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How Does Novel Nanospring Patterns Impact Bacterial Growth?

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INTRODUCTION

Previous research studying the antibacterial effects of nanopatterned surfaces has found evidence showing that these patterns can reduce or increase bacterial colonization and pathogen growth. The topographical surface patterns may be applied to future medical technology and have the potential to reduce infections and bacterial growth without the use of antibacterial products. Although commercial antibiotics are commonly used to kill pathogens, the overuse of these products has resulted in bacterial resistance, which can pose a serious health threat.

Antifouling and bactericidal capabilities occur naturally on the surfaces of various plants, insect wings, and the skin of reptiles and sharks and may provide an effective solution for killing bacteria or preventing their growth without the need for antibacterial products (M. Mischalska et al). Synthetic nanopatterned surfaces may similarly retard or encourage bacterial attachment and growth.

We have collaborated with Dave McIlroy's lab in the Oklahoma State University Department of Physics to test the impact of novel silicon nanosprings on bacterial colonization and growth. These nanosprings are on the same scale as biologically patterned structures but are distinct and have never been tested for their effect on microbial growth. Thus, testing nanospring-covered surfaces for bacterial growth was an ideal opportunity to test the ability and efficiency of novel nanospring patterns to retard or encourage bacterial colonization and growth.

METHODS

To test bacterial colonization on nanosprings, we inoculate sterile control and nanospring-covered substrates with *Pseudomonas aeruginosa*, an opportunistic human pathogen. We then incubate the substrate to allow bacterial growth. We assess attachment and growth directly, via microscopy, and indirectly by dislodging the attached bacteria, serially diluting them, and growing them on agar plates.

•Innoculate plate with LB media and bacteria
•Place sterile substrate in media
•Incubate for 24 hours
•Break up the pellicle on the surface of the media
•Vortex substrate with LB media and tiny glass beads to disrupt biofilm growth (Werner et al., 2019)
•Serial dilution of media and 24 hour incubation
•Count the colony forming units

RESULTS

Data

Data: Test #1

Dilution	REF A	RANS	REF B	VANS 60
-1	lawn	lawn	lawn	lawn
-2	lawn	lawn	lawn	lawn
-3	lawn	lawn	lawn	lawn
-4	lawn	97	lawn	lawn
-5	71	9	29	51
-6	12	0	3	6

Data: Test #2

Dilution	REF VANS	VANS 30
-3	lawn	lawn
-4	87	38
-5	4	3
-6	3	1

Data: Test #3 (Blind Test)

Dilution	A (RANS)	B (RANS)	C (Control)	D (RANS)	E (Control)
-3	lawn	lawn	lawn	lawn	lawn
-4	lawn	lawn	lawn	152	96
-5	26	35	35	17	25
-6	6	2	2	3	2

Experimental Protocol Validity

Does vortexing with beads result in more CFU?

-If yes, then beads are important to protocol

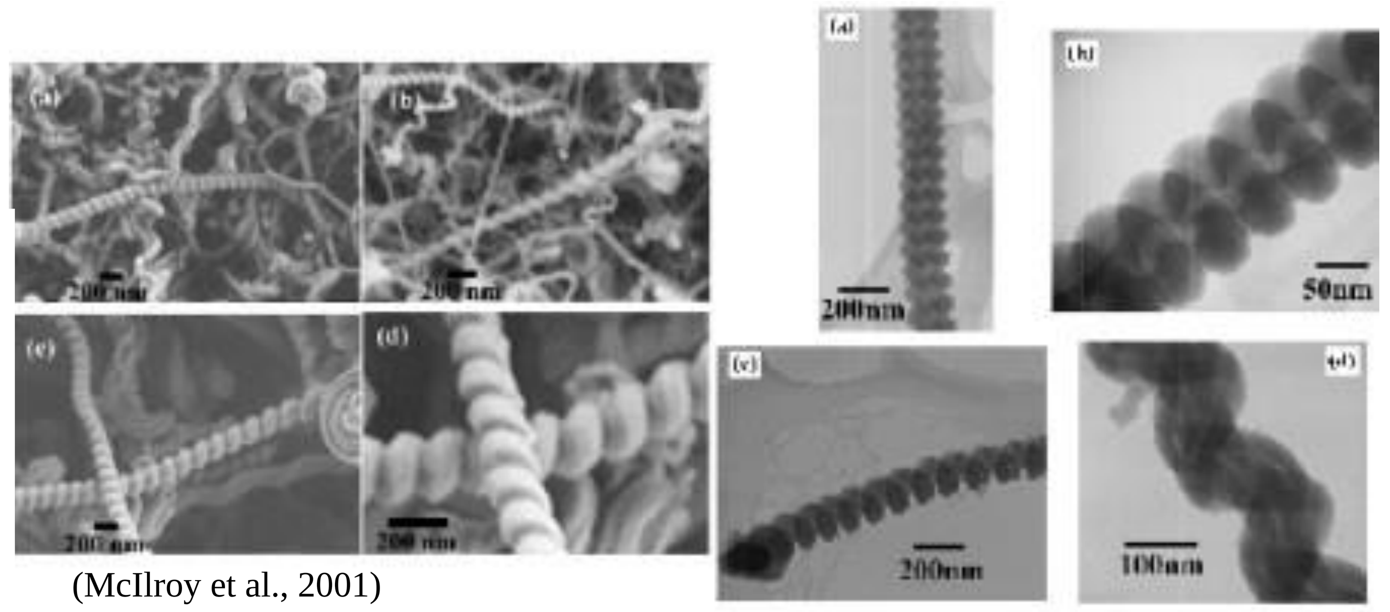
-If no, then no risk of damage to substrates

Does vortexing with beads damage nanospring surface?

-If yes, then substrates can only be used once

-If no, then substates can be used multiple times

Photos of Individual Silica Nanosprings



(McIlroy et al., 2001)

Pseudomonas aeruginosa

- Opportunistic human pathogen
- Creates biofilms
- Increasingly antibiotic resistant

RANS v. VANS

RANS = Randomly Arranged Nano Springs

VANS = Vertically Arranged Nano Springs

•30 minute, 60 minute and 90 minute samples

CONCLUSION

At the time of this presentation, I do not possess enough data to make a definite conclusion on the impacts of nanosprings on bacterial growth, but I have made significant progress in determining the most effective experimental protocol. Further research may include testing additional species of bacteria and/or human stem cells.

Studying the nanospring substrates for the retardation or absence of *Pseudomonas aeruginosa* bacteria's biofilm formation will yield instructive scientific applications. Although commercial antibiotics commonly serve to kill pathogens, the misuse of these products has resulted in bacterial resistance, posing a severe health threat. The topographical surface patterns on the nanospring substrates may be applied to future medical technology, reducing infections and bacterial growth without the administration of antibacterial products.

Alternative means of reducing bacterial growth and biofilm formation is increasingly important as many strains of bacteria resist traditional antibiotics. Assessing untested nano-patterned surfaces is one promising avenue for discovering new ways to prevent bacterial growth.

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ACKNOWLEDGEMENTS

Thank you to the Lew Wentz Foundation for funding this project and to Dr. Matthew Cabeen for his excellent mentorship