Longevity of Implant-Associated Infectious Biofilms

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Statement of Purpose: From titanium hip implants to bioprosthetic porcine heart valves, biofilm-related infections on implanted devices result in significant risk for patient morbidity and mortality. It has been shown that these biofilms impede antibiotic therapy [1], prevent normal immune response [2], and complicate clinical outcomes [3]. Current treatment includes high dose antibiotic therapy, surgical debridement, and device removal.

In general, the extracellular matrix of the body is primarily proteinaceous, whereas bacterial biofilms consist of insoluble polysaccharides and extracellular DNA. The fate of this polysaccharide matrix after treatment with high dose antibiotics is unclear. Because of immune system hindrance [2], it is possible that antibiotics decellularize the biofilm but leave behind the polysaccharide matrix, permitting later reseeding by pathogens. This theory may partially explain the frequency of recurrence in implant-associated biofilm infections.

In order to test whether antibiotics or lack of microorganisms had any effect on biofilm stability, biofilm was grown *in vitro* using *Staphylococcus aureus* as a model organism and decellularized with a bacteriocidal antibiotic, a bacteriostatic antibiotic, and a sterilization agent (cefazolin, doxycycline, and ethanol respectively). The biofilm was allowed to remain after decellularization and later recovered for analysis by biomass assay and supernatant incubation to assess biofilm and pathogen viability.

Methods: *Staphylococcus aureus* strain 29213 (ATCC) was incubated in 3-5mL Mueller Hinton broth overnight. The culture was diluted to 1:100 of 0.5 McFarland standard (as determined by absorbance at 625 nm) and 100µL was plated in 96 microwell tissue-culture treated plates for each sample. Five groups were tested: broth alone (B), doxycycline (DXC), cefazolin (CFZ), 95% ethanol (EtOH), and a positive control with broth and bacteria but no decellularization agent (NML). Each group had 6 replicates. These groups were tested at 3 time points: Days 3, 5, and 10. These microwell plates were kept in static conditions at 37°C with daily media replacement. At Day 3, all groups had their decellularization agent added to their respective media (4mg/mL of antibiotic, 95% ethyl alcohol). On Day 4, all wells were washed with 200µL broth three times and 100µL of normal broth was replaced. At the given time point, 50µL from 3 wells of each group was added to 1mL of sterile broth and incubated for 24 hours to test for bacterial viability. The plate was stained with 0.1% crystal violet dye, incubated at room temperature for 10 minutes, and measured by UV spectroscopy at a wavelength of 600nm to assess biomass by a modified protocol developed by Merritt *et al.* [4].

Results: At the Day 5 time point, B, EtOH, and DXC were the only groups which showed no cellular viability. By Day 10, only B and EtOH remained sterile.

As shown in Figure 1, the biomass of all groups increased compared with the Day 3 NML group by Day 10. Both antibiotic groups had significant decreases between Day 3 and Day 5 time points. As expected, NML continued increasing over time. EtOH also continued increasing in biomass across time points.

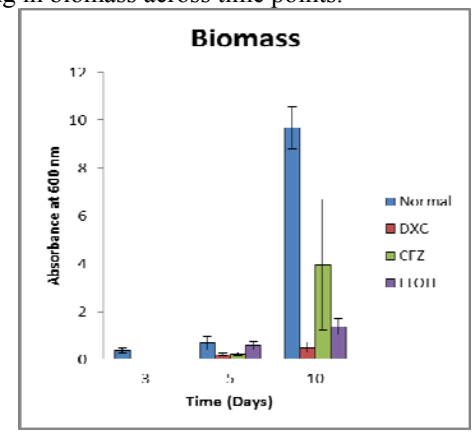

Figure 1. Biomass Assay Results

Conclusions: This study suggests that even at concentrations thousands of times higher than thought necessary for bacterial destruction [1], cefazolin and doxycycline both fail at decellularizing biofilm *in vitro* after 1 week of further incubation. Ethanol is successful at decellularizing biofilm as demonstrated by lack of cellular viability, but contrary to expectations, biomass continued to increase. As this biomass assay depends on nonspecific electronegative interactions of crystal violet with substrate, this may suggest that without microorganisms present, biofilm begins a degradation process which involves exposing additional electronegative groups.

These findings suggest that when an implant-associated infection is treated with antibiotics, the extracellular matrix of the pathogen may remain even after removing a majority of microorganism. This extracellular matrix, besides possibly interfering with material function, may encourage reinfection. The material itself may need to be removed and replaced to entirely eradicate the polysaccharide matrix. These findings reaffirm the importance of developing novel surface coatings for the prevention of bacterial adherence.

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References:

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