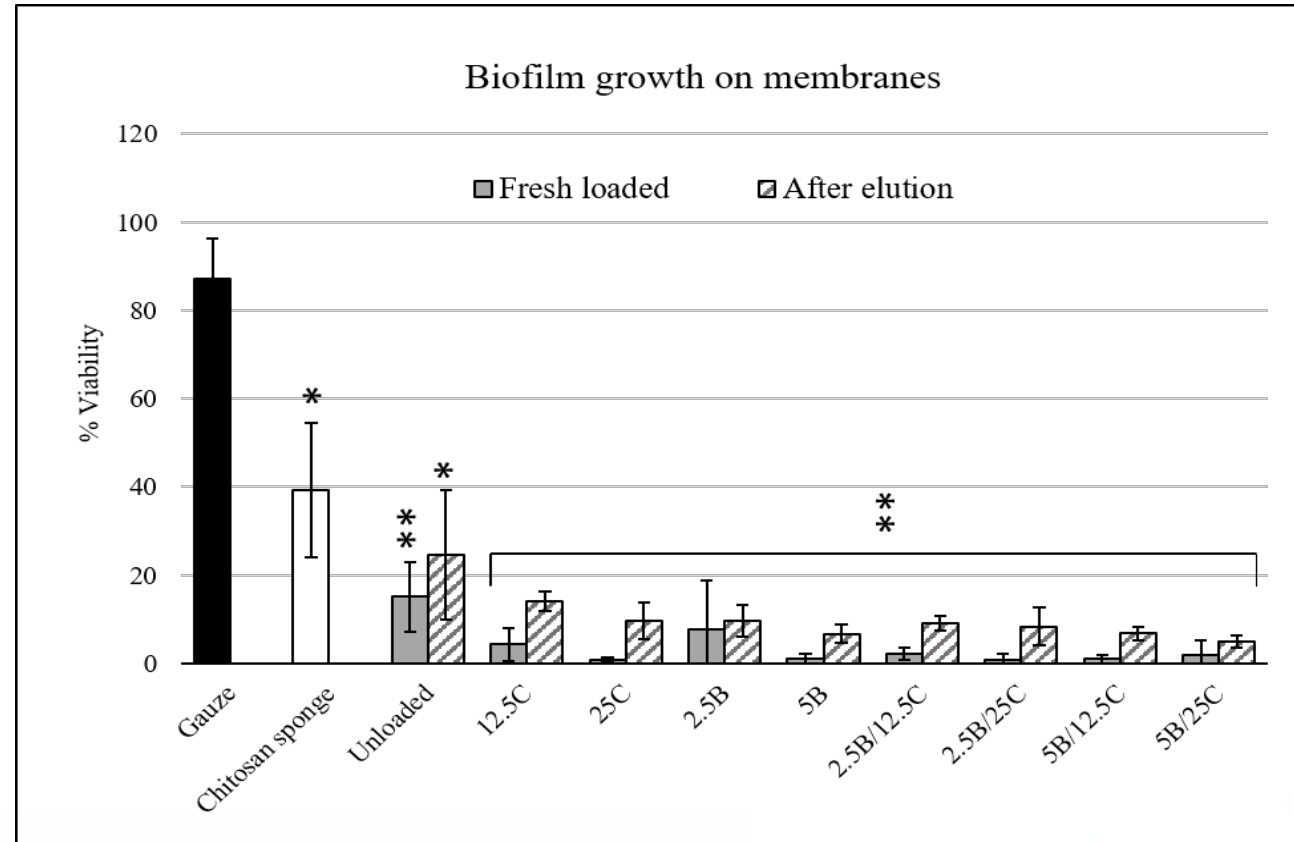


All loaded membranes had significantly lower biofilm growth on membrane compared to controls



** = significantly lower bacterial viability than both the gauze and chitosan sponge ($p < 0.05$)

* = significantly lower bacterial viability the gauze only ($p < 0.05$)

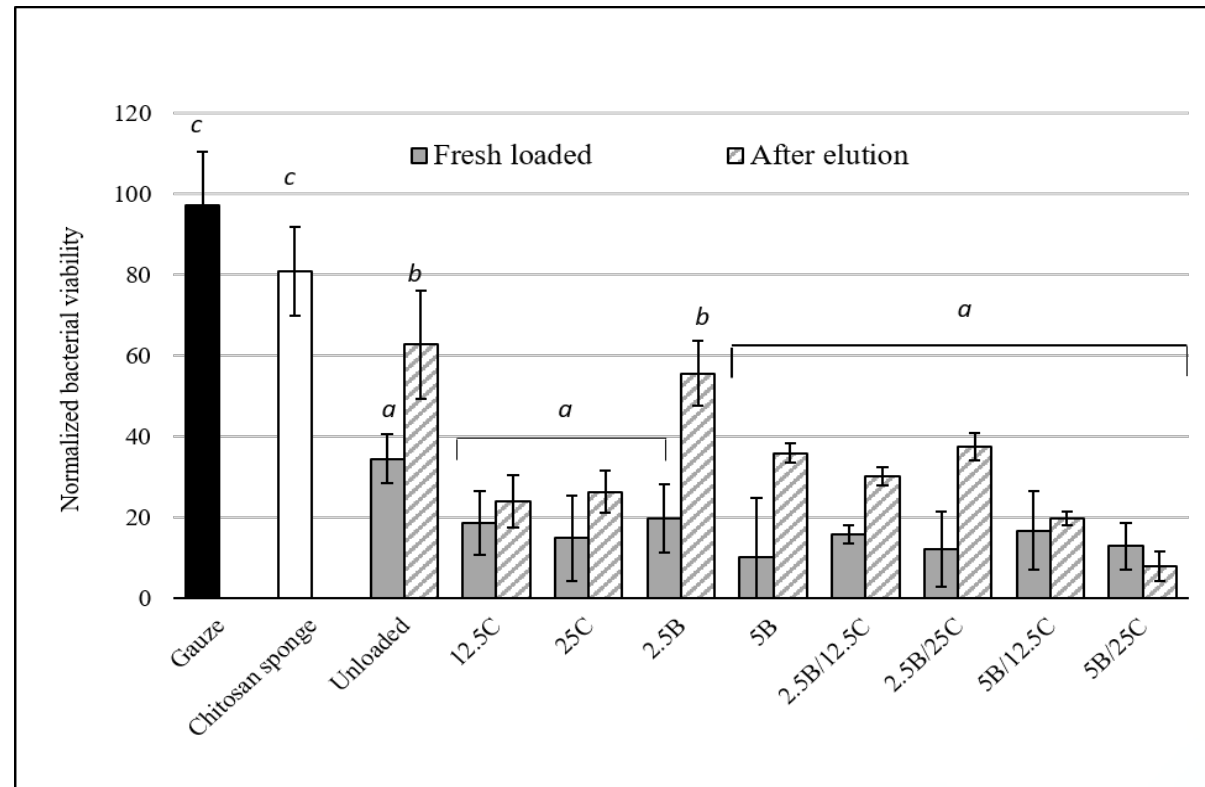


Released C2DA reduces biofilm off site of membrane

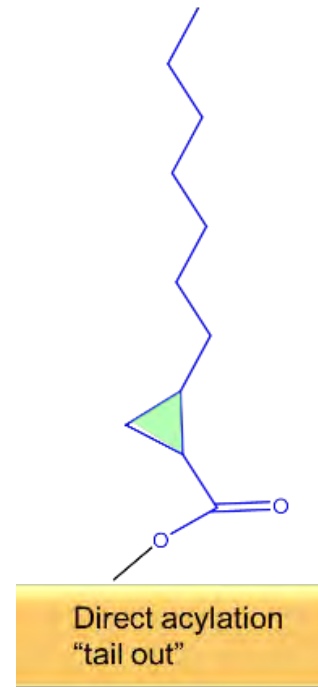
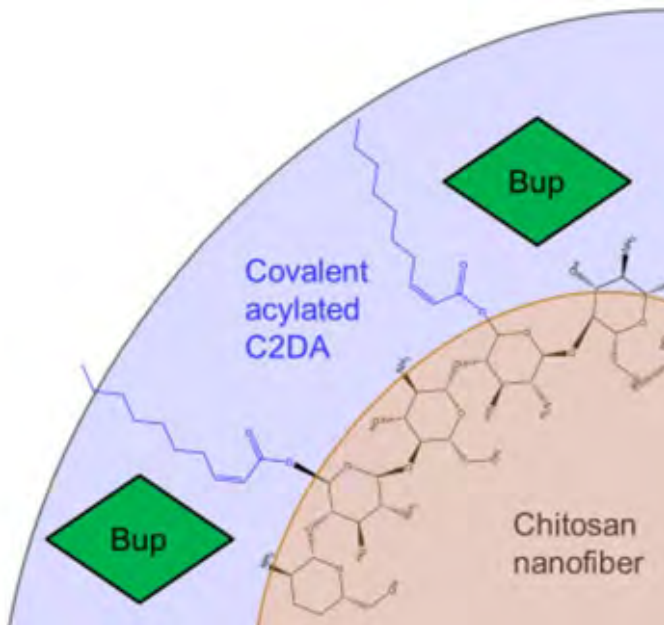


Biofilm growth surrounding membranes

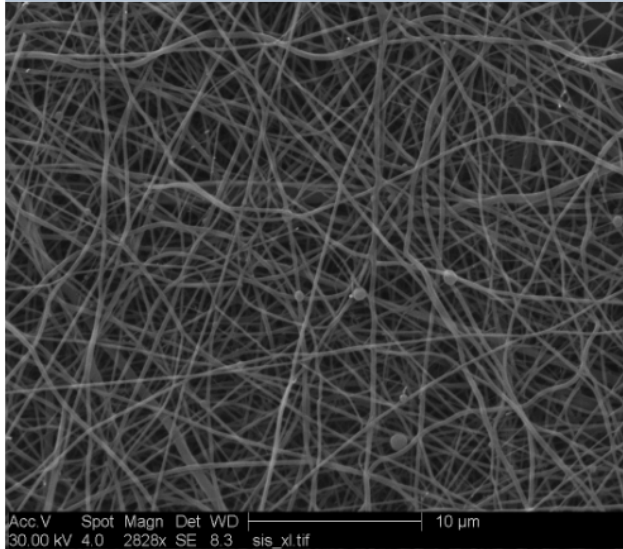
S. aureus biofilm in wells after 24 hour incubation with membranes



Next steps: Acylate with 2DA and analogs



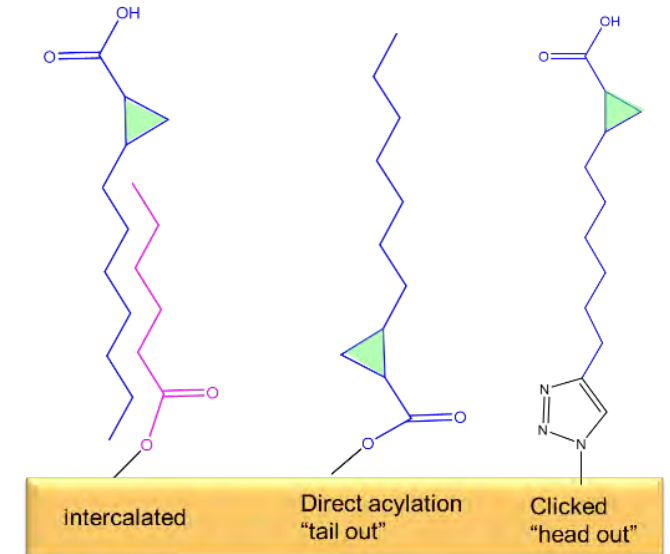
Outline



Nanofibers

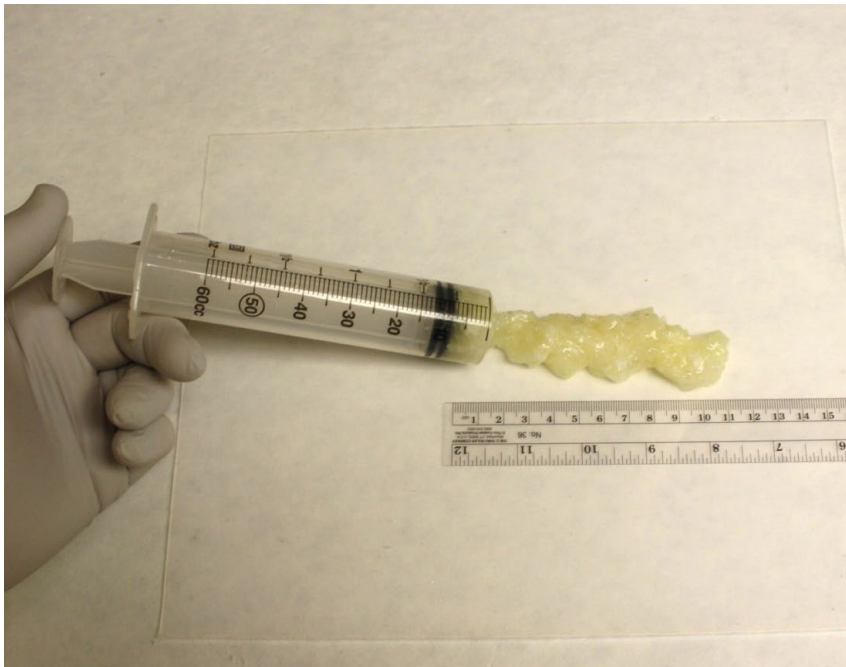


Injectable
paste

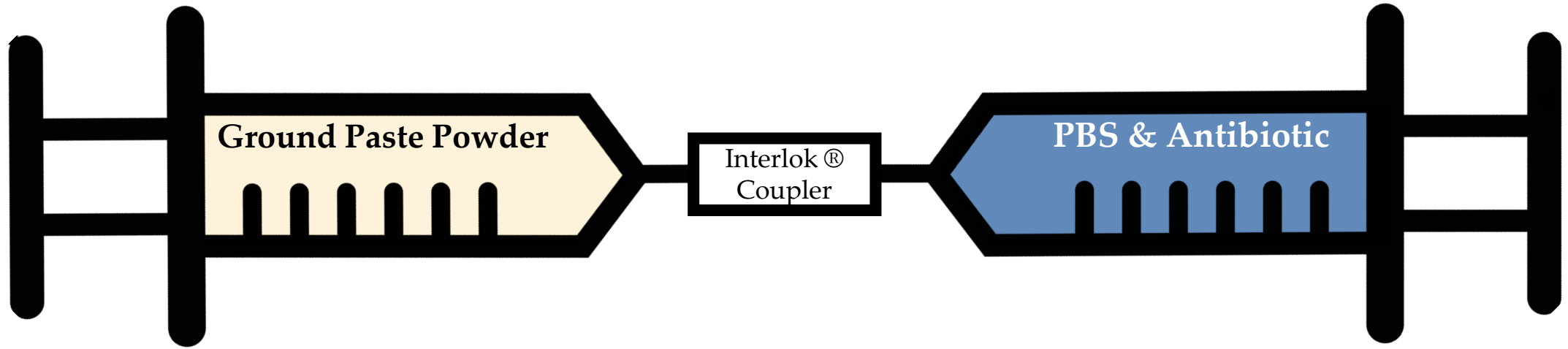


Material
functionalization

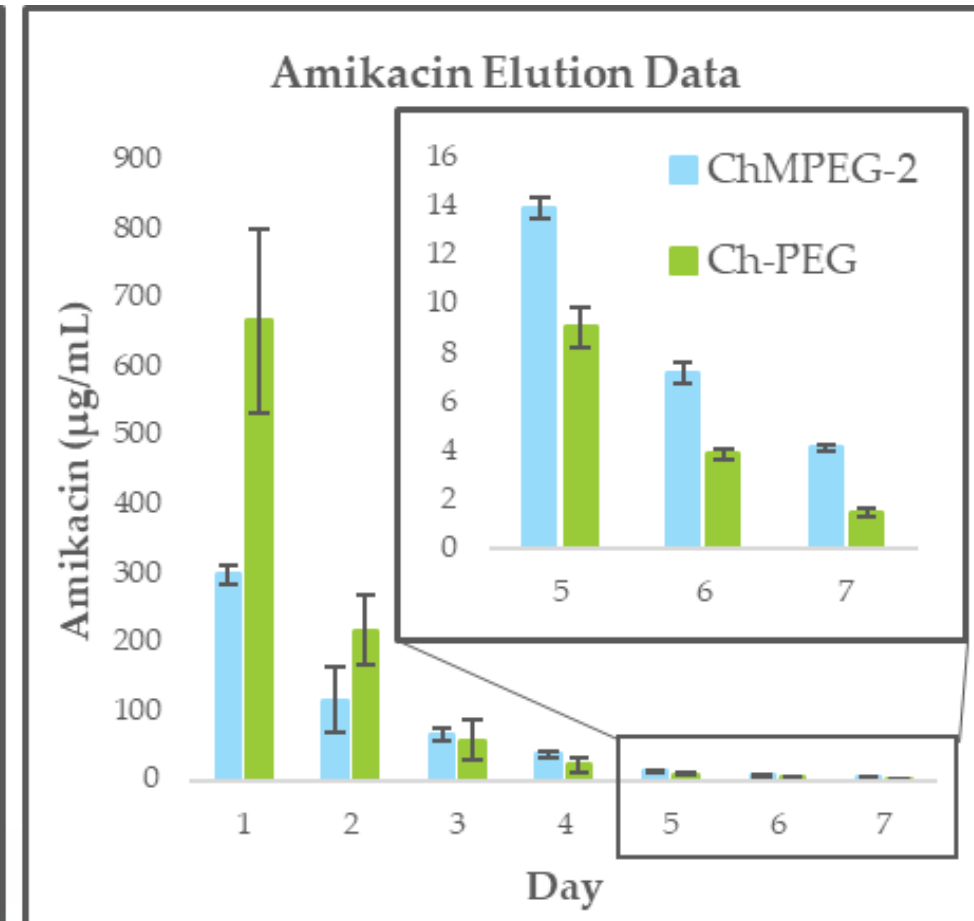
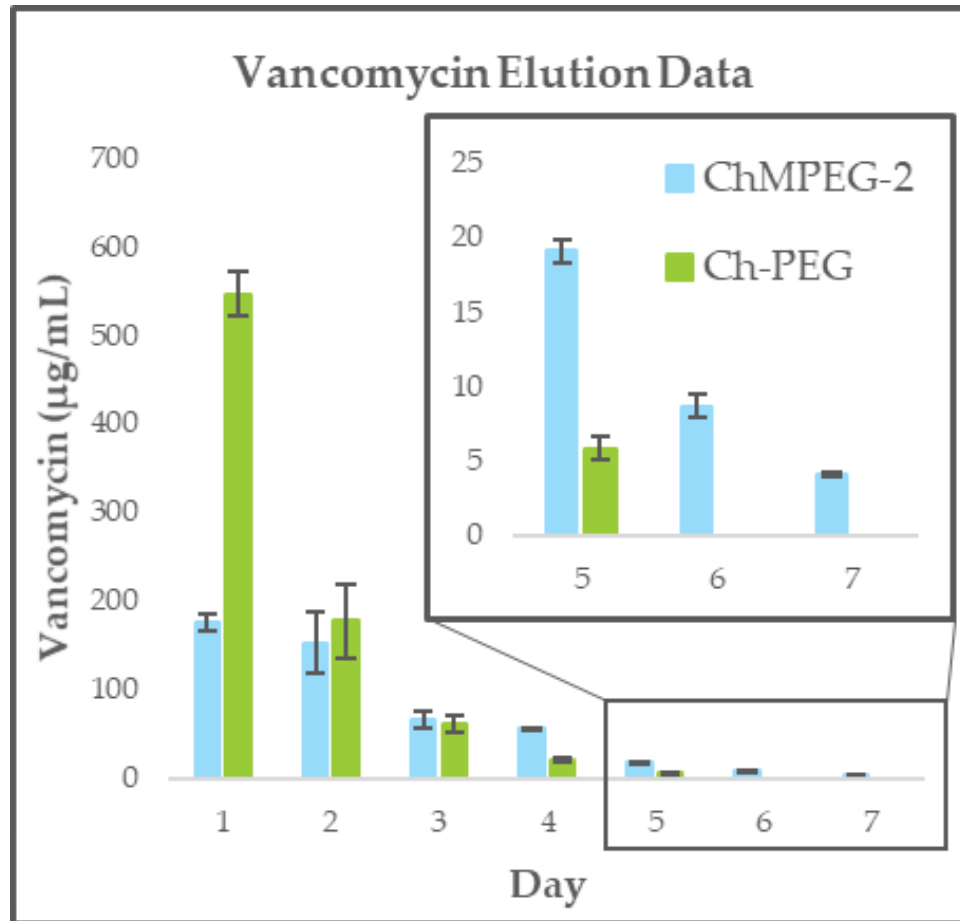
Injectable pastes have advantages for complex orthopaedic defects



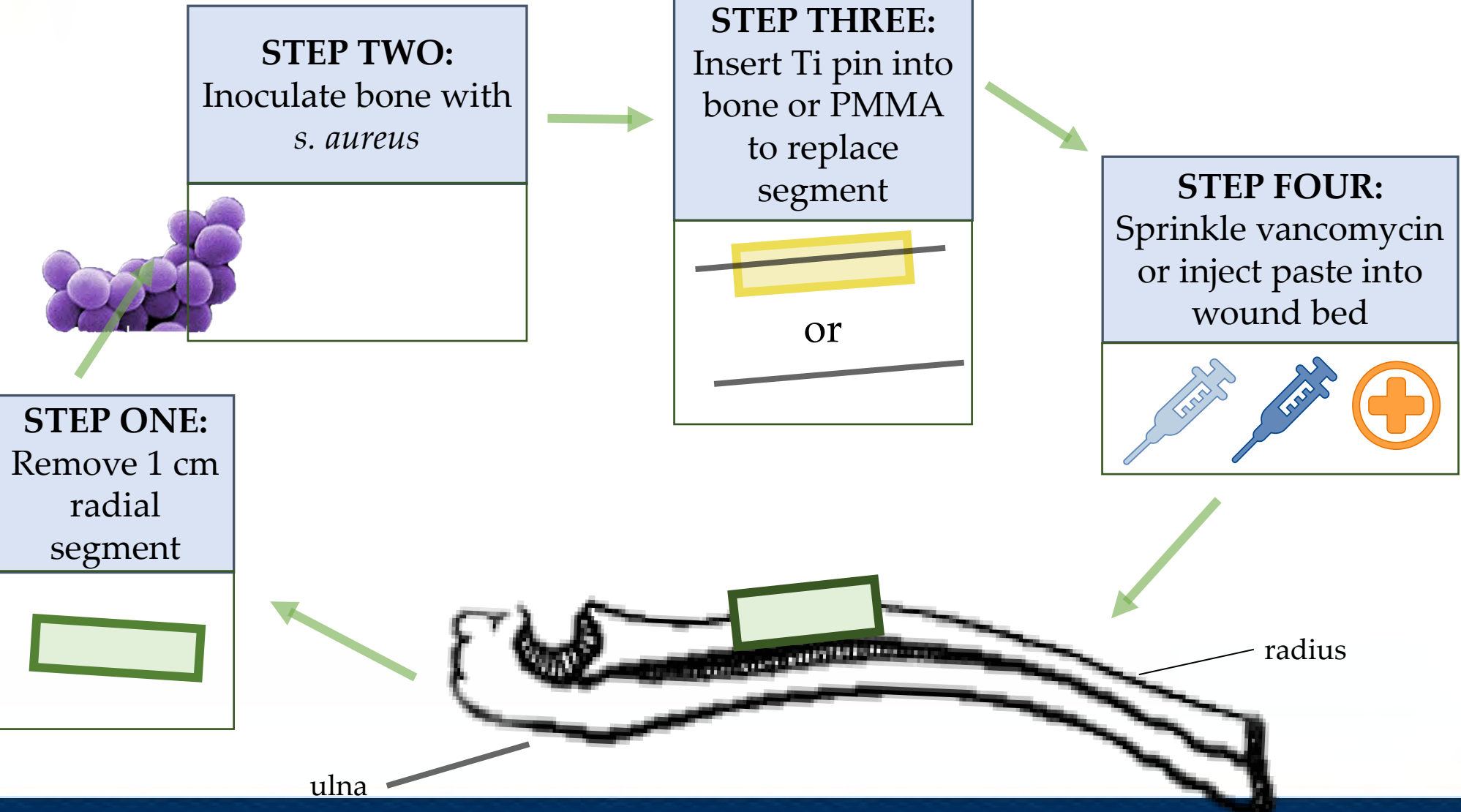
Paste Mixing Method



Chitosan-mannitol paste has been shown to deliver hydrophilic antimicrobials

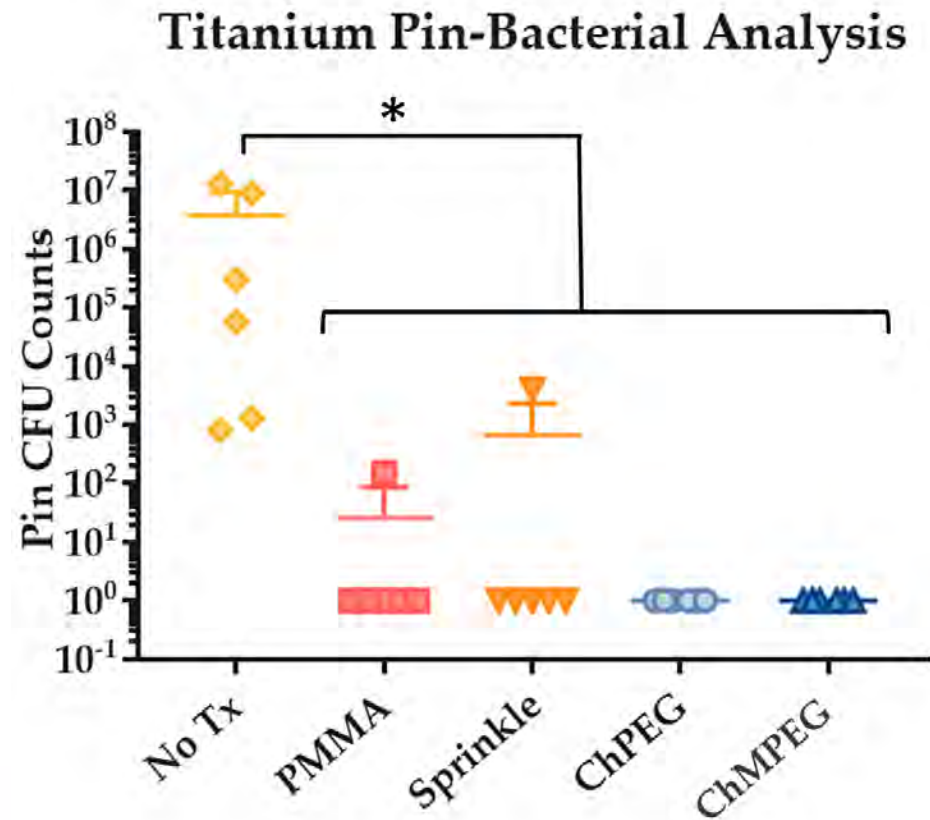


Osteomyelitis Rabbit Model



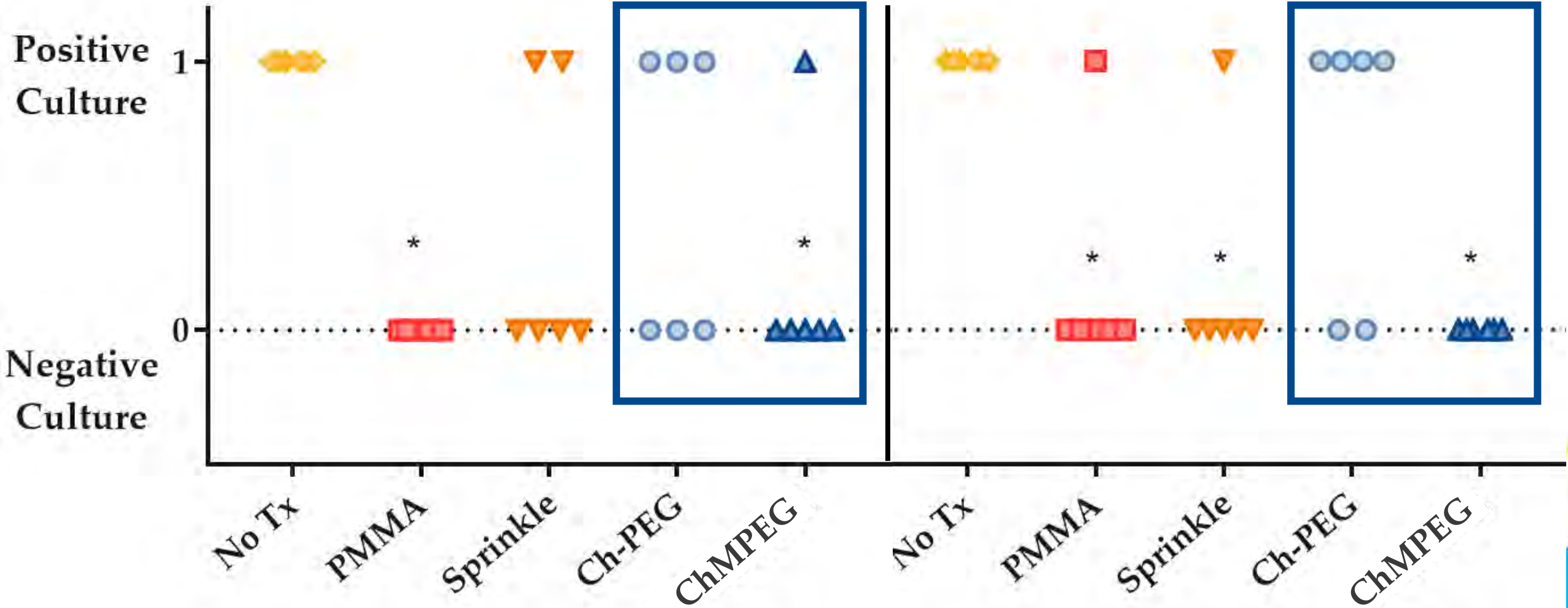
KEY	
	1 cm radial segment
	PMMA segment
	Ti Wire
	Ch-PEG paste
	ChMPEG-2 paste
	Vancomycin sprinkle

Paste groups capable of preventing biofilm formation on Titanium Pin

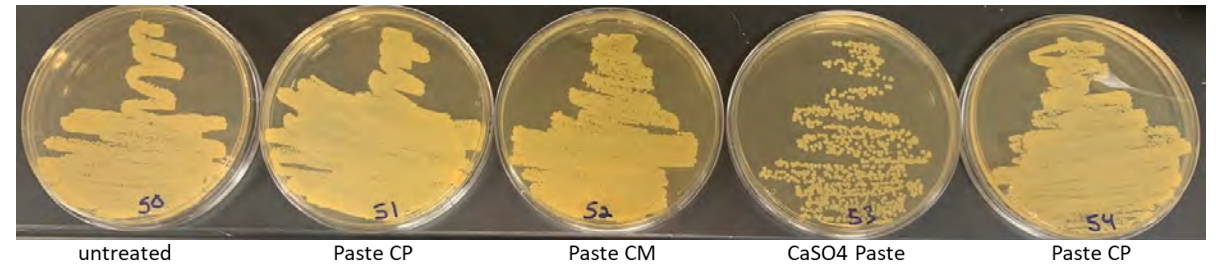
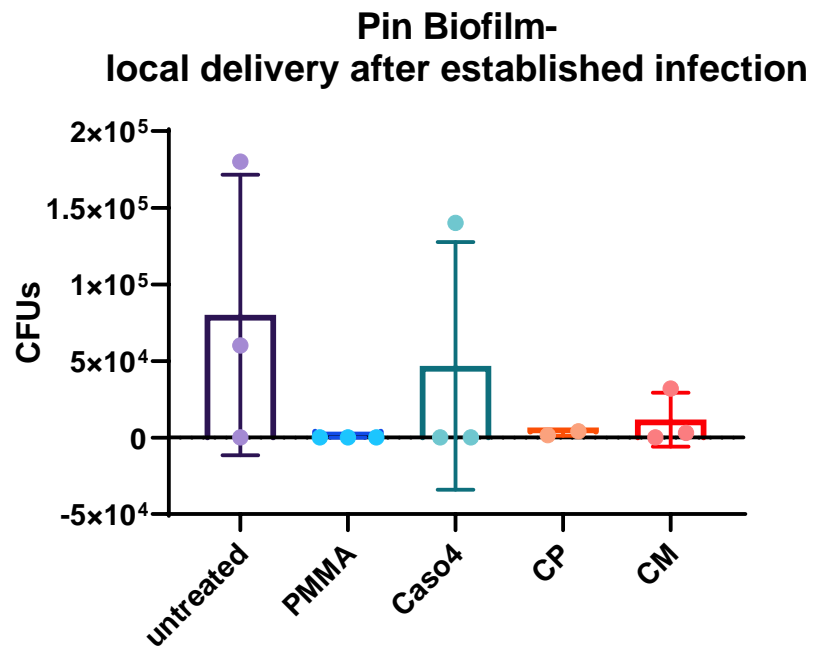


Bone- Culture Swab

Soft Tissue - Culture Swab

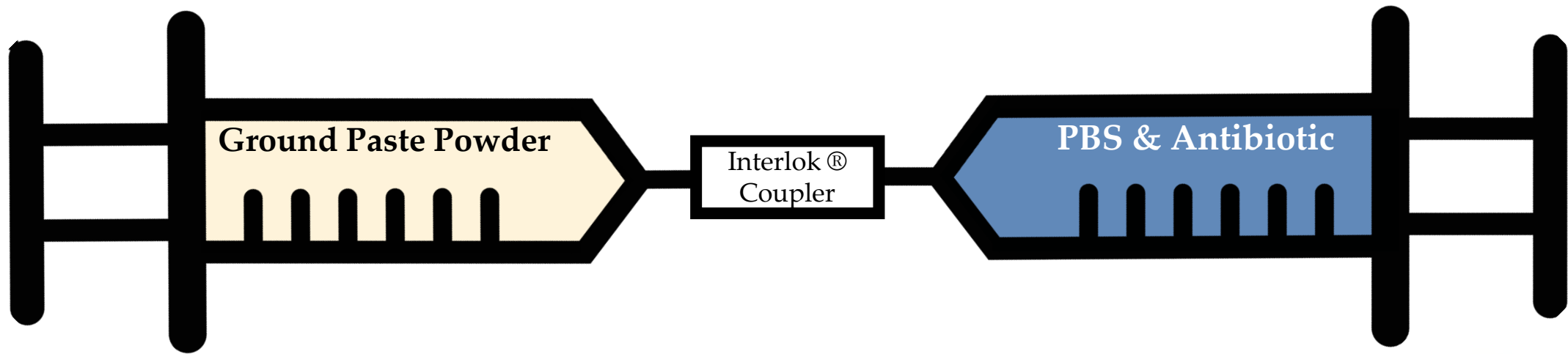


Established infection presents more of a challenge

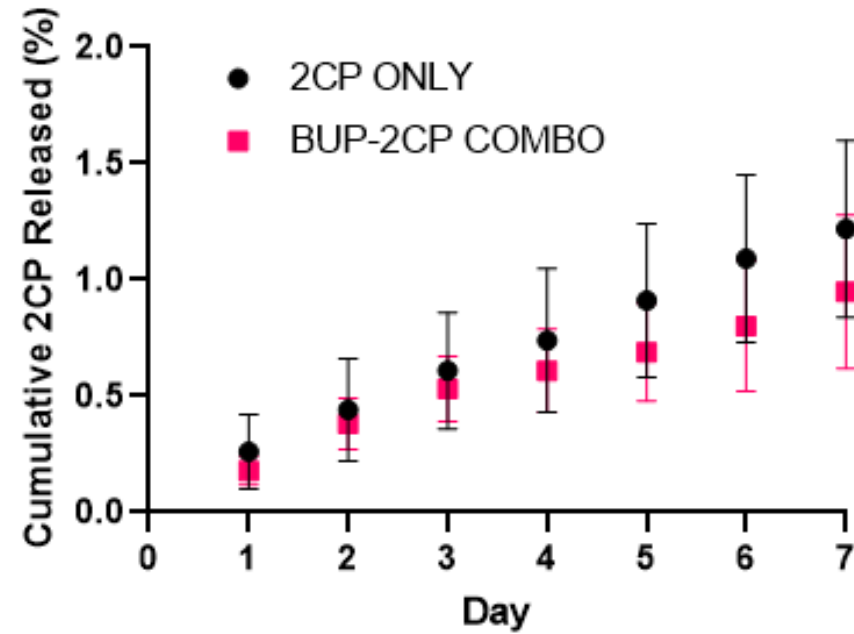
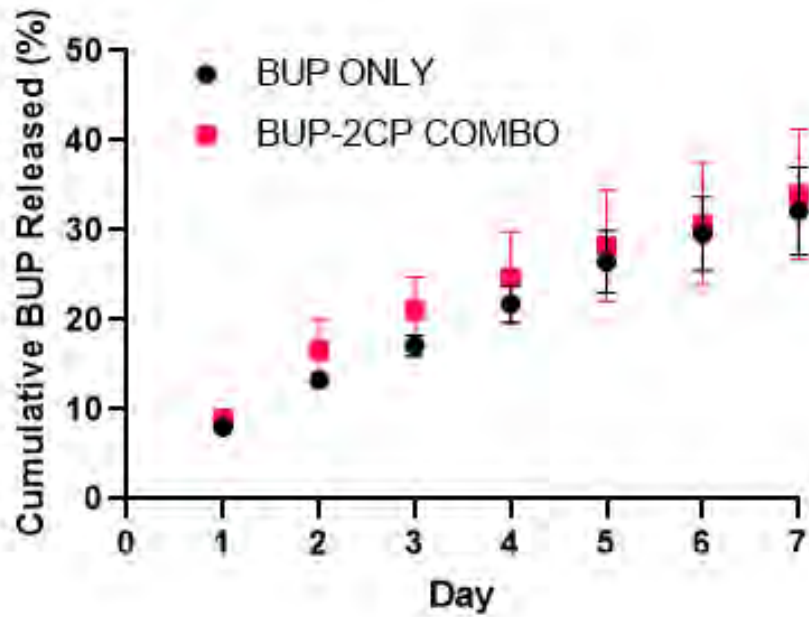


Bacteria still present in bone

Hydrophobic molecules are difficult to load using these methods

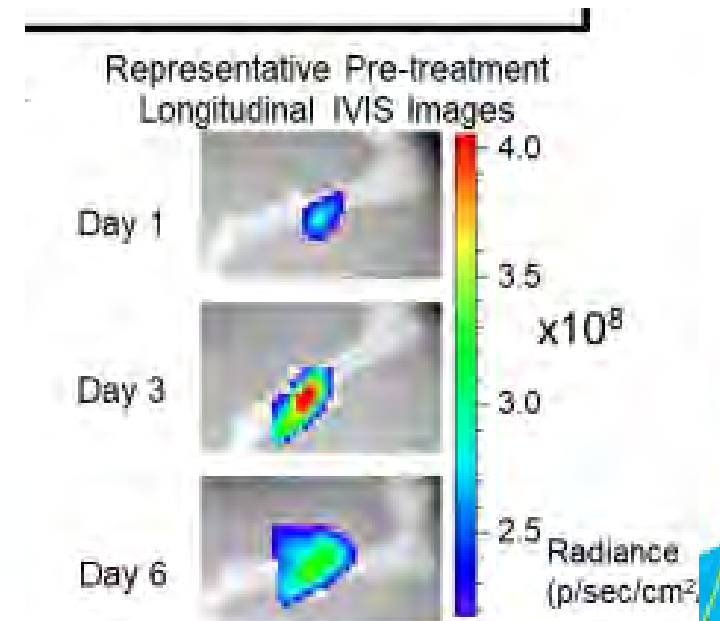
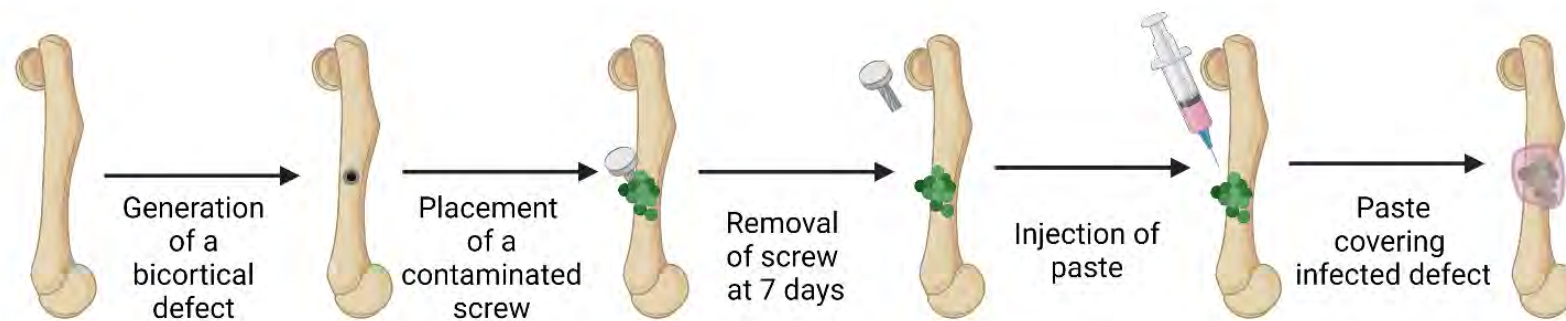


Bupivacaine elutes over 7 days, but most 2CP is retained

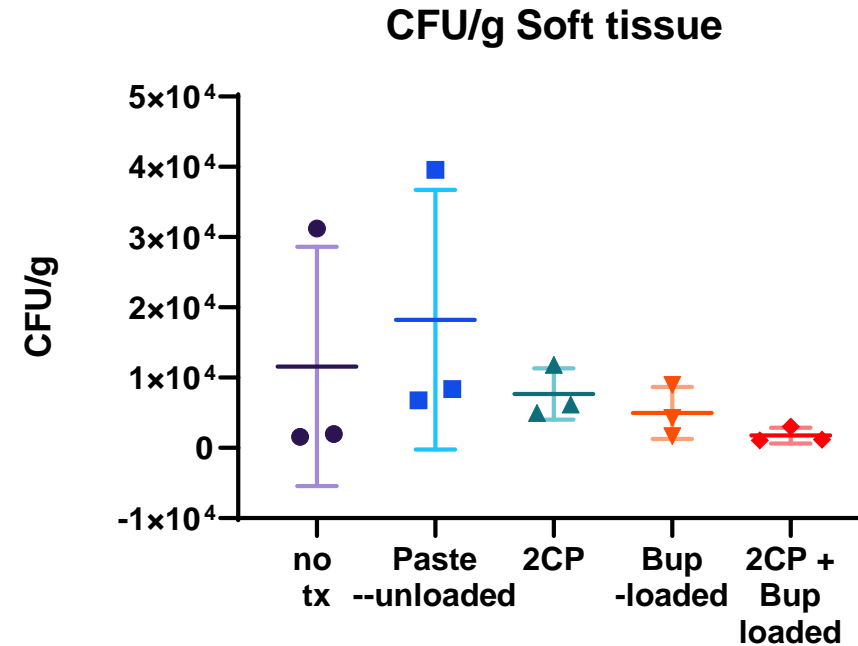
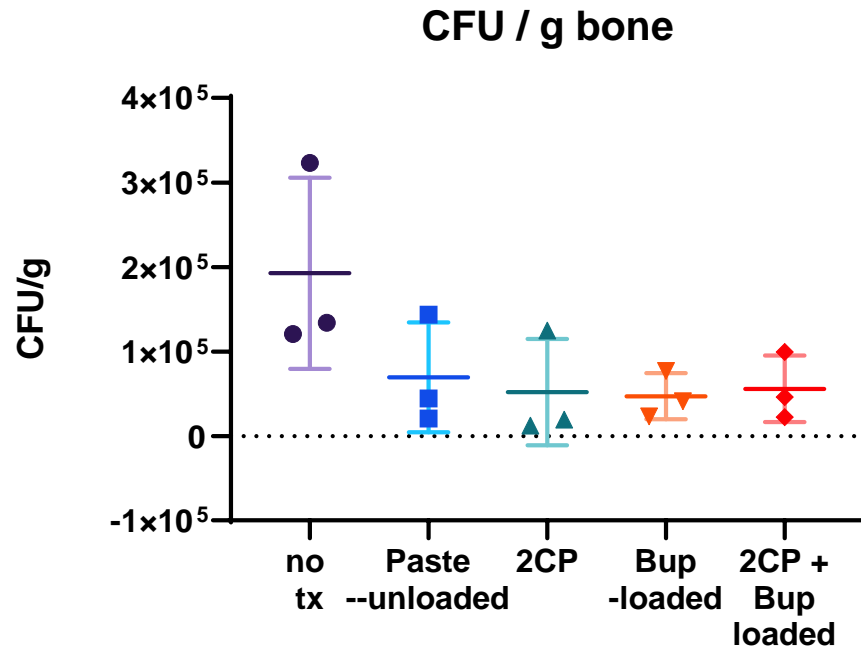


Preliminary animal study: methods

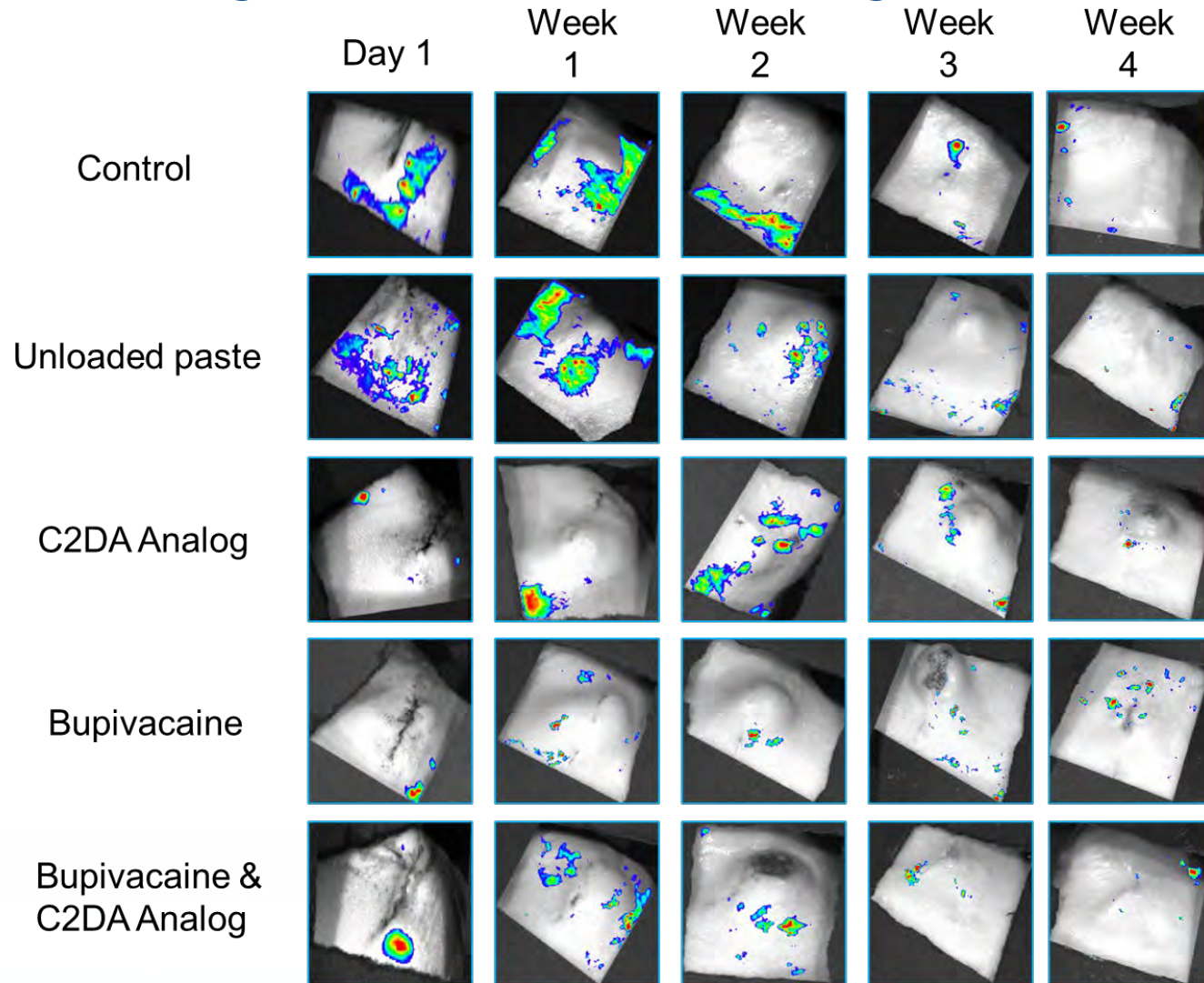
Animal	Groups	Duration	Process
Sprague Dawley Female Rats	5 (No treatment, material control, Bupivacaine loaded, 2CP loaded, and 2CP & Bupivacaine loaded)	28 days	Create infection, Remove infected hardware after 1 week; apply treatment, Euthanize and evaluate after 21 days



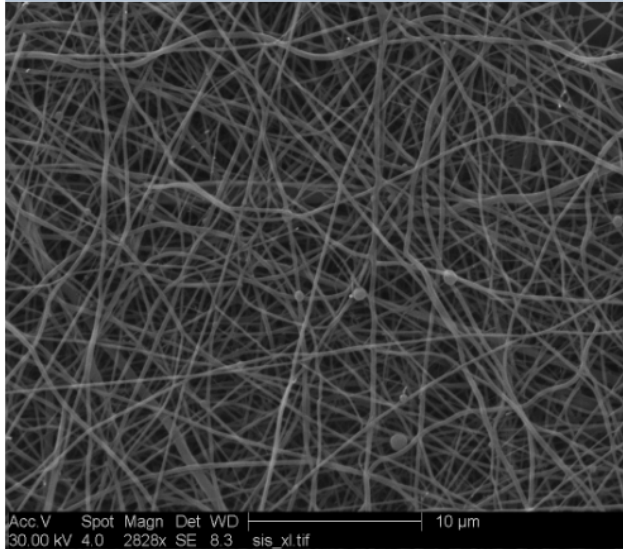
Preliminary animal study: CFU counts



Preliminary animal study: IVIS



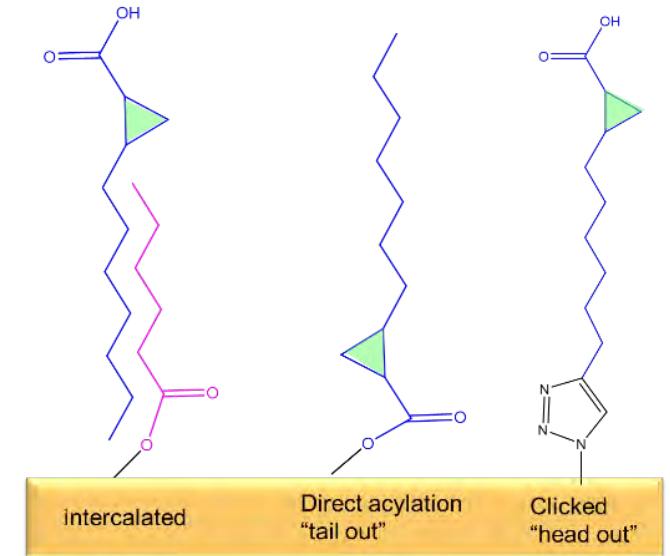
Outline



Nanofibers

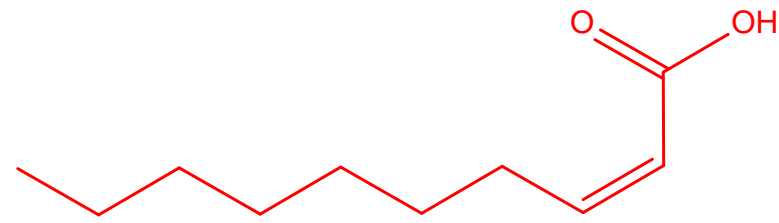


Injectable
paste

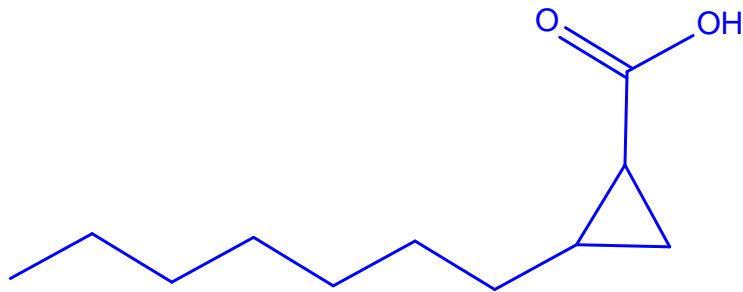


Material
functionalization

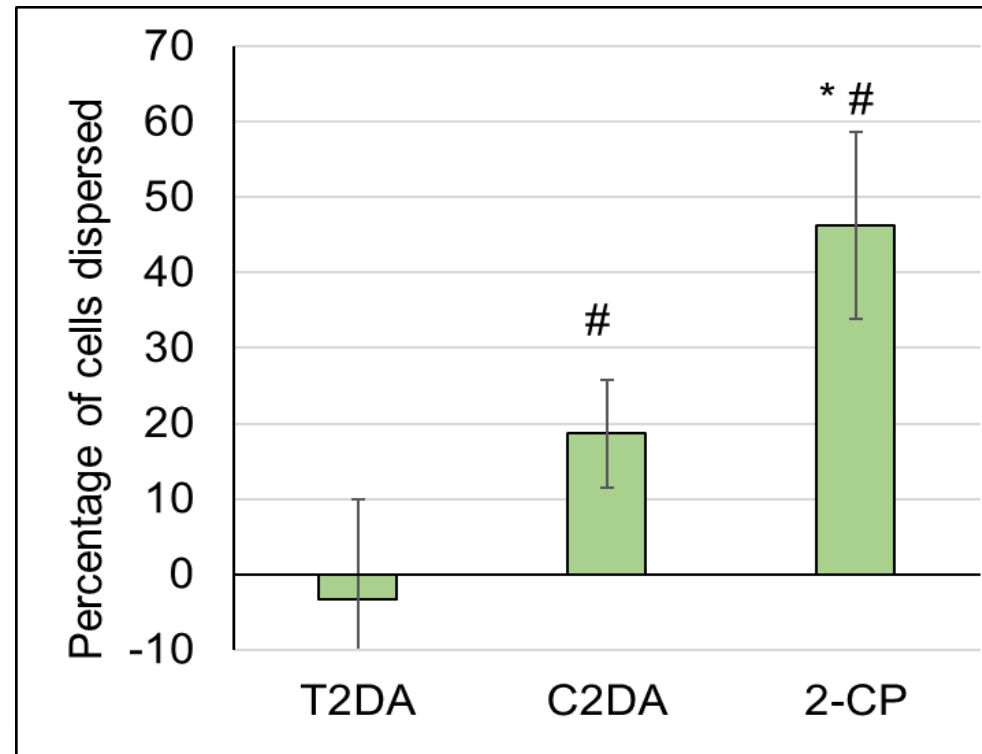
2-Heptylcyclopropane -1-carboxylic acid inhibits and disperses biofilm



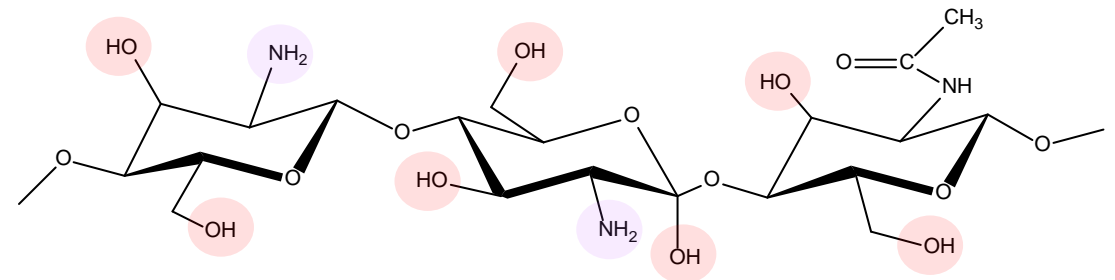
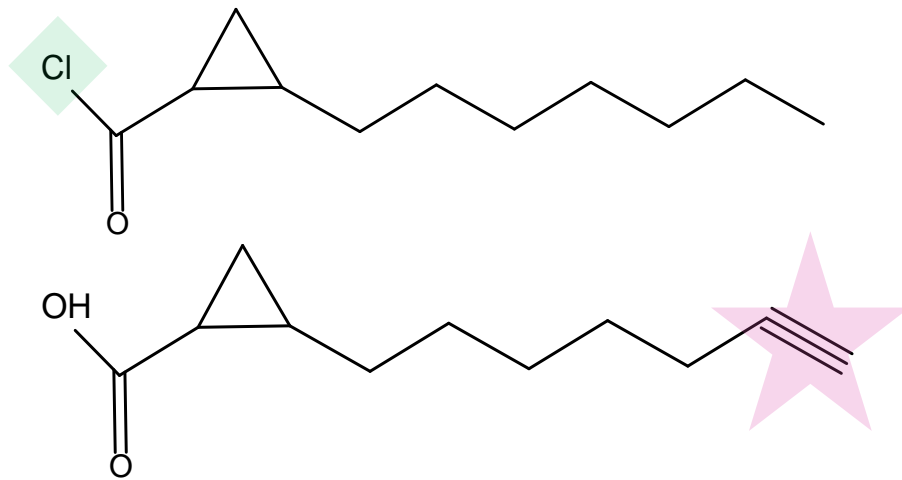
cis-2-decenoic acid



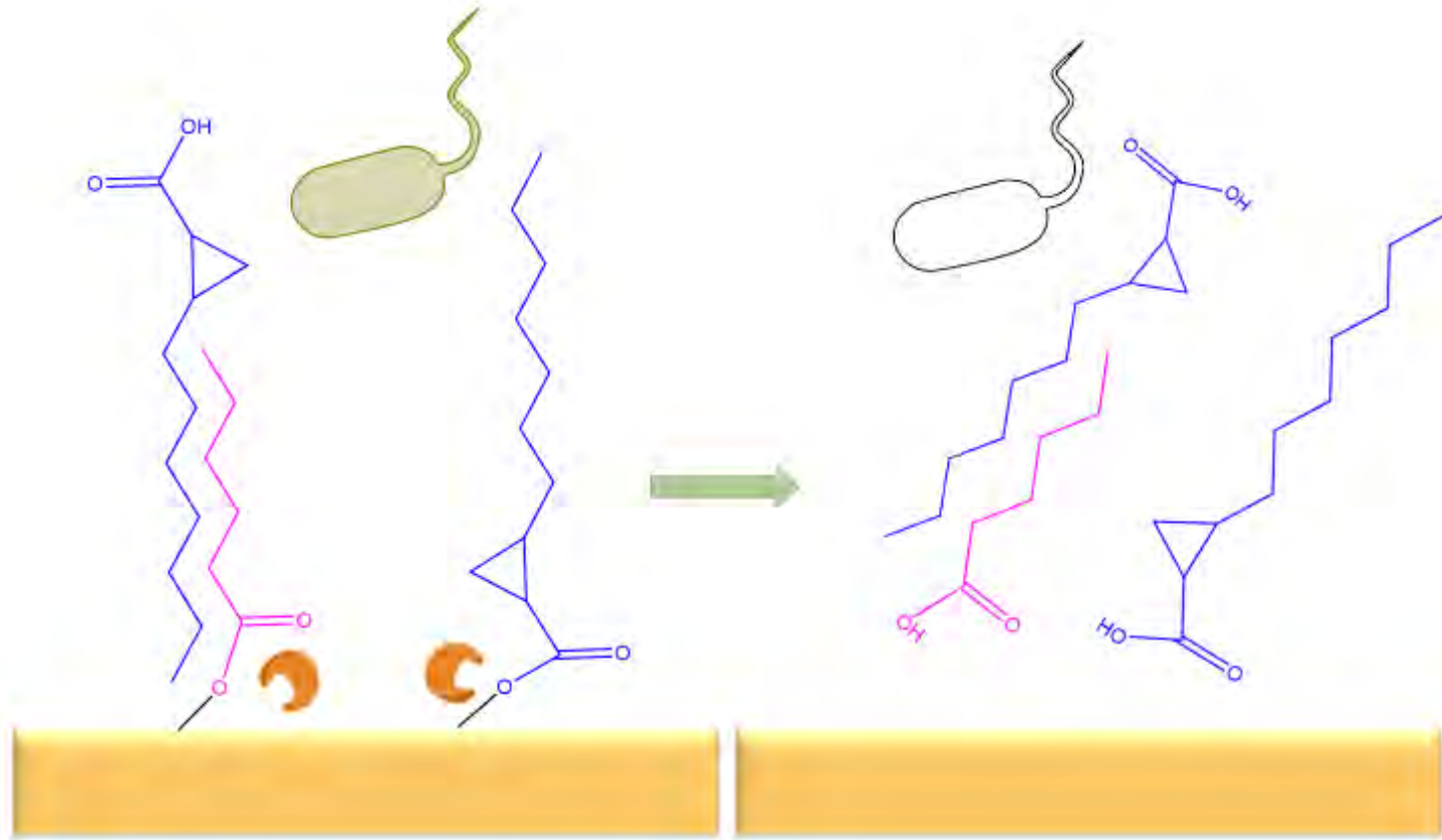
2-heptylcyclopropane-1-carboxylic acid



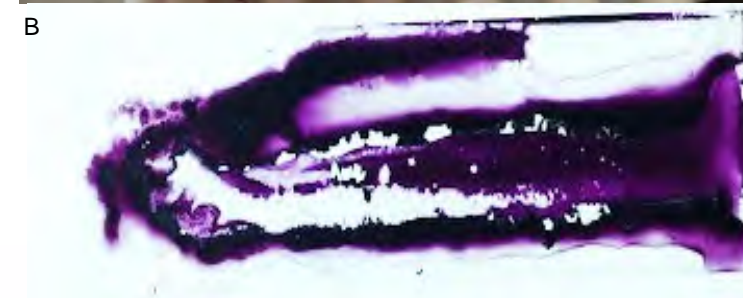
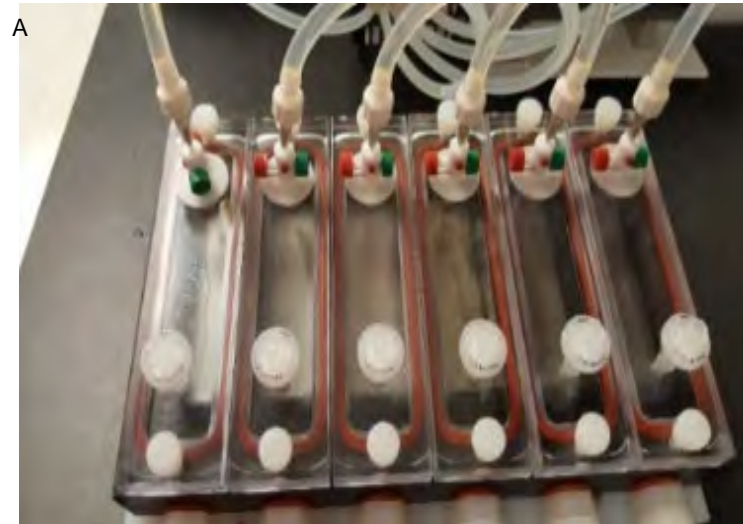
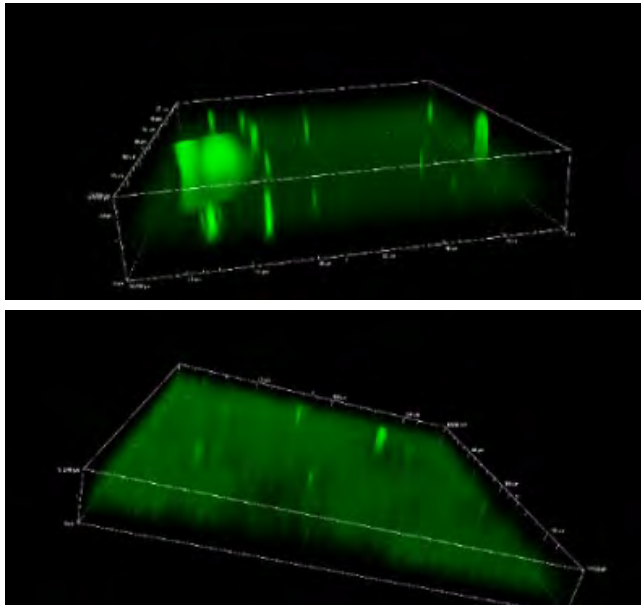
Reactive chlorides or alkynes could be used to covalently link 2CP



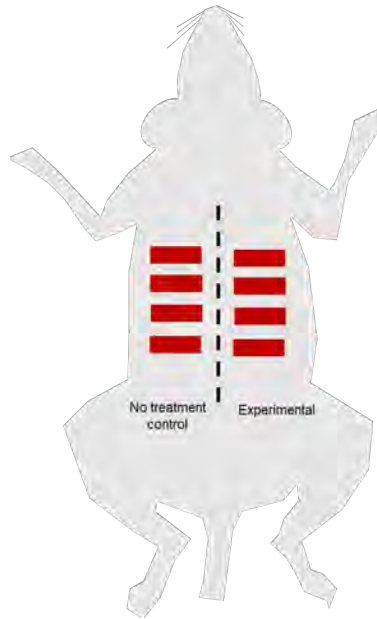
Cleavage of ester-linked molecules may be enhanced by bacterial enzymes



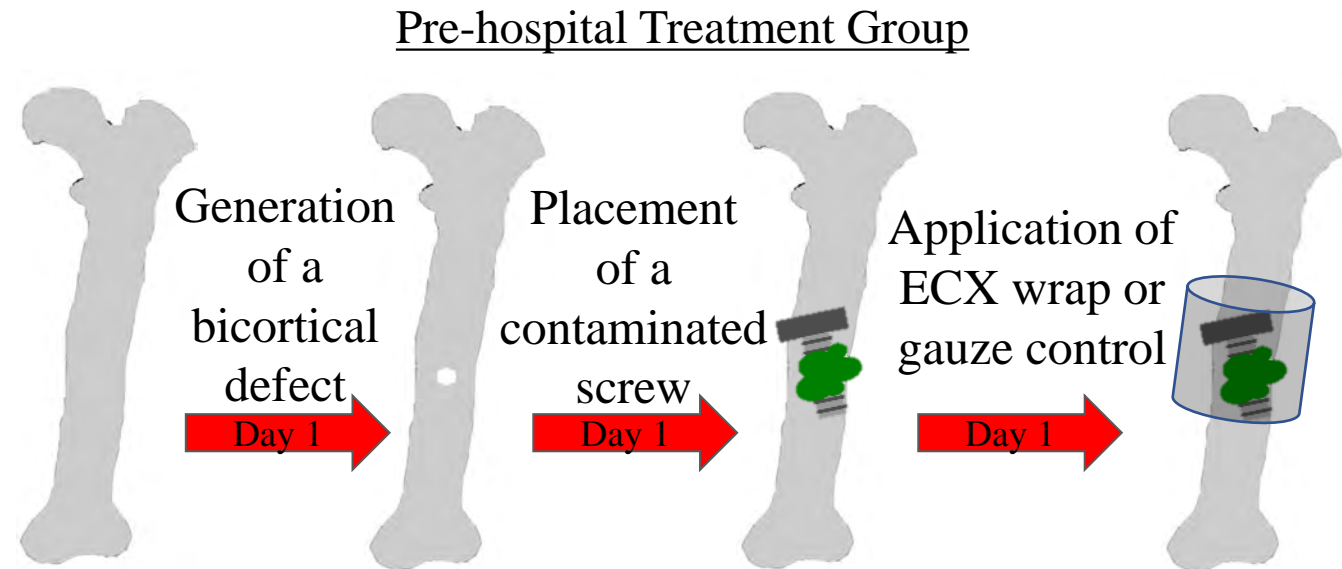
Next steps: evaluate antimicrobial properties



Next steps: evaluate materials in infected wound models



Comb scald model





Opportunities for expanding research



THE UNIVERSITY OF
MEMPHIS

Project grants

Major system/theme



TENNESSEE INSTITUTE OF
REGENERATIVE MEDICINE

Disease 1

Disease 2

Disease 3

Disease 4

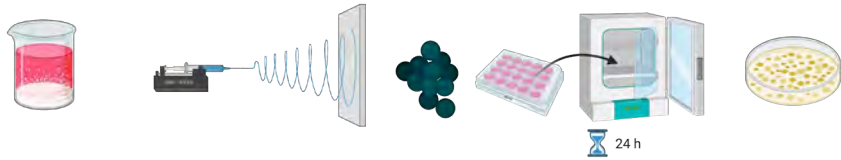
Foundational/ Basic

Therapeutic discovery/
application

Imaging/
modeling

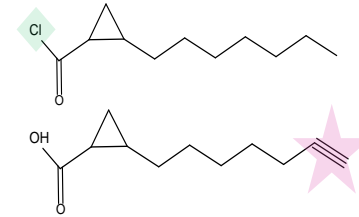
Clinical/
translation

What we can do.....and what we need

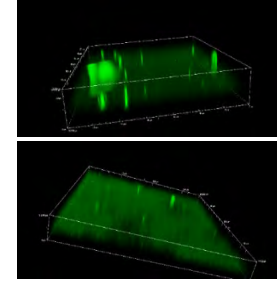


Chemical synthesis
and material
fabrication

Microbiological
evaluation



Drug/therapeutic
discovery



Detection



Biocompatibility



Data analysis/
computation



Smart materials/
translation

In summary, modified chitosan materials may be advantageous for infection prevention

