Summer 8-12-2021

Dissolvable microneedles and nanofiber dressings to eradicate biofilms and improve wound care

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Summer Undergraduate Research Program

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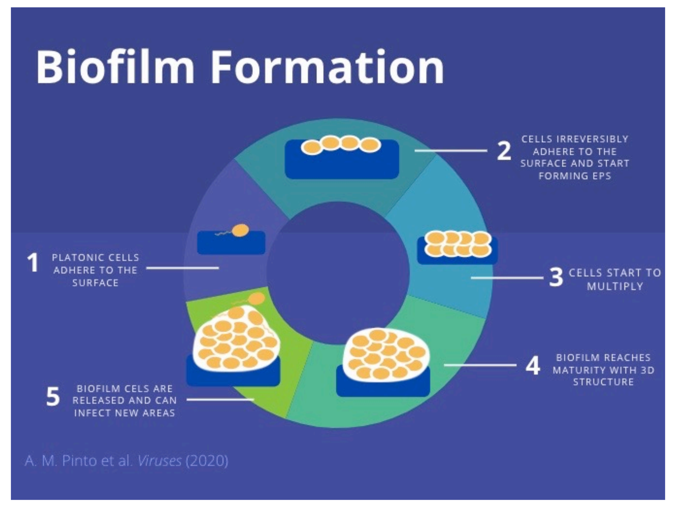
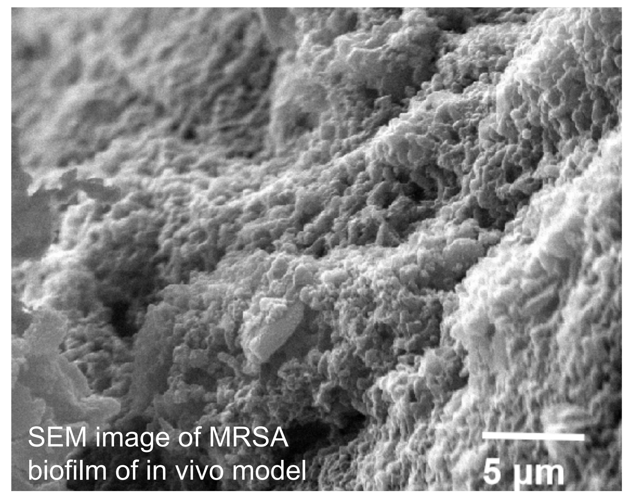
Background

- Biofilms are communities of microorganisms consisting of one or more bacterial species that attach to a biotic or abiotic surface
- They can consist of a single bacterial species or multiple species, although mixed-species biofilms are more common
- The formation of these microbial communities consists of multiple steps
  1. Platonic (free-swimming) cells attach to a surface
  2. Cells become irreversibly attached to the surface and begin to form extracellular polymeric substances (EPSs) that form the structure of the biofilm
  3. The biofilm becomes more layered
  4. When the biofilm reaches maturity, it contains a three-dimensional (3D) structure
  5. Cells from this 3D biofilm can then be released and infect other areas



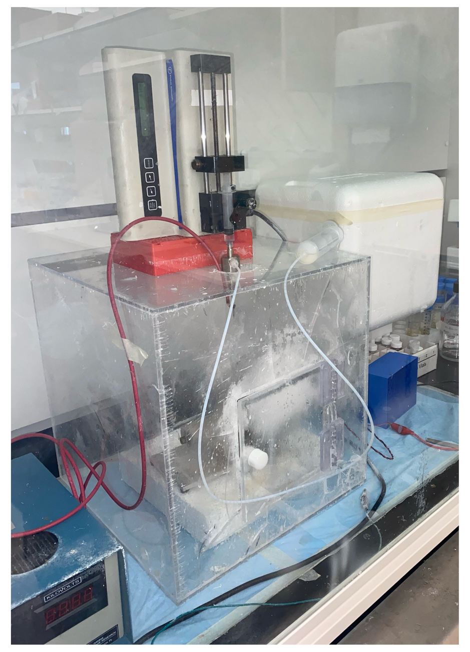


SEM image of MRSA biofilm of in vivo model

- Biofilms are highly common in chronic wounds
- These biofilms often consist of several species of multidrug-resistant bacteria, which make their eradication extremely difficult and therefore causes challenges for wound care
- In diabetic foot ulcers, failure to prevent or properly treat these infections can lead to amputation, sepsis, and sometimes death. Biofilm removal currently is done by sharp/surgical debridement, which causes discomfort for patients

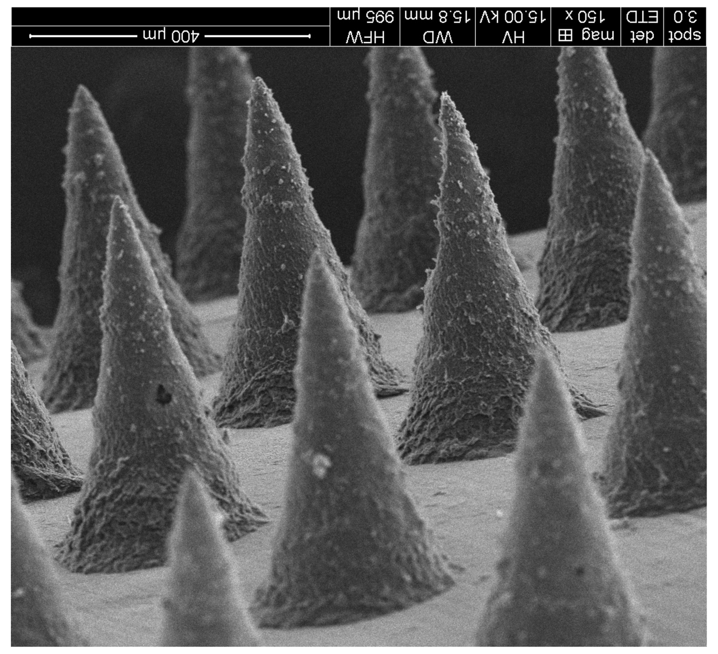
Electrospinning

- Electrospinning is a technique used to develop fibers with nanoscale diameters
- Consists of a power supply, a syringe pump, a spinneret, and a conductive collector
- Liquid comes from the spinneret, is electrified to generate a jet, and then stretched to create fibers which accumulate on the collector
- Electrospun nanofibers can easily incorporate drugs
- The diameters of fibers can be controlled by the applied voltage, flow rate of the solutions, and distance between the spinneret tip and collector
- Coaxial spinning was used to create nanofibers in the present research with a peptide core and a polymer shell



Janus-type dressings

- To improve wound care treatment, a Janus-type antimicrobial dressing was created to eradicate biofilms in chronic wounds
- The dressing consists of two layers: a top layer of electrospun nanofibers and a bottom layer of dissolvable microneedles
- The top layer is intended to destroy surface-layer bacteria while the microneedles could penetrate beneath the surface of the biofilm to kill bacteria beneath the surface
- When loaded with antimicrobial peptides, these dressings could provide an initial burst of peptides followed by a sustained release of peptides
- Electrospun nanofibers consisted of a F127 + peptide core and a polycaprolactone (PCL) shell
- PCL is a biocompatible and biodegradable polymer
- Microneedle arrays were made of solutions containing polyvinylpyrrolidone (PVP) with peptide
- PVP is a water-soluble material which allows it to penetrate beneath the biofilm surface



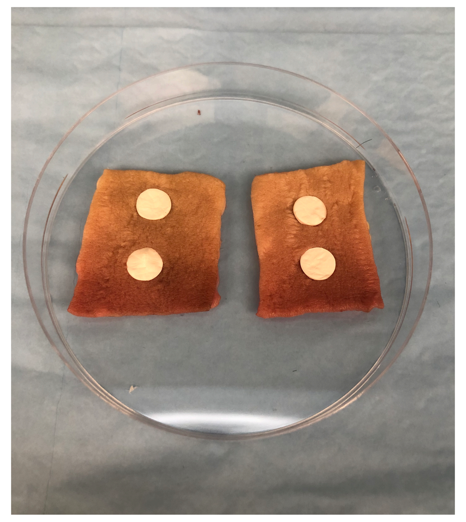
Methods

Electrospun Nanofibers
- PCL was dissolved in DCM and DMF to create the shell solution
- F127 and peptide were dissolved in water to create core solution
- Fibers were made through coaxial electrospinning

Janus-type Dressings
- Microneedle patches used for control and experimental groups were created using a polydimethylsiloxane (PDMS) micromold
- A 20 % polyvinylpyrrolidone (PVP) solution loaded with peptide was added to the molds and then placed in a vacuum
- The nanofiber mat was placed onto the microneedle patch and allowed to dry to attach the two layers of the dressings before the mold could be removed

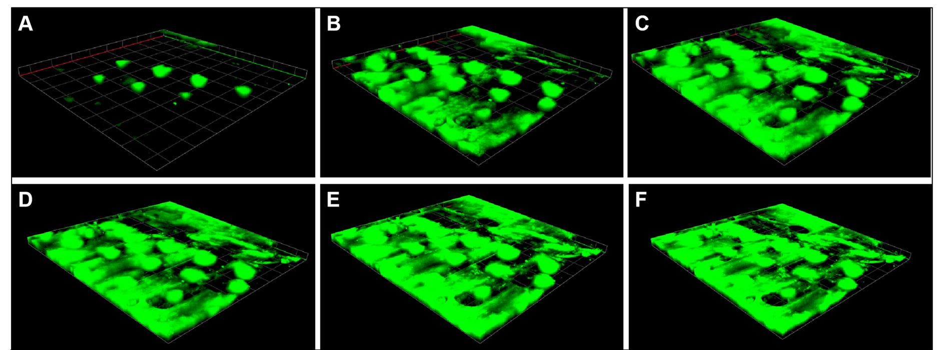
Ex Vivo Model
- Human skin wound model was used with *A. baumanni*, *P. aeruginosa*, and MRSA biofilms
- Janus dressings were applied to wounds and CFUs were counted to determine effectiveness of dressing

In Vivo Model
- Wound model in type II diabetic mouse with MRSA biofilms
- Janus dressings were applied to wounds and CFUs were counted to determine effectiveness of dressing

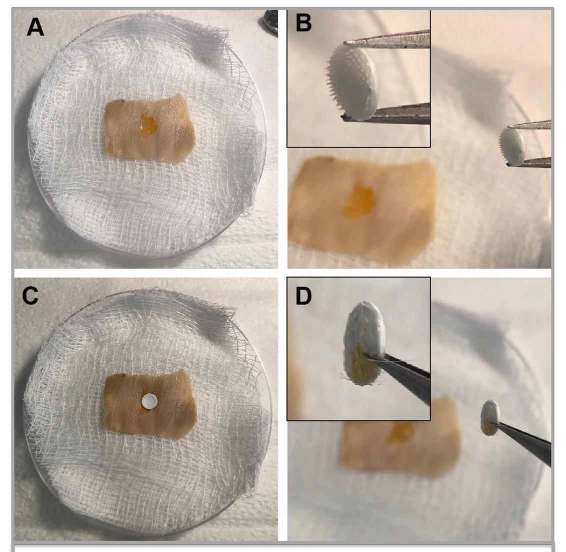


Results

Ex Vivo Model
- Dressings with peptide-loaded nanofibers and peptide-loaded microneedles were more effective than dressings where only nanofibers were loaded with peptide
- After changing dressings once a day for three days, MRSA biofilms were completely eradicated

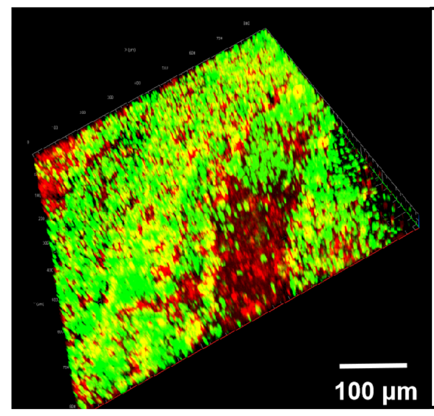


Fluorescein isothiocyanate was used in microneedles to show peptide distribution in MRSA biofilms on skin wounds. Confocal microscope photos A-F show distribution after 0, 20, 40, 60, 80 and 100 min, respectively.



Photos A and B show dressing before application to human skin wound, photos C and D show dislodged microneedles after dressing was applied to wound for 3 min.

In Vivo Model
- Findings consistent with the ex vivo model results
- As in ex vivo model, after changing dressings once a day for three days, there was no MRSA found on the wounds



Shows live/dead staining on SEM image of MRSA biofilm to confirm its formation, where green indicates live cells and red indicates dead cells.

Conclusion

- Janus-type dressings with loaded nanofibers and microneedles proved to be effective in destroying biofilms
- Peptide-loaded microneedles effectively penetrated the biofilm and resulted in more efficient eradication of biofilm
- More studies on human wound models should be conducted to better evaluate the dressings' use for real patients
- The results show that Janus-type dressings could be an effective method to destroy biofilm in wounds to prevent initial as well as delayed infection and could therefore promote better wound care treatment

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