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TITLE: A Novel Bandage Contact Lens Against Resistant Fungal Infections with Ocular Drug Delivery

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14. ABSTRACT

Nanostructured surface topographies have been successfully fabricated on chitosan and synthetic polymer such as polymethyl methacrylate surfaces. Due to the large surface area, chitosan nanopillars would quickly lose water and shrink in size. The rate of water loss can be retarded by crosslinking the polymer. Genipin is chosen for this purpose and found to retain the shape of the nanopillars. However, the aspect ratio of nanoimprinted chitosan pillars was found to be around 1, which may be too small for effective antimicrobial activity. An alternative technique, electrohydrodynamic patterning, which has the potential for producing much higher aspect ratio pillars from polymers that cannot easily be imprinted, is being developed. Initial results show that extremely large aspect ratio nanofibers can be fabricated. The antibacterial and antifungal functions of these nanotopographic surfaces have been evaluated and found to be effective in certain size ranges against the fungal species *Fusarium* and *Aspergillus*, and the bacterium *Pseudomonas Aeruginosa*. A biocomposite material of chitin:chitosan 1:9 ratio was fabricated and satisfactory transparency was established. The release rate of an antifungal drug natamycin from polyvinyl alcohol, chitosan, and poly(lactic-co-glycolic acid) as the matrices, and poly(2-hydroxyethyl methacrylate) as the outer layer were all studied. Certain compositions were found to meet the target of 10 days for complete release. Finally, a prototype drug releasing contact lens was designed and built, and the drug release profile has been found to be satisfactory.

15. SUBJECT TERMS

Bandage; contact lens; antifungal drug delivery; antibacterial surface; biomimetic nanoarchitecture

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

We aim to address the prevention of fungal and bacterial biofilm formation, and the facilitated delivery of antifungal drugs to a wound site. Wound dressings consisting of inherently antifungal and antibacterial materials that also deliver controlled, consistent dosages of traditional antifungal medications could present an effective, field-deployable, shelf-stable and inexpensive solution that would be beneficial for use in military zones, clinical facilities, developing world/rural care facilities and disaster sites. Here, we propose to develop a prototype bandage contact lens (BCL) that would serve to simultaneously *prevent* the development of a biofilm and *continuously deliver antifungals* to the site of the injury for up to two weeks. To do so, we have designed a therapeutic composite bandage device that harnesses advanced nanotechnology to combine (1) inherently antimicrobial bio-derivatives; (2) bio-mimetic antimicrobial nanoarchitectures; and (3) an innovative nanocomposite modality for antimicrobial drug delivery.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Bandage contact lens; antifungal drug delivery; antibacterial surface; biomimetic nanoarchitecture

3. ACCOMPLISHMENTS:

What were the major goals of the project?

We propose to design and evaluate in vitro a novel, multi-functional antifungal material (chitin-chitosan) with both inherent antifungal properties and antifungal drug-releasing characteristics.

Specific Aim (specified in proposal)	2017	2018	2019
Aim 1: Design and implement composite prototype fabrication			
Task 1.1: Synthesis of biocomposite core material and optimization of material properties			
Subtask 1.1a: Create central chitin-hydrogel layer	90%		
Subtask 1.1b: Fabricate lens-shape biocomposite	50%	50%	
Subtask 1.1c: Characterize biocomposite core material	90%	50%	
Task 1.2: Fabrication of surface layer: lubricating antimicrobial hydrogel with biomimetic antimicrobial nanostructures			
Subtask 1.2a: Nanostructures on synthetic and biopolymer surfaces (r-NIL)	100%		
Subtask 1.2b: Nanostructure characterization (AFM, SEM)	100%		
Subtask 1.2c: <i>In vitro</i> antifungal experiments (washing assay, Live-Dead assay)		100%	X
Task 1.3: Fabrication of the lens-shaped three-layer antifungal, ophthalmologic bandage device			
Subtask 1.3a: Fabricate three-layer bandage sandwich (r-NIL)		30%	X
Subtask 1.3b: Nanostructure characterization (SEM, AFM)		10%	X
Subtask 1.3c: <i>In vitro</i> antifungal experiments (washing assay, Live-Dead assay)		50%	X
Aim 2: Develop drug delivery material with desired drug release characteristics			
Task 2.1: Fabricate different systems of polymers with embedded anti-fungal drugs			
Subtask 2.1a: Make chitosan nanoparticles	10%		
Subtask 2.1b: Embed chitosan nanoparticles in hydrogel	20%	20%	
Subtask 2.1c: Add other antifungals in nanoparticles	10%	25%	X
Task 2.2: Characterize physical properties, drug release, and <i>in vitro</i> functionality of the polymer-drug systems			
Subtask 2.2a: Characterize chitosan nanoparticles, hydrogel	10%	25%	
Subtask 2.2b: Evaluate drug release kinetics (HPLC with UV spectroscopy, LC/MS)		70%	X
Subtask 2.2c: Evaluate <i>in vitro</i> functionality of polymer-drug system (agar diffusion)		10%	X
Subtask 2.2d: Assess cytotoxicity of polymer-drug system (MTT assay)		X	X

Aim 3: Determine the role of the antifungal bandage contact lens on fungal keratitis in established murine and rabbit models of <i>Fusarium</i> and <i>Aspergillus</i> keratitis			
Task 3.1: Murine model of contact lens associated fungal keratitis			
Subtask 3.1a: Local IRB/IACUC Approval	100%		
Subtask 3.1b: Fabricate BCL prototype for mouse model	X	X	X
Subtask 3.1c: Cornea analysis for fungal keratitis (opacity, CFU count)			X
Task 3.2: Rabbit model of contact lens associated fungal keratitis			
Subtask 3.2a: Local IRB/IACUC Approval	100%	X	
Subtask 3.2b: Fabricate BCL prototype for rabbit model	X	X	X
Subtask 3.2c: Cornea analysis for fungal keratitis (opacity, CFU count, PASH staining)			X

What was accomplished under these goals?

Aim 1: Design and implement composite prototype fabrication

- 1.1a: Synthesize biocomposite material
 - We have attempted to use hydrogels and their combinations with other polymers for the bandage contact lens. These materials include poly(ethylene glycol) (PEG), chitosan, PEG/chitosan, chitosan/gelatin, chitin, chitosan/chitin and poly(2-hydroxyethyl methacrylate) (pHEMA).
 - The PEG containing hydrogels obtained by UV-polymerization generally show poor mechanical stability, and could not be used to obtain a thin, *smooth* film. The calcium-methanol-gelation synthesis route was used to prepare chitin hydrogels but we had difficulty in solvent exchange with methanol and water due to the formation of an insoluble chitin-calcium complex. Finally, chitosan/gelatin hydrogels were obtained with different compositions by drop casting but the resulting films are semi-transparent. These hydrogels are therefore not further considered in the project.
 - We found that chitosan itself can form a mechanically strong and transparent hydrogel. Physical crosslinked chitosan hydrogel was fabricated with chitosan 1-4

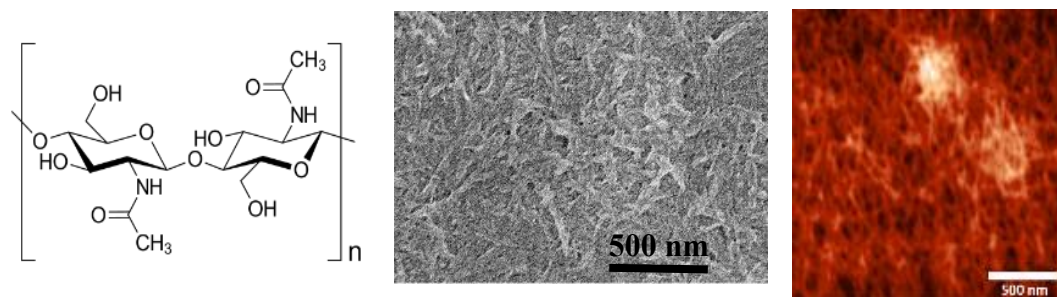


Figure 1. From left to right: molecular structure of chitin, SEM and AFM images of chitin nanofibers.

wt.% in acetic acid. Films were prepared at different compositions (0.5 wt% - 0.0125 wt% chitosan) using crosslinkers (5 wt% NaOH 5 min – 10 min). The time to neutralization after crosslinking with NaOH was investigated. Films were washed with Milli-Q DI water until neutral pH (7.4). It was found that washing for 1 minute was sufficient. High weight ratio (0.5 wt%) chitosan solution was found to have good film-forming ability but poor penetration of nanopillar mold, perhaps due to high viscosity. Low weight ratio (0.0125 wt%) chitosan solution however resulted in films too fragile to characterize. An optimal concentration, 0.25 wt% chitosan solution was finally chosen to fabricate pillared surfaces and the detailed experiments can be found in Section 1.2a.

- Chitin nanofibers were used to reinforce the chitosan matrix. Typically, a 2 wt% chitin solution was prepared by dissolving chitin powder in a saturated calcium-methanol solution (85% w/v CaCl₂ solution) under reflux condition for 6 hours. The viscous chitin-methanol gel was then immediately dispersed into 2.0 L of Milli-Q DI water to exchange the solvent. After collection by filtration, the chitin nanofibers (Fig. 1) were dialyzed using regenerated cellulose to remove residual ionic species. Chitin nanofibers themselves were difficult to cast into a transparent

film but can be used to strengthen the chitosan matrix at different amounts of additions. A low volume fraction of chitin nanofibers is preferred for obtaining a transparent composite hydrogel. A biocomposite material of chitin:chitosan 1:9 ratio was fabricated. Several more ratios will be fabricated using multiple compatible solvents for best results.

- Poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogels were also considered because they can provide us with good comparison samples, and further may allow the rapid integration of our design concept into soft contact lenses currently in production. pHEMA hydrogel was fabricated via UV-polymerization with various cross-linker ratios (0.9, 1, 1.5 wt%), water contents (40-60 wt%) and UV cure time (1 min – 2 min) in a PDMS mold (thickness ~0.5 mm) covered by a glass slide. UV-photopolymerization for 2 min was found to be optimal. Reaction purge with inert gas increases transparency of the film, which detaches from the glass slide after peeling off from PDMS mold and immersion in water. The film shows good transparency and mechanical robustness. The improved synthesis of pHEMA hydrogel for the creation of sandwich structure is detailed Section 1.3a.
- 1.1b: Fabrication of lens shaped biocomposite
 - Physical crosslinked chitosan hydrogels of various weight ratios were cut into lens shape.
 - pHEMA hydrogels were injected into lens mold and subsequently cured with UV light.
- 1.1c: Characterization of biocomposite material
 - pHEMA hydrogel films exhibit good transparency and the surface is also very smooth as confirmed by SEM observations (Fig. 2).

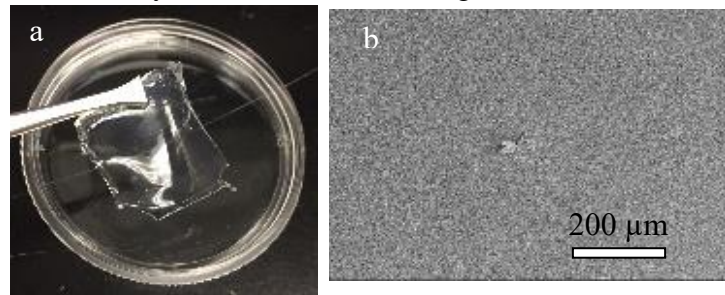


Figure 2. a) Optical and b) SEM images of pHEMA hydrogel film, respectively.

- 1.2a: Fabricate nanostructures on chitosan and composite surface
 - Different geometries of the nanopillars (P500, P300) were fabricated using chitosan dissolved in 0.1 M acetic acid. The solution was stirred at 120 rpm until just dissolved. Films were stabilized with sodium hydroxide (NaOH) or chemically crosslinked with genipin (Fig. 3).



Figure 3. From left to right: macroscopic images of chemically crosslinked P500, stabilized P500, P300, and flat biopolymer film.

- To fabricate chitosan films with nanopillars, the chitosan solutions were stabilized with 5 wt% NaOH and then cast into the nanohole negative mold using dropcast-NIL. The samples were dried at 110 °C for 3 h to remove the acetic acid. The film was covered with 1 mL of 5 wt% NaOH to neutralize the chitosan. Coverage for 5 min with neutralization solution was found to be sufficient (Fig. 4). After neutralization, the films were washed with Milli-Q DI water. The films were then dried at 100 °C with an applied pressure of 3.9 kPa.
- Due to the large surface area, chitosan nanopillars would quickly lose water and shrink in size. The rate of water loss can be retarded by crosslinking the polymer. Genipin, a natural plant extract, is well known for its ability to crosslink chitosan, and is chosen for this purpose. A stock genipin solution was prepared by dissolving genipin in ethanol. The solution was drop cast into nanohole mold to crosslink and left unperturbed.

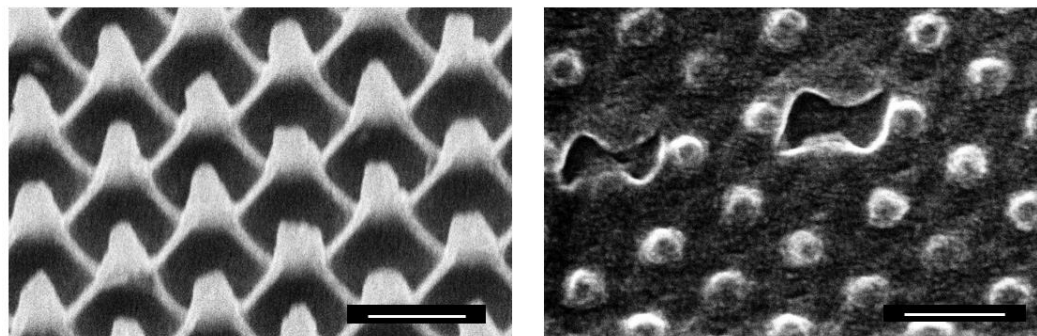


Figure 4. P500 films neutralized for 5 min (left) and 1 min (right). Scale bar 500 nm.

- The reaction rate of 200 μ L 0.25% chitosan 0.5 mM genipin solution was monitored using ultraviolet-visible spectroscopy (UV-Vis). The results are shown in Fig. 5, where the chitosan solution (0.275% chitosan) represents the chitosan solution prior to genipin addition. The absorption at 240 nm decreases due to the genipin ester to amide conversion, while an absorption increase at 280 nm indicates the formation of a heterocyclic amino compound (Fig. 5). The reaction appears sensitive to oxygen, future work includes purging the reaction with nitrogen to remove residual air.

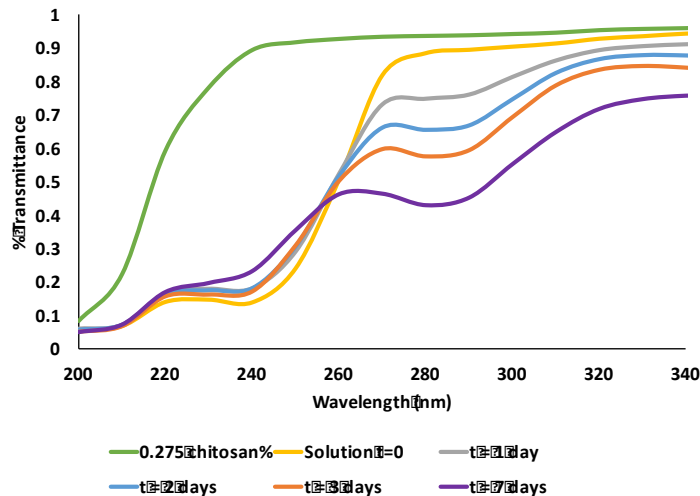


Figure 5. UV-Vis spectra of P500 films neutralized for various time periods.

- From previous work, P200 is found to have strong bactericidal yet weak fungicidal effect, while P500 shows the opposite. Therefore, an irregular pattern with nanopillars in a distribution of sizes could show both antibacterial and antifungal effects compared with regularly spaced nanopillar arrays due to random clustering of the pillars. An electrohydrodynamic system was developed to fabricate nanopillars with positional randomness and high aspect ratio to achieve higher antimicrobial efficiency. A system (Fig. 6) was assembled to test the concept. The system consists of a set of parallel electrodes made from doped silicon. A voltage is applied across the electrodes to generate a high electric field. A molten polymer film deposited on the lower electrode will respond to the field and form undulations due to the field fluctuations from a highly uneven upper electrode, which is formed by using black silicon (Fig. 6), which has a scalloped surface, unlike a typical flat silicon wafer. The polymer in this experiment is a thin film of polyvinylidene fluoride-co-trifluoroethylene P(VDF-trFE). It is chosen for its molecular backbone, which has a high dipole moment and can be piezoelectric. 8 wt% P(VDF-trFE) in 2-butanone was spin coated at 2000 rpm for 30 s. A 2 μm film of P(VDF-trFE) was obtained and deposited onto a polished doped silicon wafer substrate serving as the bottom electrode. Another silicon wafer treated with heptadecafluorodecyl trimethoxysilane for antistick property was mounted as the upper electrode. The scalloped black silicon produces electric field concentrations that would result in irregularly spaced nanopillars. A thin layer of polydimethylsiloxane (PDMS) was spin coated at 6000 rpm for 150 s on top of the P(VDF-trFE) film and cut as spacer between two electrodes, leaving a 5 μm air gap. The assembly was heated to 165 $^{\circ}\text{C}$ for 20 minutes in a vacuum oven to ensure temperature equilibrium. Then a voltage (42 V) was applied to create a high electric field ($\sim 4 \times 10^6$ V/m) between the electrodes. After 100 minutes, the assembly was rapidly cooled to room temperature to freeze the polymer structure while maintaining the electrical bias. Electric field was switched off after 150 min. Current across the assembly was recorded over the entire process. SEM

image (Fig. 6) shows that scalloped nanostructure on the black silicon surface is in size scale from 300 nm to 500 nm, which is ideal to serve as upper electrodes to induce nanopillars in different size scale with high efficiency to kill microorganisms

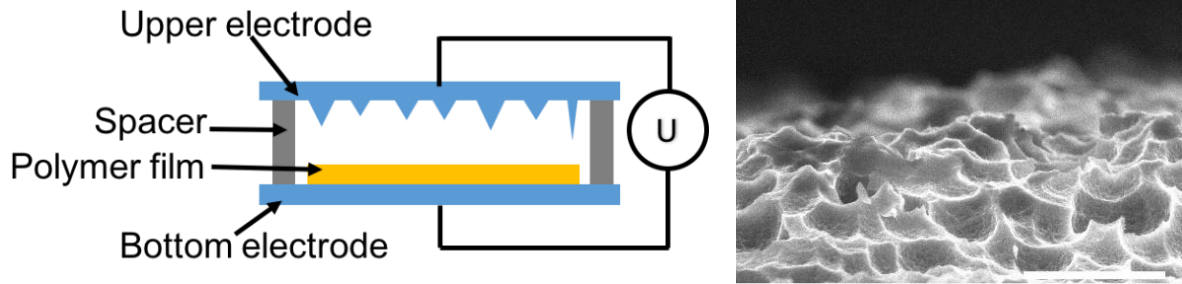


Figure 6. Left: Schematic illustration of irregular nanopattern fabrication with electrohydrodynamic system. Right: SEM of profile of black silicon nanostructure used for upper electrode. Scale bar is 1 μm .

- 1.2b: Characterize nanostructures on chitosan surface and composite surface
 - o Scanning electron microscope (SEM) images show high fidelity of periodic structures on chitosan (Fig. 7).

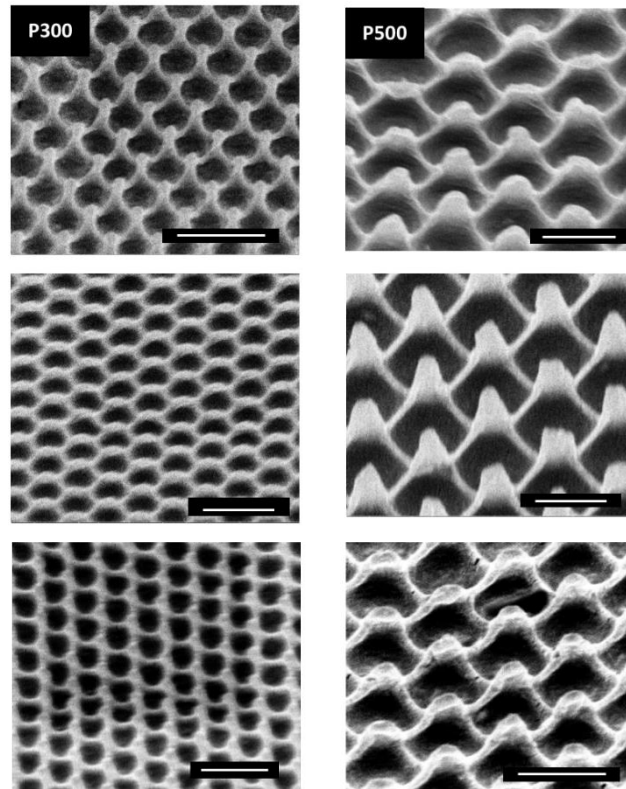


Figure 7. SEM images of unneutralized (top), neutralized (center), and crosslinked (bottom) chitosan films. Scale bars are 500 nm.

- Hydrated films were observed using environmental SEM (ESEM) at 60%-90% relative humidity. The P500 hydrogel nanopillars do not bend or collapse after hydration, which indicates that genipin was successful in maintaining a stable feature geometry for applications in an aqueous environment (Fig. 8).

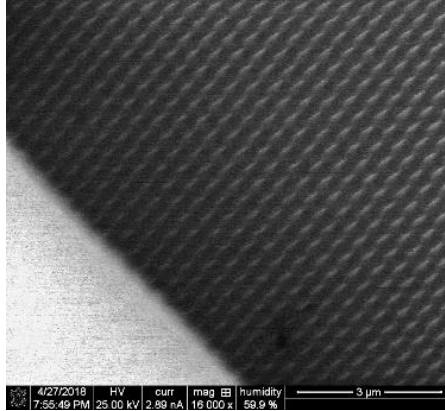


Figure 8. Environmental SEM image of chitosan film imprinted with nanopillars at 65% relative humidity showing that nanopillars remain on the humidified surface. Scale bar is 3 microns.

- The aspect ratio of the nanopillars is expected to change with the swelling of the hydrogel film in liquid. An aspect ratio of at least 1 is desirable to maximize antimicrobial properties. We evaluated the properties of hydrated biopolymer surfaces in environmental atomic force microscopy (AFM) at different humidity levels to characterize the morphology change. Further studies include liquid AFM on samples incubated in PBS to determine nanopillar stability over longer time periods. The hydrated neutralized P500 surface has < 10% taller nanopillars at relative humidity 80% compared to ambient humidity (~47%). These findings confirm previous environmental SEM observations. Similarly, the neutralized P300 surface is 3.5% taller pillars at relative humidity 95% compared to ambient humidity (Fig. 9).

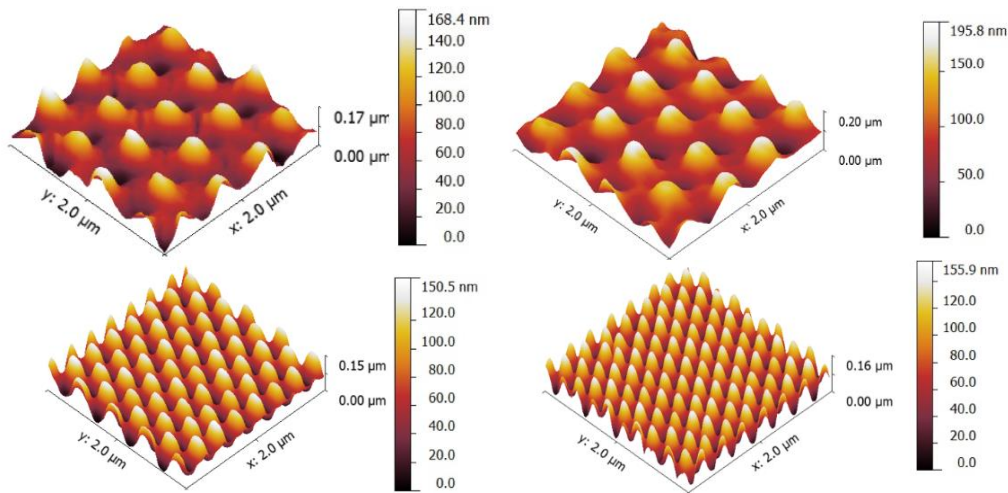


Figure 9. AFM images of nanopillared chitosan films at ambient relative humidity (~47%) (left) and increased relative humidity (80-90%) (right). Images are 2 μm by 2 μm .

- The chemically crosslinked biopolymer films were evaluated with AFM at ambient humidity (~47%). The genipin crosslinked features were found to be of similar height to unneutralized chitosan features (Fig. 10).

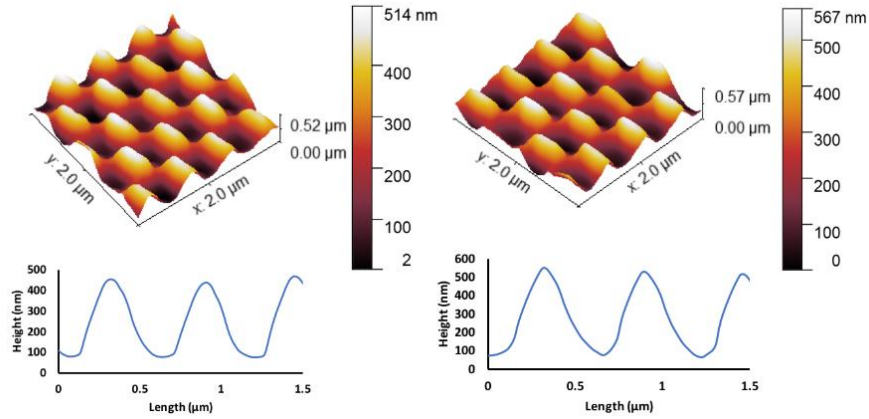


Figure 10. AFM images of nanopillared unneutralized chitosan film (left), and chemically crosslinked film (right). Images are 2 μm x 2 μm.

- We evaluated the transparency of biopolymer (chitosan) using ultraviolet-visible spectroscopy (UV-Vis). Different geometries of chitosan nanopillars are used (P300, P500). The chitosan nanopillars have greater than 90% transparency when hydrated in phosphate buffered saline (PBS 10X, pH 7.4) (Fig. 11), which is very close to the flat film. The chitosan transparency is however dependent on swelling and crosslinking. It is found that the genipin crosslinked chitosan film showed much decreased transparency.

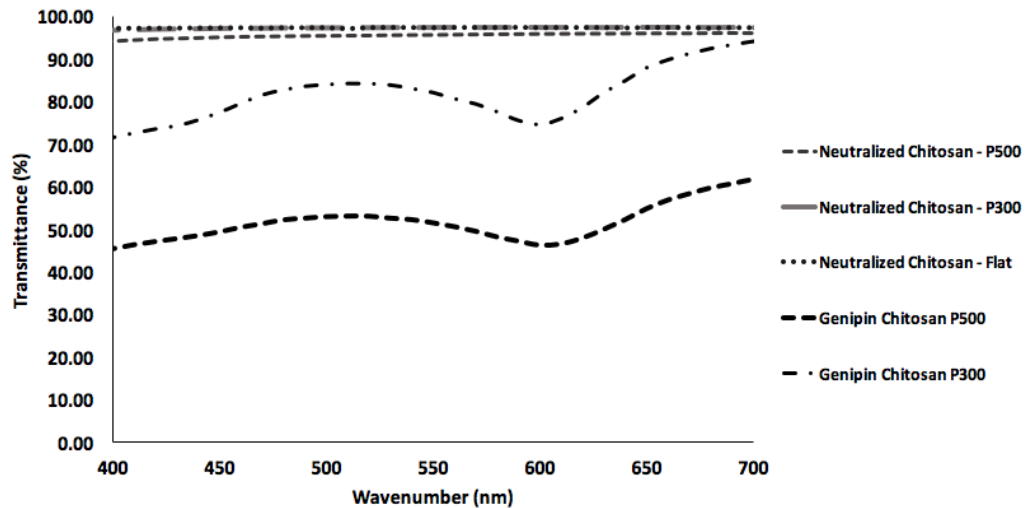


Figure 11. UV-Vis spectrum of nanopillared biopolymer films.

- SEM image (Fig. 12) of P(VDF-trFE) after EHD process shows no pillars perpendicular to the surface formed. Instead, the image shows a stack of collapsed

ligaments less than 100 nm in size. The ligaments likely began as very high aspect ratio slender pillars. The aspect ratio of these pillars is estimated to be >50 based on the geometry. These results suggest that the nanopillars detached from the electrode in the cooling process and collapsed. In future work we plan to use a smaller air gap to create much smaller aspect ratios, and to use a higher cooling rate to freeze in the as-formed structures.

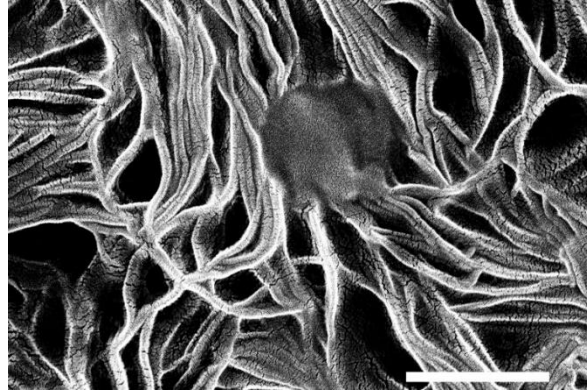


Figure 12. P(VDF-trFE) film after EHD process. The approximately round and flat structure near the center of the micrograph may be the point where a bundle of fine fibrils detached from the upper electrode. Scale bar is 1 μm .

- 1.2c: In vitro antifungal experiments (washing assay, live-dead stain)
 - A robust test methodology was established to accurately evaluate the antimicrobial properties of different surfaces. The previously well-developed PMMA pillared surfaces were used. Not only antifungal but also antibacterial properties were evaluated. These established assays will be implemented for evaluating the antimicrobial properties of pillared chitosan, pHEMA or chitosan/chitin surfaces.
 - The antifungal properties of surfaces consisting of PMMA nanopillars were evaluated using both *Fusarium oxysporum* and *Aspergillus fumigatus*. Different geometries of PMMA nanopillars (P200, P300, P500 and P600, Fig. 13) were prepared using nanoimprinting.

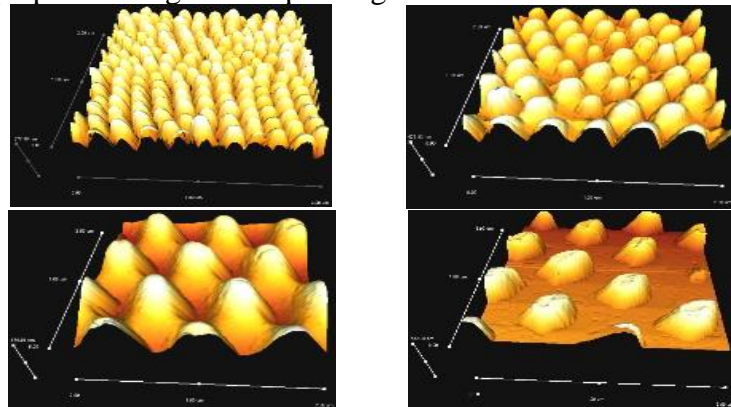


Figure 13. AFM images of PMMA pillars (P200, P300, upper row, and P500 and P600, lower row). The images are 2 μm by 2 μm .

- Compared with flat surfaces of PMMA, the cell growth of *A. fumigatus* on P300 was inhibited after a 24-h incubation (Fig. 14). SEM observations showed that the presence of surface pillars can induce the rupture of *A. fumigatus* (Fig. 15).

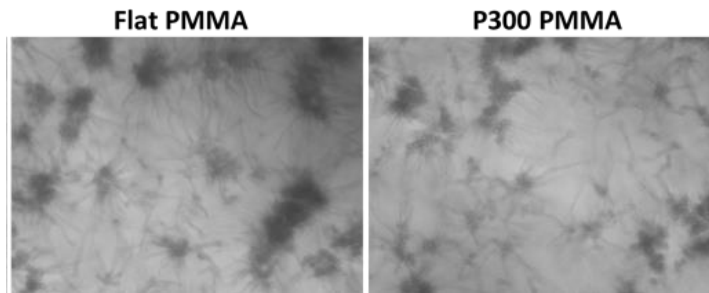


Figure 14. Optical images of *A. fumigatus* growth after 24 h on flat and P300 PMMA surfaces.

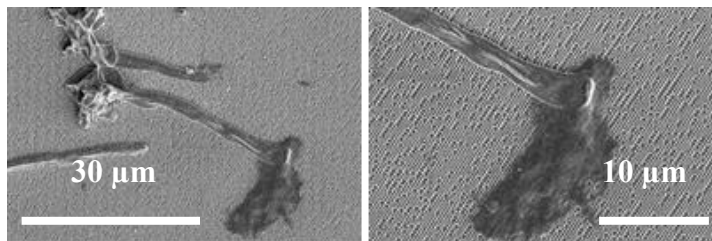


Figure 15. SEM images showing the ruptured *A. fumigatus* on P300 PMMA surface.

- Similar results were observed for *F. oxysporum*. Compared with flat surfaces of PMMA, the cell growth of *F. oxysporum* on P300 was inhibited after a 24-h incubation (Fig. 16). SEM observations showed that *F. oxysporum* is capable of sensing and adhering to P300 surface (Fig. 17).

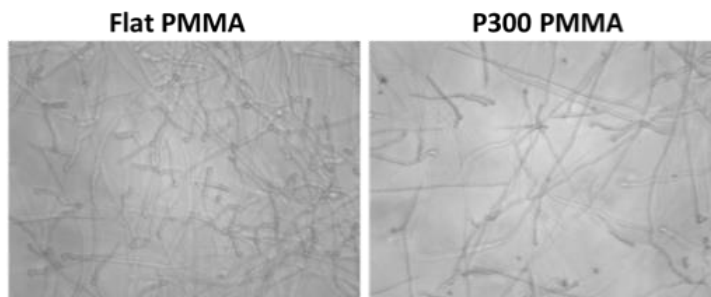


Figure 16. Optical images of *F. oxysporum* growth after 24 h on flat and P300 PMMA surfaces.

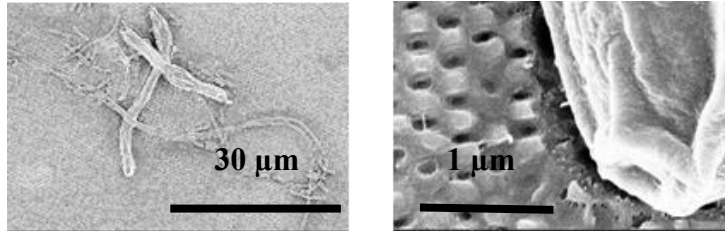


Figure 17. SEM images showing *F. oxysporum* capable of sensing and adhering to P300 PMMA surface.

- Fungi cells were grown on all surfaces from 0 to 24 hours with an initial 10^5 spore count. It was found that all the pillared surfaces can inhibit the growth of *F. oxysporum* and *A. fumigatus* (Fig. 18). This is consistent with the results of fungal mass absorbance (Fig. 19). These results have confirmed the geometry effect of nanopillars on the antifungal properties of surfaces. It can be expected that pillared surfaces made from materials with intrinsic antifungal properties including chitosan and chitin should have improved performance compared with PMMA surfaces. Further studies include advanced fluorescence microscopy on specific growth proteins of the fungi strains to deduce the mechanism of the inhibition of cell growth on nanopillars.

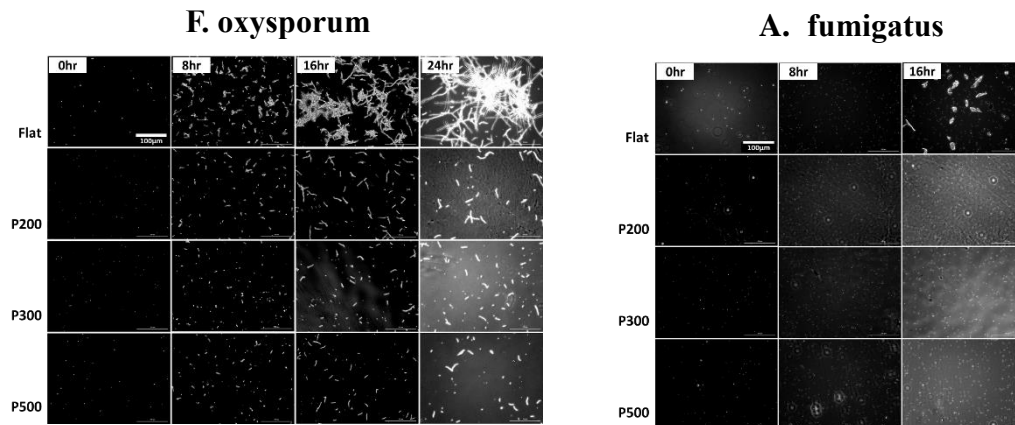


Figure 18. Optical images of left) *F. oxysporum* and right) *A. fumigatus* growth after different lengths of time on all surfaces.

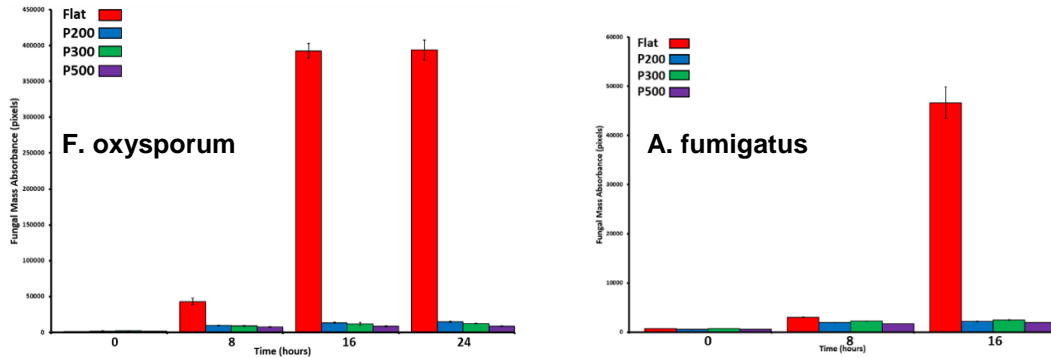


Figure 19. Fungal mass absorbance after different lengths of time on all surfaces: left) *F. oxysporum* and right) *A. fumigatus*.

- A live/dead stain was also established for *Fusarium oxysporum* with green fluorescent protein (Fox-GFP). The fungi culture was grown in Sabourad Dextrose (SD) broth with propidium iodide (PI) stain (Fig. 20). Natamycin was used as an antifungal drug. The top row are phase images of fungi cell growth at the 0, 6, 12, and 18th hours. The second row shows the red fluorescence of fungi cells successfully killed by the Natamycin over time. The two rows with Natamycin do not show fungal hyphae growth, indicating cells death. Row 3 are phase images of fungi cell growth at the 0, 6, 12, and 18th hours without antifungal present. The 4th row shows no red fluorescent readings from fungal hyphae (red fluorescence shown are autofluorescence from background contamination).
- Antibacterial properties of pillared film (P200) against *P. aeruginosa* growth were evaluated using live/dead stain. Compared with flat surfaces of PMMA, the cell growth of *P. aeruginosa* on P200 was clearly inhibited after a 12-h incubation as revealed by the presence of massive green fluorescence from live cells on the flat surface but dominant red fluorescence from dead cells on P200 (Fig. 21).

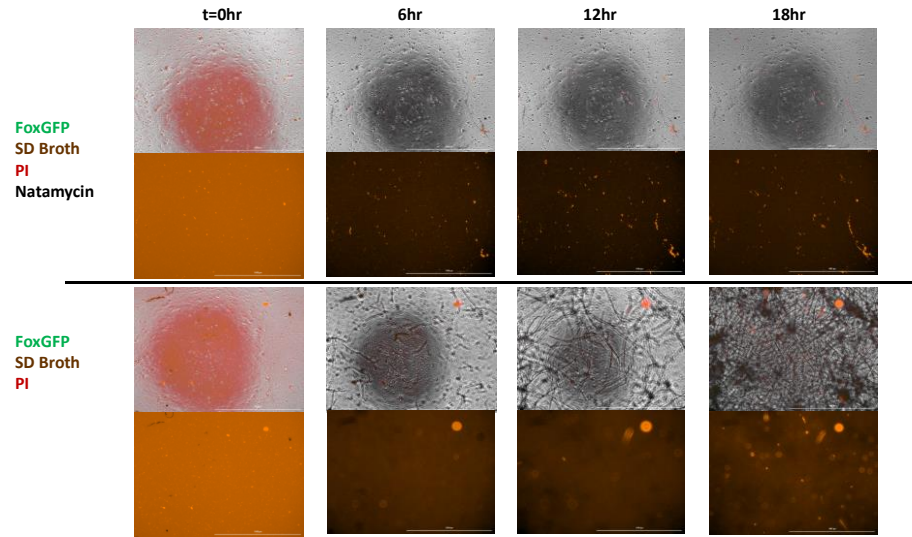


Figure 20. Live-dead assay of *F. oxysporum* with natamycin (top two rows) and without antifungal present.

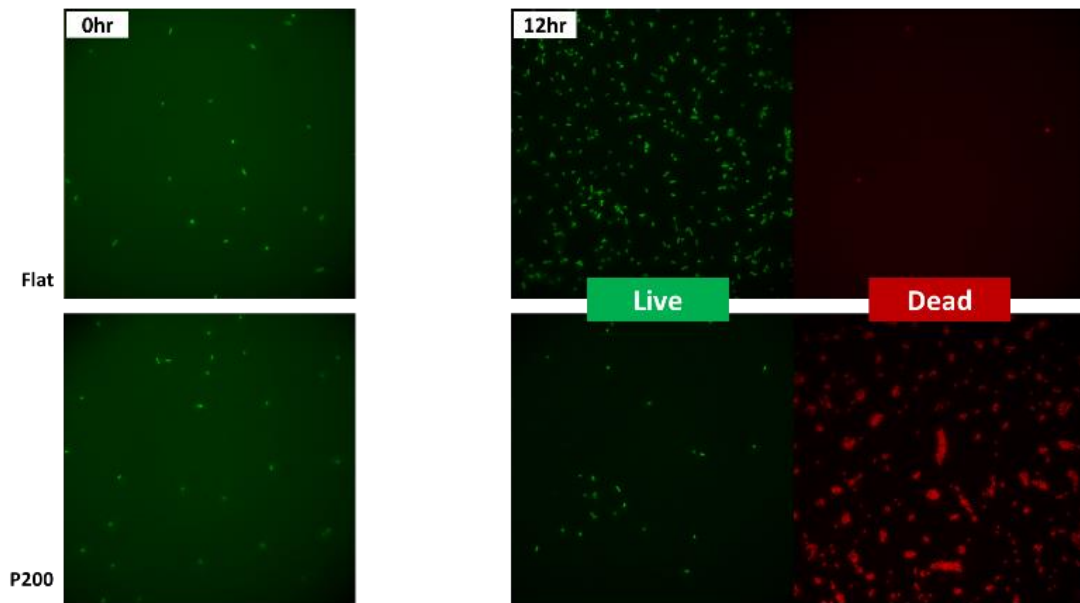


Figure 21. Live-dead assay of *P. aeruginosa*.

- 1.3a: Fabricate three-layer bandage sandwich
 - o pHEMA hydrogel was first used in the creation of three-layer bandage sandwich as the outer layer because it is a well-established material for soft contact lenses. PLGA was selected as the reservoir for antifungal drug particles (natamycin) (see the section of 2.1c) in the central layer for its ability in controlling the release properties (see the section of 2.2 b). The chitosan and chitosan/chitin biopolymer

hydrogels will be integrated into this prototype system by either attaching to or replacing pHEMA hydrogels in the future work.

- The hydrogel was made of crosslinked poly (2-hydroxyethyl methacrylate) (pHEMA) using UV polymerization. To make pHEMA, 2-hydroxyethyl methacrylate (HEMA) was the monomer, 2-hydroxy-2-methylpropiophenone (Darocur 1173) was the photo initiator and ethylene glycol dimethacrylate (EGDMA) was the crosslinker. HEMA was pre-polymerized using UV light to increase the viscosity, making it easier to be cast into the mold forming the pHEMA film.
- The mold for making the pHEMA film was composed of glass slides, isolating films (polypropylene) and spacer (polyethylene terephthalate) (Fig. 22). The hydrogel film thickness was controlled by the spacer. The pre-polymerized HEMA was mixed with EGDMA and cast into the negative mold.

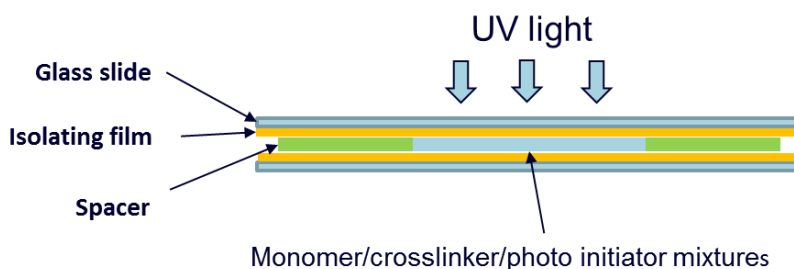


Figure 22. Schematic illustration of the pHEMA film fabrication using the mold.

- The mixture was exposed to UV light for 60 seconds to cure the hydrogel. Most of the unreacted HEMA, EGDMA and Darocur 1173 in the pHEMA film can be removed by dialyzing 3 times against DI water with time interval of 2 h at 34 °C (Fig. 23). This short dialysis time can minimize the drug being released during the dialysis.

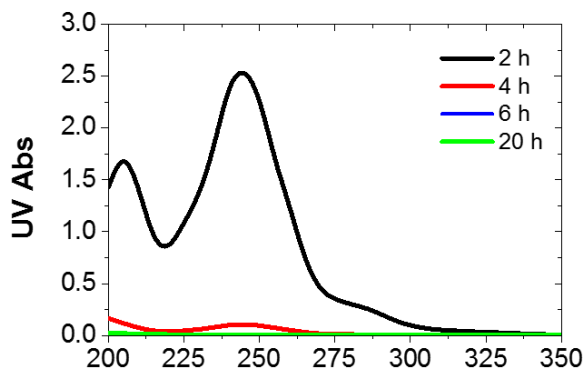


Figure 23. UV absorbance of the unreacted components released from pHEMA film after dialyzing against DI water.

- The swelling of pHEMA film in water reached an equilibrium within 2 h, corresponding to a water content of 33% (Fig. 24A). After the swelling, both the diameter and thickness of the pHEMA film increased by approximately 13% (Fig. 24B), with the thickness reaching about 90 μm .

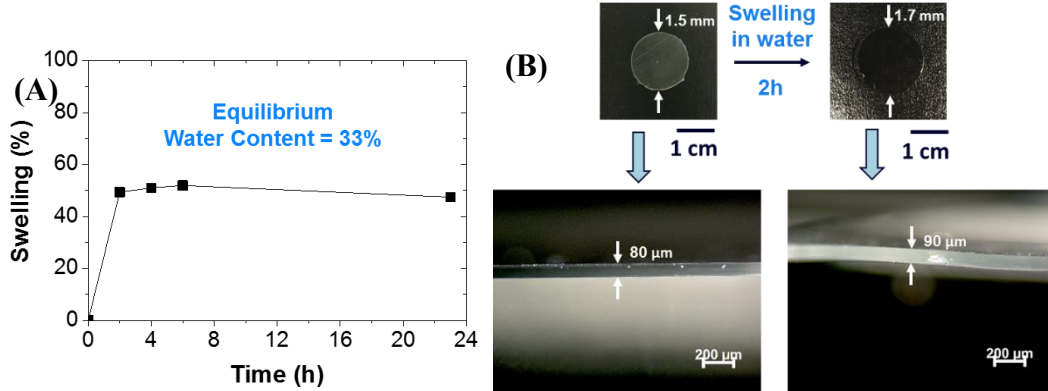


Figure 24. (A) The swelling of pHEMA film over time and (B) the diameter and thickness changes of pHEMA film after swelling.

- The water content of hydrogels can be increased by introducing methacrylic acid (MAA) as a second monomer, which increased from 32 to 73 % when 0 – 5 % v/v of MAA was added. Correspondingly, the increase of diameter improved from 13 to 60 % (Fig. 25).

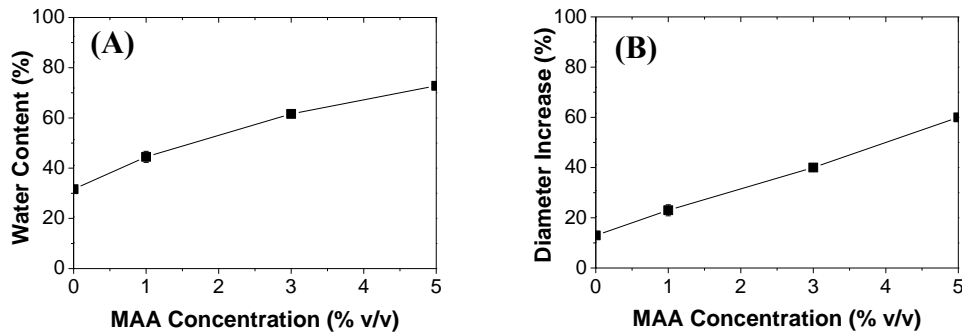


Figure 25. The effect of MAA concentration on the (A) water content and (B) diameter of the contact lenses (90 μm , no natamycin/PLGA ring imbedded) during the immersion in PBS solution.

- The pHEMA hydrogel layers with different thicknesses were obtained by using different spacers (PP films) during the UV polymerization. The swelling behavior in PBS solution however did not change significantly when varying the thickness (Fig. 26).

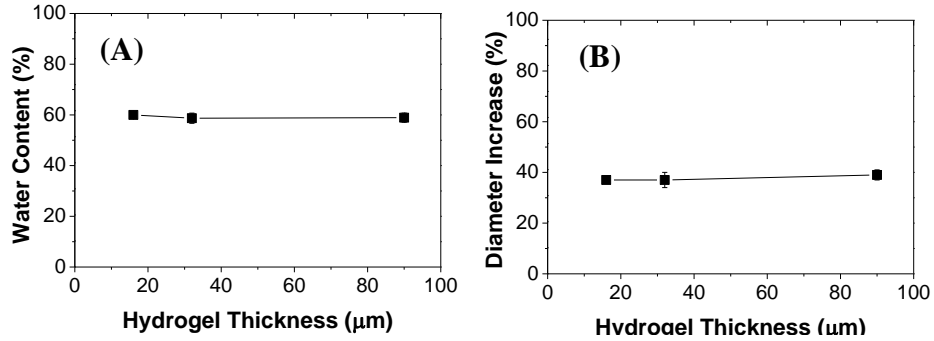


Figure 26. The effect of hydrogel thickness on the (A) water content and (B) diameter of the contact lenses with MAA concentration of 3 % v/v (no natamycin/PLGA ring imbedded).

- To make the three-layer structure of the contact lenses, the bottom layer of pHEMA was first made as the negative mold. The mold has inner and outer diameters of 6 and 10 mm, respectively. The PLGA/natamycin suspension in ethyl acetate was cast in the pHEMA negative molds. The samples were dried in the air for 1 day and in the vacuum for another day to remove the ethyl acetate. Then, top layers of pHEMA were made. The top and bottom layers were sealed together by a thin layer of the pre-polymerized monomers using UV light (Fig. 27).

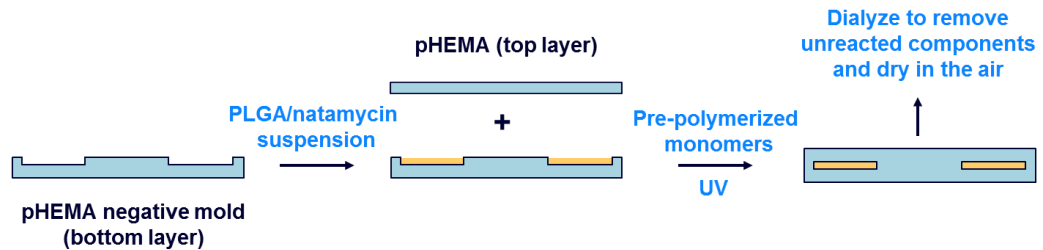


Figure 27. Schematic illustration of the fabrication of three-layer contact lenses.

- PLGA/natamycin rings were made using similar polypropylene mold as a control for the drug release experiment. The contact lenses that loaded with PLGA/natamycin rings (Fig. 28A) were dialyzed against DI water three times with time interval of 2 h to remove unreacted components. The thickness of the contact lenses is about 0.3 mm (Fig. 28B).

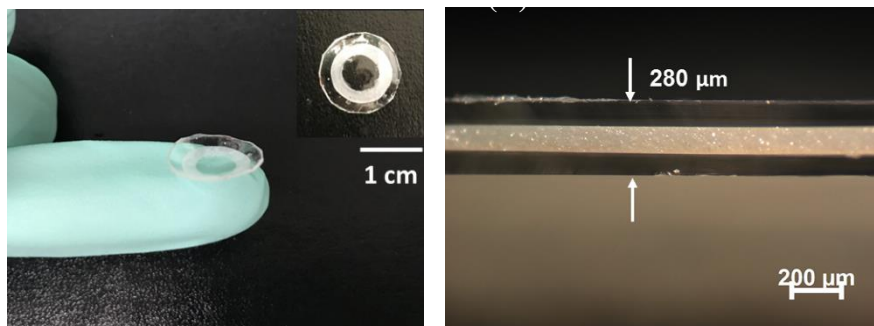


Figure 28. (A) An optical image and (B) the dark-field micrograph of the cross-section of the contact lenses.

Aim 2: Developing drug delivery material with desired drug release characteristics

- 2.1a: Make chitosan nanoparticles
 - Chitosan nanoparticles have been fabricated.
- 2.1c: Add other antifungals in nanoparticles
 - The solubility of natamycin in PBS solution was determined to be 36 $\mu\text{g}/\text{mL}$ at 37 $^{\circ}\text{C}$, similar with that in water.
 - Natamycin particles were prepared by an anti-solvent precipitation method. The particles were characterized by SEM and exhibited flake-like structures with broad size distributions on the order of tens of micrometers (Fig. 29). These were smaller than natamycin powder from the vendor and were used in the following experiment.

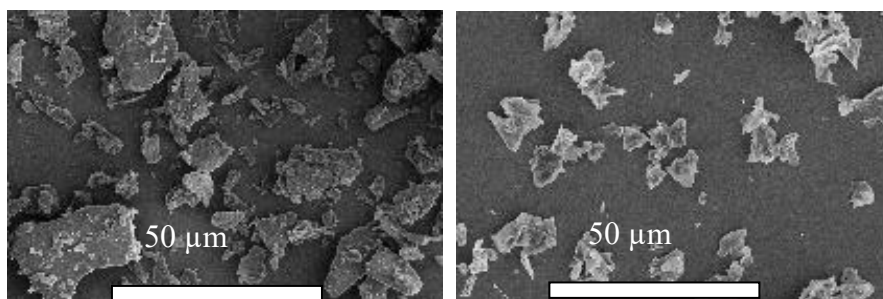


Figure 29. SEM images of (left) original natamycin powder and (right) natamycin particles obtained from anti-solvent precipitation (methanol:water = 1:4)

- Dissolution profile of natamycin particles was studied. Natamycin particles dissolved rapidly in PBS at 37 °C. Most of natamycin dissolved within 2 hours, regardless of the anti-solvents were used (e.g. water, ethyl acetate and hexane). This indicates that the natamycin particle dissolution will not be a limiting parameter for the drug release from contact lenses.
- 2.2a: Characterize chitosan nanoparticles, hydrogel
 - Chitosan nanoparticles have been characterized for their size and uniformity using dynamic light scattering. The particles have been imaged with scanning electron microscopy and atomic force microscopy. The particles will be imaged using transmission electron microscopy.
- 2.2b: Evaluate drug release kinetics
 - Three polymers (polyvinyl alcohol, chitosan and PLGA) were used to prepare polymer/natamycin composite films as controlled-release implants within the contact lens hydrogel. These films were prepared by a drop casting method and their drug release kinetics in PBS at 37 °C were tested and compared.
 - Most of natamycin were released from the polyvinyl alcohol (PVA)/natamycin within 2 hours (Fig. 30). This relatively fast release is likely a result of weak matrix/natamycin interactions and matrix swelling. Natamycin release was extended to 8 hours when the drug was encapsulated into chitosan films, and this release period was increased to 1 day when the chitosan films were crosslinked by tripolyphosphate (TPP) (Fig. 30). No significant swelling or dissolution was observed for the chitosan films both with and without TPP crosslinking.

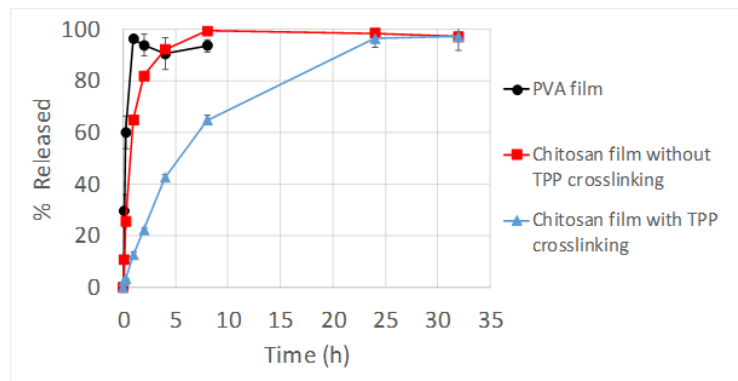


Figure 30. Release profile of natamycin from PVA film and chitosan film with and without TPP crosslinking.

- Natamycin can be sustainably released from poly(lactic-co-glycolic acid) (PLGA) (L:G 75:25, ester terminated) film for more than two weeks (Fig. 31). By fitting the release profile to the Korsmeyer-Peppas equation, the n value was calculated ($n = 0.51$) and suggested that the drug release was controlled by Fickian diffusion. The apparent diffusivity of natamycin within the PLGA film was $2.5 \pm 0.4 \times 10^{-12} \text{ cm}^2/\text{s}$.

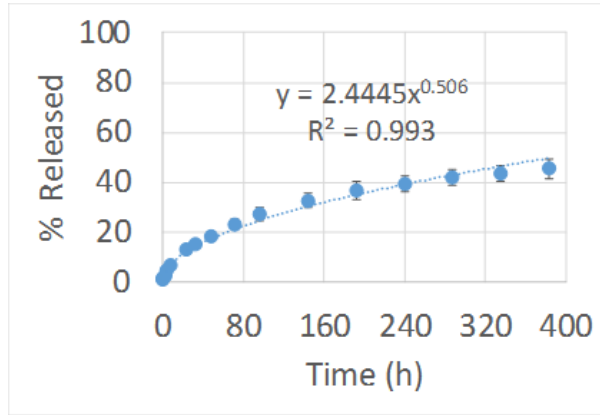


Figure 31. Release profile and curve-fit of natamycin from PLGA film (L:G 72:25)

- The influence of molecular weight (M_w), terminal groups, and L:G (lactide: glycolide) ratio of PLGA on natamycin release behavior was investigated (Fig. 32). For PLGA with acid-terminated groups, the drug release tends to be faster when the PLGA molecular weight is lower. For PLGA with ester-terminated groups and lower molecular weight, the release rate was initially faster and plateaued after 8 h. The drug release tends to be faster when PLGA has acid-terminated groups compared with ester-terminated groups. When the $L:G$ ratio is smaller, drug release rate tends to be slower. These release profiles appeared to be affected by non-uniform particle and polymer distribution in the films, and this effect will be explored further.

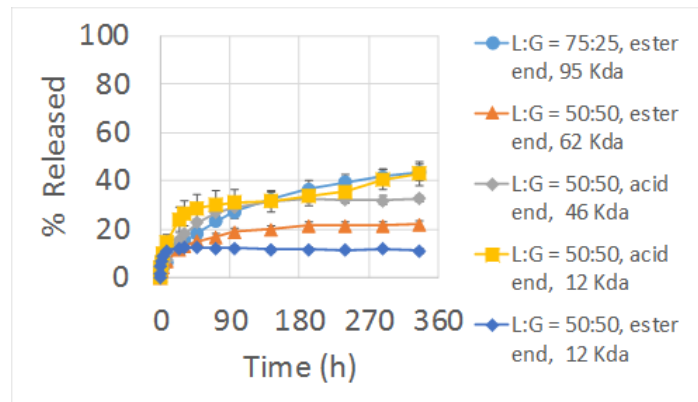


Figure 32. Release profiles of natamycin from PLGA films with different molecule weights, terminal groups and L:G ratios

- PLGA with L:G ratio of 50:50, acid end and M_w of 7-17 kDa was used in the following experiments. The drug release experiment of contact lenses or PLGA/natamycin rings as a control was conducted by the solvent replacement method. Specifically, the contact lenses or PLGA/natamycin rings were placed in 20 mL vials with 5 mL of PBS. The vials were shaken at 100 rpm at 34 °C. At specific time intervals, the supernatants were collected for UV-vis measurements and the PBS solution was completely replaced with 5 mL of fresh PBS.
- All films contain about 1.5 mg of natamycin. For the PLGA/natamycin ring without pHEMA layers, the release rates strongly depended on the fraction of natamycin in the PLGA/natamycin film (Fig. 33A). When 25 wt% of natamycin was in the film, only 16% of the natamycin was released after 8 days; however, when the natamycin fraction went higher, the release rate increased greatly (Fig. 33A).
- After embedding the PLGA/natamycin ring within the pHEMA layers, the release rate decreased dramatically (Fig. 33B). Although the release rate increased with the fraction of natamycin in the rings, less than 12% of natamycin were released after 13 days release (Fig. 33B). The slow release of natamycin in the sandwich structure may be related to the low swelling ratio of pHEMA, which may be adjusted by the control over crosslinking density of pHEMA hydrogel layer.

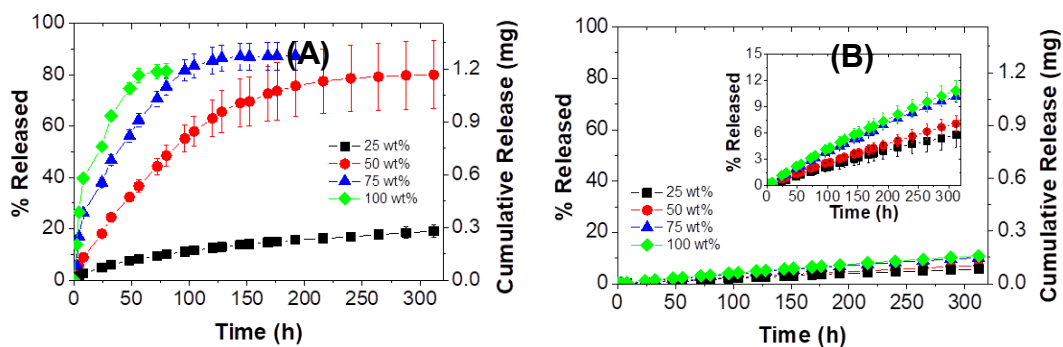


Figure 33. Release profiles of (A) PLGA/natamycin rings as a control and (B) the sandwich contact lenses.

- With the increase of MAA concentration during the UV polymerization, the drug release rate increased significantly (Fig. 34A). The release profiles before reaching plateaus are linear, indicating a zero-order release kinetics. This zero-order release may be due to the supersaturation of natamycin within the central layer, where the dissolution rate of natamycin particle is much higher than the diffusion rate.
- Despite the apparent retardation of drug release upon sandwiching the PLGA/natamycin rings inside the pHEMA hydrogels, the decrease of the hydrogel thickness has a much smaller effect on the release rate compared with MAA concentration (Fig. 34B).

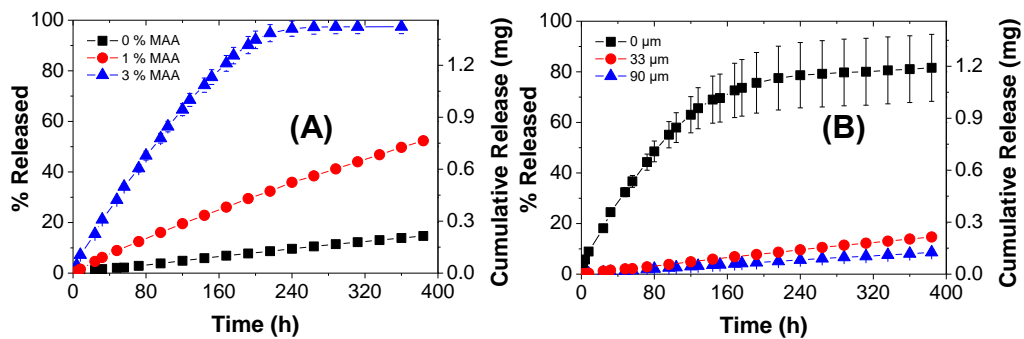


Figure 34. The effect of (A) MAA concentration (thickness of 33 μm) and (B) hydrogel thickness (0% MAA) on the drug release profiles from the contact lenses.

What opportunities for training and professional development has the project provided?

This interdisciplinary project has provided substantial opportunities for training students and postdoctoral researchers. The study of antimicrobial properties of different surfaces has promoted an extensive training for Rachel Rosenzweig, a PhD student from the department of Material Science and Engineering, on the biological assays for assessing antimicrobial activities. During this first year, she has acquired the necessary techniques for washing and live/dead assays for evaluating the antifungal and antibacterial properties of various pillared surfaces. The interesting results obtained by Rachel has granted her many presentation chances in the academic and technical conferences to report our new findings and exchange ideas, which help further strengthen her expertise. The need to understand the aqueous stability of nanopillars has also led to a technical training on the advanced in-situ characterization methods such as environmental SEM and AFM imaging for PhD student Sara Heedy. A third PhD student, Xin Fu, has learned to use a novel electro-hydrodynamic technique to fabricate high aspect ratio polymeric nanopillars, a technique essential for fabricating chitosan, which, due to its high molecular weight, is difficult to fabricate into high aspect ratio nanopillars using nanoimprinting. Separately, a master's student, Christie Sutanto, attempted to directly grow nanopillars of polyethyleneimine onto polymer surfaces, thus bypassing nanoimprinting. She succeeded in growing nanopillar crystals onto polyurethane, and has demonstrated initial success in antifungal behavior. An undergraduate student Van Ly, who assisted Rachel in the preparation of pillared PMMA surfaces, has gained the robust training for acquiring the nanoimprinting and drop casting techniques. Van's training has allowed her to quickly obtain an entry level position with St. Gobain, a supplier of materials and components for medical instruments. A postdoctoral researcher, Dr Yuhang Cai, who is mainly responsible for the fabrication of drug-eluting sandwich structure, has learned many new characterization techniques such as HPLC. All these training opportunities will play an important role in helping the project participants develop their future personal career in either academic or industrial environment.

How were the results disseminated to communities of interest?

We presented the results in five conferences:

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, “Inhibiting Upstream Motility of *Pseudomonas Aeruginosa* via Nanopillared Surface Structuring,” Materials Research Society (MRS) Spring Meeting & Exhibit, Phoenix, AZ, April 2018 (Oral).

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, “Inhibiting Upstream Motility of *Pseudomonas Aeruginosa* on Nanopillared Surfaces,” American Physical Society (APS) March Meeting, Los Angeles, CA, March 2018 (Poster).

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, “Inhibiting Upstream Motility of *Pseudomonas Aeruginosa* on Nanopillared Surfaces,” Biophysical Society (BPS) Annual Meeting, San Francisco, CA, February 2018 (Poster).

R. Rosenzweig, V. K. Ly, M. Marshal, K. Perinbam, E. Pearlman, A. F. Siryaporn, A. F. Yee, “Investigating Antimicrobial Properties of Nanotextured Surfaces for Bandages Against Resistant Infections,” Military Health System Research Symposium, Kissimmee, FL, August 2018 (Poster)

R. Rosenzweig, V. K. Ly, M. Marshal, K. Perinbam, E. Pearlman, A. F. Siryaporn, **A. F. Yee**, “Investigating Antimicrobial Properties of Nanotextured Surfaces for Bandages Against Resistant Infections,” American Chemical Society National Meeting, Boston, Aug. 2018. (Invited talk at Industrial Polymer Scientist Award Symposium)

What do you plan to do during the next reporting period to accomplish the goals?

First, the biological assays we established will be used to evaluate the antimicrobial properties of biopolymer pillared surfaces. Second, the mechanism of the geometry effect on the inhibition of microbial growth will be elaborated. Third, the nanotextured surface will be implemented on the sandwich structure with the sustained release of natamycin and the in vitro antimicrobial performance and cytotoxicity of the bandage contact lenses will be assessed. Fourth, the murine model of contact lens associated fungal keratitis will be initiated.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Our proposed research addresses **antimicrobial resistance**, targeting *identification and evaluation of novel antifungals against resistant fungal infections, particularly topical therapies for wounds, surgical, and post-surgical therapies*. There is an unmet urgency seeking both prevention and treatment against resistant fungal infection for military service members and veterans caused by combat conditions and traumatic injury.

We proposed to address the critical problem of antimicrobial resistance in fungal infection by designing a dual-functional technology/therapeutic development device that 1) prevents fungal infections in an injured eye and 2) treats infection by dispensing antifungal medication at controlled rates. The short-term impact of our research involves fabrication of the antifungal preventative nanopillars on the lens surfaces composed of an inherently antifungal biocomposite material. We have found in the first year of research that ideal pillar dimensions on an antifungal surface are around 500 nm, while those pillars most effective for bacteria such as pseudomonas as around 200 – 300 nm. This suggests that the ideal might be a mix of the two pillar densities, while randomness in the placement of the pillars might improve transparency. We are developing a novel technique known as electrohydrodynamic patterning to address this problem. Another short-term impact involves facilitating continuous delivery of antifungal drugs to a wound site. Current treatment methods for ocular fungal infection are inefficient due to the antimicrobial resistance caused by ineffective, low, or short dosage rates. We have developed device designs that can deliver antifungal drugs steadily and continuously over a period of over 10 days. Validation by animal models will determine the efficacy of these designs.

This bandage provides a continuous long-term treatment and shields the eye from the environment, significantly diminishing the risk of infection while allowing damaged tissues to heal. We envision that this device could be applied to an injured eye in the field to protect the eye, tiding a military service member over until surgery. Additionally, we envision this device could be used to aid personnel, veterans and civilians for post-operative care, providing a solution for infection prevention, treatment, and overall quality of life. The long-term research and clinical care impact of our novel dual-use prevention and treatment device against resistant fungal infections- a technology envisions by the military and congress -paves a way to translate the antimicrobial ocular technology to treat fungal infection in other injured limbs of military service members, veterans, and the civilian population. Evaluation of these surfaces on catheters is also underway as a separate project for the prevention and treatment against resistant fungal infection for military service members and veterans caused by combat conditions and traumatic injury.

What was the impact on other disciplines?

The imprint methods for creating pillared surfaces out of both synthetic and natural polymer sheets represent an important technical guidance for the design of functional nanotextures with distinct mechanical and physicochemical properties. The stability study of the pillared hydrogels helps extend the potential application of antimicrobial nanopillars from dry to wet conditions, which is critical for implantable biomedical devices. In addition, the successful control of drug release from the sandwich structure provides a practical way to achieving zero-order release profile, which may facilitate continuous delivery of antifungal drugs to the infected area. The extension of electrohydrodynamic patterning into the nanometer range provides a critical pathway for nanopatterning polymers that do not lend themselves to thermal imprinting methods.

What was the impact on technology transfer?

U.S. Provisional Patent Application has been filed on “NOVEL ANTI-BACTERIAL ANTI-FUNGAL NANOPILLARED SURFACE”.

What was the impact on society beyond science and technology?

The antimicrobial activity of our pillared surfaces against pathogenic fungi promises our bandage contact lenses a superior prevention and treatment device against resistant fungal infections. Its application to an injured eye in the field to protect the eye can tide a military service member over until surgery, avoiding the impairment and disfigurement. The translation of our design into other infection treatment is also quite probable and will in general improve people’s living conditions and loosen the burden of medical care.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

In the proposal, we planned to use chitosan as the main components for both central and outer layers. In this period of the project, instead, PLGA was chosen as a reservoir for natamycin in the central layer and pHEMA hydrogel with controlled compositions was used as the outer layer. The main advantage of using these two synthetic polymers over chitosan at the beginning of the project is they allow the readily control over antifungal drug release. It has been well-established that PLGA is a good matrix for slowing down the diffusion of natamycin. In addition, the synthesis of pHEMA with different selections of monomers provides extra control over the release kinetics of natamycin in the sandwich structure. Indeed, the prototype bandage contact lenses have been fabricated out of these materials showing very promising release profiles. The current design can be integrated with chitosan or chitosan/chitin layers to further improve the mechanical and antimicrobial properties of the pillared surfaces whenever needed. We note that such change does not affect the general plan of the project but the better ability to control polymeric matrix may further the implementation of our prototype products into the market considered that both PLGA and pHEMA are already approved by FDA.

Actual or anticipated problems or delays and actions or plans to resolve them

A potential anticipated problem is associated with the soft nature of pHEMA or chitosan hydrogels, which may have adverse effect on the antimicrobial properties of pillared surfaces. To mitigate this, we plan to first study the antimicrobial properties of chitosan or pHEMA hydrogels under different swelling conditions. This will help us establish the relationship between the mechanical/surface properties of hydrogels and their antimicrobial activities, based on which an optimal condition can be found by carefully tuning the crosslinking density.

Changes that had a significant impact on expenditures

Delays in hiring post-doctoral staff, thus causing us to fill the positions with a part-time senior scientist, and several graduate students.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, "Inhibiting Upstream Motility of Pseudomonas Aeruginosa via Nanopillared Surface Structuring," Materials Research Society (MRS) Spring Meeting & Exhibit, Phoenix, AZ, April 2018 (Oral).

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, "Inhibiting Upstream Motility of Pseudomonas Aeruginosa on Nanopillared Surfaces," American Physical Society (APS) March Meeting, Los Angeles, CA, March 2018 (Poster).

R. Rosenzweig, K. Perinbam, V. K. Ly, S. Ahrar, A. Siryaporn, A. F. Yee, "Inhibiting Upstream Motility of Pseudomonas Aeruginosa on Nanopillared Surfaces," Biophysical Society (BPS) Annual Meeting, San Francisco, CA, February 2018 (Poster).

R. Rosenzweig, V. K. Ly, M. Marshal, K. Perinbam, E. Pearlman, A. F. Siryaporn, A. F. Yee, "Investigating Antimicrobial Properties of Nanotextured Surfaces for Bandages Against Resistant Infections," Military Health System Research Symposium, Kissimmee, FL, August 2018 (Poster)

R. Rosenzweig, V. K. Ly, M. Marshal, K. Perinbam, E. Pearlman, A. F. Siryaporn, **A. F. Yee**, "Investigating Antimicrobial Properties of Nanotextured Surfaces for Bandages Against Resistant Infections," American Chemical Society National Meeting, Boston, Aug. 2018. (Invited talk at Industrial Polymer Scientist Award Symposium)

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

UC Case No. 2017-421-1, R. Rosenzweig, M. N. Dickson, E. I. Liang, S-W. Wang, A. F. Yee. "Novel Anti-Microbial Bandage Contact Lens with Ocular Drug Delivery," *UCI Record of Invention (Filed)*.

UC Case No. 2017-420-1, R. Rosenzweig, M. N. Dickson, E. I. Liang, A. F. Yee. "Novel Anti-Bacterial Anti-fungal Nanopillared Surface," *UCI Record of Invention (Filed)*.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Albert F. Yee
Project Director/PI
12

Dr. Yee held meetings, managed the team, hired staff, and contributed experimental planning and data analysis

Szu-Wen Wang
PI
9

Dr. Wang held meetings, managed her post-doc and cross disciplinary graduate students, hired staff, and contributed experimental planning and data analysis

Eric Pearlman
PI
12

Dr. Pearlman held meetings, managed cross disciplinary graduate students, and contributed experimental planning and data analysis

Marjan Farid
PI
12

Dr. Farid prepared animal trial documents and contributed experimental planning and data analysis

Ming Yang
Post-Doc
10

Dr. Yang planned meetings, monitored project progress, prepared report materials and contributed experimental planning

Yuhang Cai
Post-Doc
11

Dr. Cai has performed work in the area of fabricating and characterizing drug crystals for the hydrogel core layer

Rachel Rosenzweig
Graduate Student
12

Ms. Rosenzweig has performed work in the area of fabricating and characterizing flat and nanopillared materials for the exterior of the bandage contact lens as well as evaluating the *in vitro* antifungal and antibacterial properties

Serena Abbondante
Associated Specialist
12

Ms. Abbondante has performed work in the area of creating a live/dead stain protocol and assessing the stain using fluorescence imaging

Sara Heedy
Graduate Student
12

Ms. Heedy has performed work for fabricating and characterizing flat and nanopillared biomaterials materials for the exterior of the bandage contact lens and evaluating the antifungal and antibacterial properties.

Xin Fu
Graduate student
3

Ms. Fu has performed work on the fabrication of nonuniform pillared surfaces.

Van Ly
Undergraduate Student

Ms. Ly has performed work in the area of fabricating and characterizing flat and nanopillared materials for the exterior of the bandage contact lens.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nanofabrication work was performed with support from staff of the Lawrence Berkeley National Laboratory in Berkeley, CA. They provided no financial support; but they provided facilities to support our research. Personnel in the Molecular Foundry of LBNL also provided training for fabrication of imprinting molds. The specific facility is the reactive ion etcher in the Biological Nanostructures Facility of the Molecular Foundry. Future collaboration in actual research has been discussed.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS

A Novel Bandage Contact Lens Against Resistant Fungal Infections With Ocular Drug Delivery
 PR161453 Year 1 Quarter 4
 W81XWH-17-1-0355

PI: Albert F. Yee

Org: University of California, Irvine

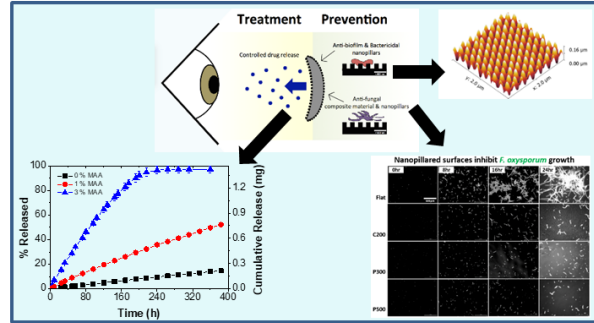
Award Amount: \$2,518,291.00

Study/Product Aim(s)

- Our product aims to serve as a bandage contact lens that has antimicrobial surface structures and an ocular drug delivery system.
- Year 1 (8/2017-8/2018)
- Year 2 (8/2018-8/2019)
- Year 3 (8/2019-8/2020)

Year 1, Quarter 4 Approach

- Established biological assays for testing antimicrobial activities on pillared surfaces
- Confirmed stability of pillared chitosan hydrogels
- Sustained drug release achieved from sandwich bandage contact lenses with PLGA as central reservoir and pHEMA as outer layer



High-fidelity nanopillared surfaces have been made out of both synthetic and biopolymers, showing excellent antifungal and antibacterial properties. The three-layer contact lenses consisting of the drug-eluting layer in between the soft hydrogel layers exhibit controlled release profiles of natamycin for more than 10 days.

Timeline and Cost

Activities	CY	2017	2018	2019	2020
Create central hydrogel lens material					
Fabricate nanostructured surface					
Fabricate & embed drugs into lens					
Test in murine and rabbits trials					
Estimated Budget (\$K)		\$630K	\$630K	\$630K	\$630k

Updated: 1 August 2018

Goals/Milestones

- CY17 Goal** – Create central hydrogel lens material
 - fabricate composite materials capable of drug loading
- CY17 Goals** – Fabricate nanostructures surface
 - Fabricate several blends of biocomposite material
- CY18 Goal** – Test anti-fungal properties of surface
 - Test fungi on nanostructured surface to evaluate cell death
- CY18 Goal** – Fabricate anti-fungal drugs
 - Fabricate anti-fungal nanoparticles
 - Embed drugs into bandage contact lens

Budget Expenditure to Date

Projected Expenditure: \$839,430 (as of 7/31/18)
 Actual Expenditure: \$ 791,523 (as of 7/31/18)

9. APPENDICES

A Novel Bandage Contact Lens Against Resistant Fungal Infections With Ocular Drug Delivery
 PR161453 Year 1 Quarter 4
 W81XWH-17-1-0355



PI: Albert F. Yee

Org: University of California, Irvine

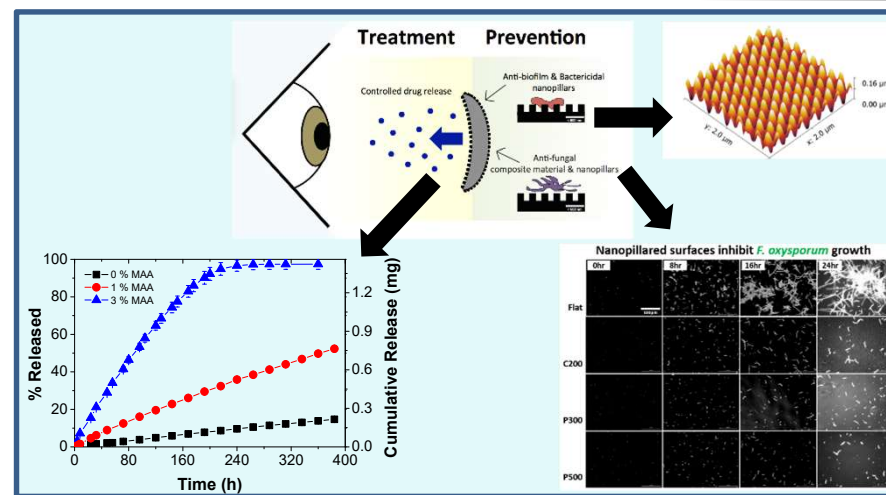
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Year 1, Quarter 4 Approach

- Established biological assays for testing antimicrobial activities on pillared surfaces
- Confirmed stability of pillared chitosan hydrogels
- Sustained drug release achieved from sandwich bandage contact lenses with PLGA as central reservoir and pHEMA as outer layer



High-fidelity nanopillared surfaces have been made out of both synthetic and biopolymers, showing excellent antifungal and antibacterial properties. The three-layer contact lenses consisting of the drug-eluting layer in between the soft hydrogel layers exhibit controlled release profiles of natamycin for more than 10 days.

Timeline and Cost

Activities	CY	2017	2018	2019	2020
Create central hydrogel lens material		█			
Fabricate nanostructured surface		█			
Fabricate & embed drugs into lens		█			
Test in murine and rabbits trials				█	
Estimated Budget (\$K)		\$630K	\$630K	\$630K	\$630k

Goals/Milestones

CY17 Goal – Create central hydrogel lens material

- fabricate composite materials capable of drug loading

CY17 Goals – Fabricate nanostructures surface

- Fabricate several blends of biocomposite material

CY18 Goal – Test anti-fungal properties of surface

- Test fungi on nanostructured surface to evaluate cell death

CY18 Goal – Fabricate anti-fungal drugs

- Fabricate anti-fungal nanoparticles
- Embed drugs into bandage contact lens

Budget Expenditure to Date

Projected Expenditure: \$839,430 (as of 7/31/18)

Actual Expenditure: \$ 791,523 (as of 7/31/18)

Updated: 1 August 2018