Antibacterial Silk Fibroin Based Biofabrics with Controlled Release

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Introduction

Pathological biofilm formation limits the functionality of implants and scaffolds, leading to the investigation of biofabrics as a promising solution. These versatile fabrics are used in medical applications such as drug delivery, wound dressings, and grafts/meshes, as well as in military clothing and bandages with embedded particles. However, most biofabrics struggle with a trade-off between antibacterial properties and mechanical strength. Combining polyethyleneimine (PEI) and silk can address this issue, providing both properties effectively. This combination, seen in SF-PEI biofabrics, is expected to offer superior mechanical strength and prolonged antimicrobial effects, showing potential in medical and military contexts. To assess this approach, we studied its impact on the antibiofilm activity of the opportunistic pathogen Pseudomonas aeruginosa, which causes challenging infections in skin, lungs, and respiratory tissues.

P aeruginosa biofilm formation

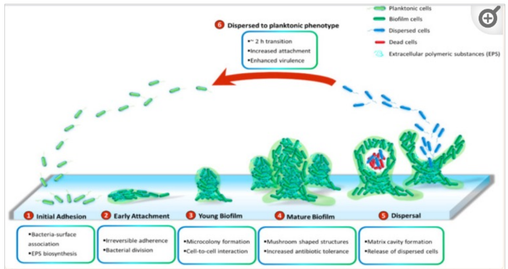


Fig. 1. *P. aeruginosa* has been shown to grow slowly as unattached call aggregated under hypoxic and anoxic conditions. Biofilms of *P. aeurginosa* can be developed on abiotic surfaces, of note being medical implants or industrial equipment. The biofilm formation of *P. Aeruginosa* can be broken down into six stages as described above.

Objectives of the Study:

- ❑ To embed SF-PEI microparticles into polymethyl methacrylate (PMMA) resins to assess the mechanical and physical properties
- ❑ To embed an SF-PEI biofabric into a PMMA resins for similar analysis as the first goal
- ❑ Compare these resins' acute and prolonged antimicrobial effect

Methods

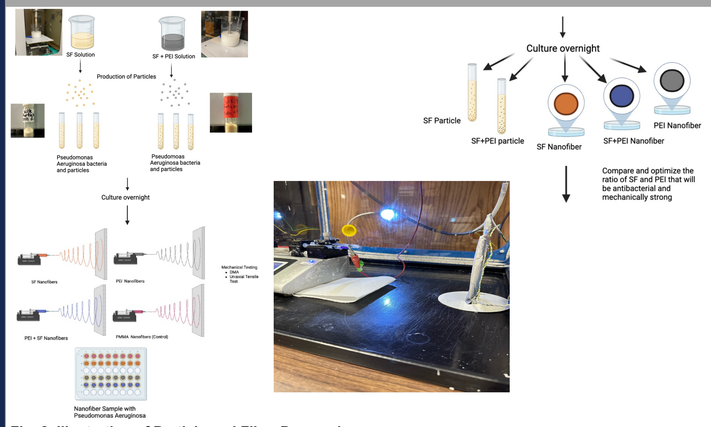


Fig. 2. Illustration of Particle and Fiber Processing

Methods

Silk Extraction

Fig. 3 A , B, C The process, as depicted in Fig. 3 A, B, C, involved mincing silk cocoons from Bombyx mori silkworms and boiling them in a sodium carbonate solution to separate sericin and fibroin. The fibroin yarn was then washed, dried, dissolved in a SF/LiBr solution, and dialyzed against ultrapure water with periodic changes. The solution concentration varied from 5.0-5.5% w/v due to batch differences.

Particle Creation

For SF microparticle formation 25 ml of 4% w/v silk solution was added dropwise to a 600 ml beaker of 250 ml acetone then sonicated using a sound enclosed Qsonica probe sonicator. This solution was sonicated for 30 minutes with a 50% amplitude with 30 seconds pulses. Afterwards, the solution was left out overnight in the fume hood and while constantly stirred. The remaining dried particles were then crushed with a mortar and pestle and set aside. Similarly, SF|PEI microparticle formation, the process is identical to the SF microparticle formation with the addition of 1% w/v PEI addition to the solution.

Biofabric Creation

PMMA, PMMA+SF, PMMA + PEI, and PMMA+PEISF biofabrics were created by electrospinning. A 10 ml plastic syringe with a metallic blunt 21 gauge needle was placed horizontally onto a syringe pump. The stationary plate collector was 20 cm from the pump and a high-voltage power supply set to 20 kV was used to induce electrostatic forces. It operated at room temperature and a humidity ranging from 12-20%. A constant flow rate of the polymer from the pump at 1.4 ml/hr.

Antibacterial Activity Assays

Pseudomonas Aeruginosa (ATCC 39327) was used to assess the antibacterial effects of SF and PEI particles using two assays. In the biofabric antibacterial test, eight 10mm samples were placed in a 64-well plate, and P. Aeruginosa was seeded into each well. For the antibacterial nutrient broth assay, three samples of 5 mg of SF|PEI were placed in 5 ml of nutrient broth, alongside three samples of 5 mg of SF particles and 5 ml of nutrient broth, as well as 5 ml of plain nutrient broth without particles. P. Aeruginosa was seeded into all vials, which were then incubated at 37°C. After 24 hours, absorbance was measured using a spectrophotometer.

Results

Particle Characterization

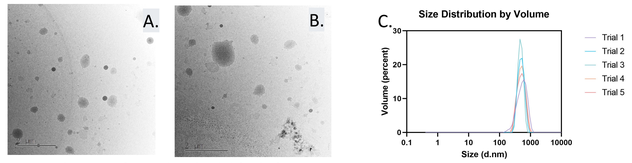
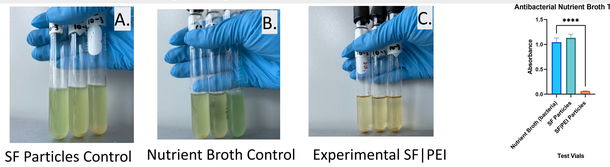


Fig 4: Particles were formed and solidified after sonication in acetone and left overnight in a tume hood. Their morphology was observed using a transmission electron microscope at 5000x magnification (See A,B), revealing spherical or egg-shaped particles with mostly smooth surfaces and no obvious pits or pores. Size distribution was measured using a dynamic light scattering machine at 25°C with a Z-average ranging from 500-700 nm, showing one consistent peak and a polydispersity index of 0.473, indicating a narrow size distribution suitable for measurement with DLS (See C). This supports the successful creation of particles within a specific range and consistency.

Particle Antibacterial Activity Assay



SF Particles Control | Nutrient Broth Control | Experimental SF|PEI

Fig. 5 shows the absorbance data collected using a spectrophotometer after 24 hours of bacterial infusion. The SF Particles control and Nutrient Broth Control exhibited green coloration indicative of bacterial growth (See A,B). In contrast, SF|PEI particles remained yellow, indicating no discoloration (See C). The SF|PEI sample had the lowest absorbency, suggesting the lowest P. aeruginosa count, likely due to PEI's antibacterial properties inhibiting bacterial growth. Statistical analysis revealed significant differences in data between SF|PEI samples (p value <0.0001), highlighting its efficacy. SF particles also showed bacterial growth but were not statistically significant compared to the control.

Results

Biofabric Characterization: SEM and Size Distribution

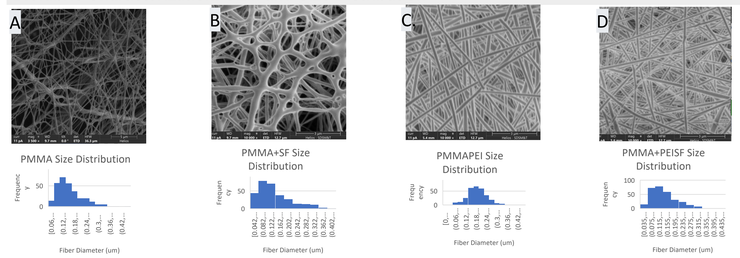


Fig. 6: Biofabric mats were characterized by a scanning electron microscope (SEM) and the size distribution graphed. Four mat types were analyzed, PMMA (A), PMMA+SF (B), PMMA +PEI (C), and PMMA+PEISF (D). PMMA, while having some melted fibers, was mostly composed of straight fibers. In PMMA+SF, more melted fibers were observed, with less defined edges. With the PMMAPEI and PMMA+PEISF mats, the fibers were straight and minimal melting was observed. The average distribution was 0.203 ± 0.058, 0.161 ± 0.067, 0.156 ± 0.076, and 0.169 ± 0.065

Biofabric Antibacterial Activity Assay: SEM

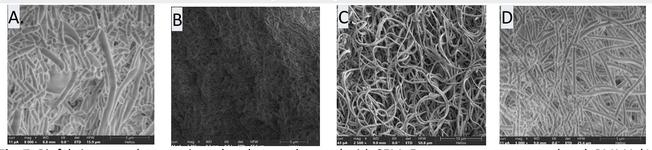


Fig. 7: Biofabric mats who were inoculated were observed with SEM. Four mat types were analyzed, PMMA (A), PMMA+SF (B), PMMA +PEI (C), and PMMA+PEISF (D). PMMA and PMMA+SF showed bacterial growth, while PMMA+PEI and PMMA+PEISF showed little or no bacterial growth.

Biofabric Antibacterial Activity Assay: Live/Dead

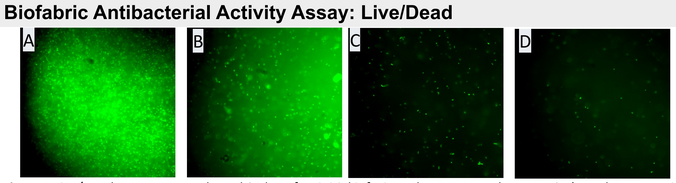
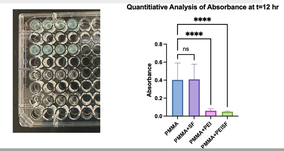


Fig. 7. A Live/Dead Assay was conducted 24 hrs after initial infusion. The PMMA and PMMA+SF (A and B, respectively) biofabric samples indicated numerous bacteria shown by the green coloration. PMMA+PEI and PMMA+PEISF (C and D, respectively) biofabric samples revealed a significant decrease in bacteria.

Biofabric Antibacterial Activity Assay: Absorbance

Fig 8 shows absorbance data collected using a spectrophotometer after 24 hours. Higher absorbance indicates lower bacterial counts, with PMMA and PMMA+SF showing higher levels compared to PMMA+PEI and PMMA+PEISF. This suggests that PMMA and PMMA+SF have higher bacterial counts due to PEI's antibacterial properties, which limit bacterial growth. Statistical analysis revealed significant differences between PMMA+PEI, PMMA+PEISF, and PMMA, with a p-value of <0.0001.



Conclusion and Future Work

- The antibacterial test in nutrient broth found that SF|PEI particles showed antibacterial activity in stopping the bacterial growth of *P. aeruginosa.*
- Both DLS and TEM analysis of the particles helped provide characteristics and proved relative consistency in size
- Create a SF|PEI biofabric using coaxial electrospinning
- To embed a SF-PEI biofabric into a PMMA resins for similar analysis as this study compare these studies acute and long-term effects.

Acknowledgements&References

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