Polymorphonuclear leukocyte enhancement of bacterial biofilms on contact lens surfaces

Naiya B. Patel, Jorge A. Hinojosa, Meifang Zhu, and Danielle M. Robertson
Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX

INTRODUCTION

Contact lens (CTL)-related microbial keratitis is the most severe and visually devastating complication associated with CTL wear, with the potential to affect more than 150 million CTL wearers worldwide. It is well established that hypoxia induced by low oxygen transmissible lens wear results in corneal epithelial surface damage and thus represents a major risk factor for lens-related infection.[1,2] Recent studies in our laboratory however, have shown that wear of ultra high oxygen transmissible lenses does not reduce the rate of infection and this finding is supported by current epidemiological evidence.[3,4] Our laboratory has further established that invasive corneal isolates of *P. aeruginosa*, when trapped under the lens surface during wear, exploit the robust subclinical inflammatory response to enhance colonization on lens surfaces and facilitate lipid raft-mediated uptake into the corneal epithelium.[5]

PURPOSE

This study investigated the capacity of five FDA test strains, all commonly associated with CTL-related inflammatory events, to form biofilms on CTL surfaces in the presence of neutrophil-derived cellular debris.

MATERIALS AND METHODS

Bacterial Strains: Unworn Lotrafilcon B silicone hydrogel CTLs (Alcon Laboratories, Ft. Worth, TX) were used in this study. Each lens was incubated overnight with one of five ATCC reference strains with or without stimulated neutrophils: *Pseudomonas aeruginosa* (9027), *Staphylococcus epidermidis* (35984), *Staphylococcus aureus* (6538), *Stenotrophomonas maltophilia* (13637), and *Serratia marcescens* (13880).

Neutrophil Isolation: Neutrophils were isolated from human blood using Ficoll gradient separation and stimulated using phorbol 12-myristate 13-acetate.

Bacterial Viability: To assess bacterial viability, inoculated CTLs were stained using a BacLight Bacterial Viability Assay and imaged using laser scanning confocal microscopy. Image stacks were reconstructed three dimensionally using IMARIS software. Viability was also assessed using standard colony counts.

Scanning Electron Microscopy: Biofilm architecture on CTL surfaces was visualized using a Zeiss Sigma VP Field Emission Scanning Electron Microscope.

RESULTS

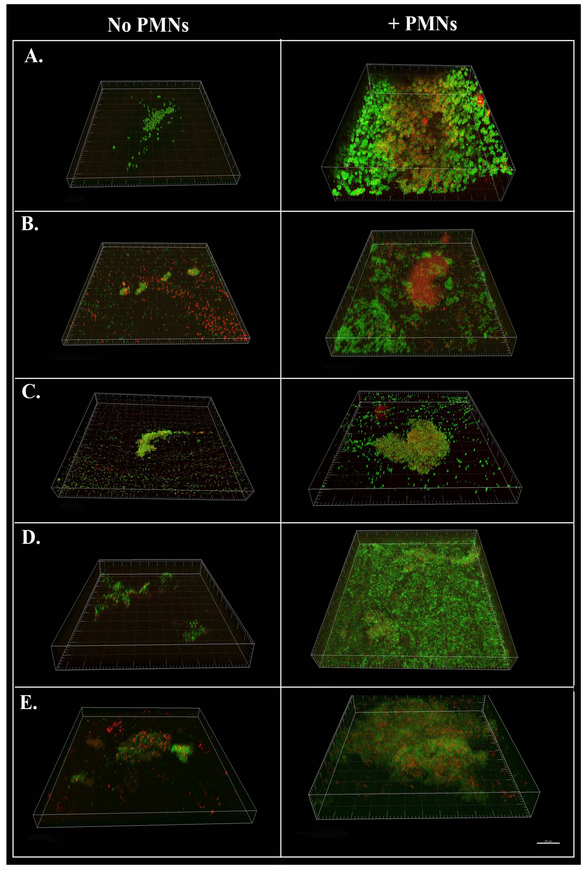


Figure 1: BacLight staining of colonized bacteria. (A-E) All five strains demonstrated increased adherence to CTL surfaces in the presence of dying neutrophils (viable bacteria shown in green; non-viable bacteria in red). *S. aureus (A), S. maltophilia* (B), *S. epidermidis* (C), *P. aeruginosa* (D), and *S. marcescens* (E). Compared to the other 4 test strains, *S. epidermidis* (C) showed the smallest increase in bacterial colonization when incubated with PMNs. Scale bar: 20 µm.

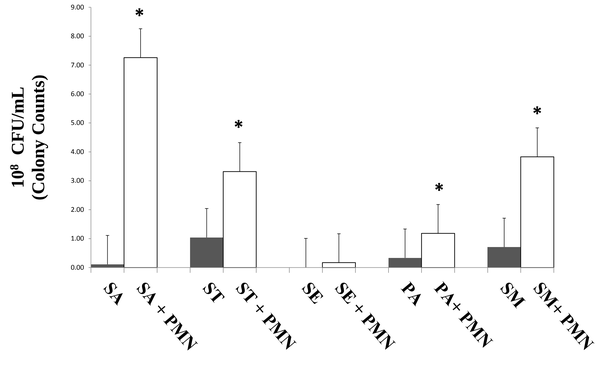


Figure 3: Colony counts of bacteria harvested from CTL surfaces after overnight culture with and without PMNs. CFU counts were significantly increased compared to the no neutrophil control group for the following bacterial strains: *S. aureus* (SA, *p<0.001, n=6), *S. maltophilia* (ST, *p<0.001, n=6), *P. aeruginosa* (PA, *p=0.030, n=6), and *S. marcescens* (SM, *p<0.001, n=6). There was not a significant increase in CFU counts compared to controls for *S. epidermidis* (p=0.659, n=6). Data expressed as mean ± standard deviation. Graph representative of three independent experiments.

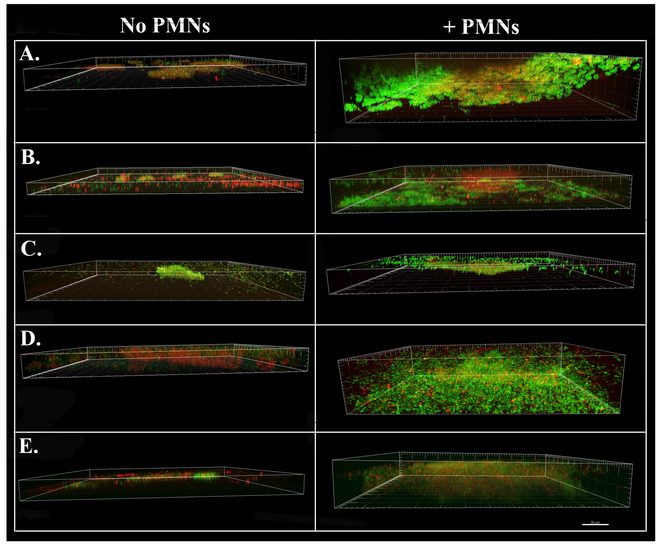
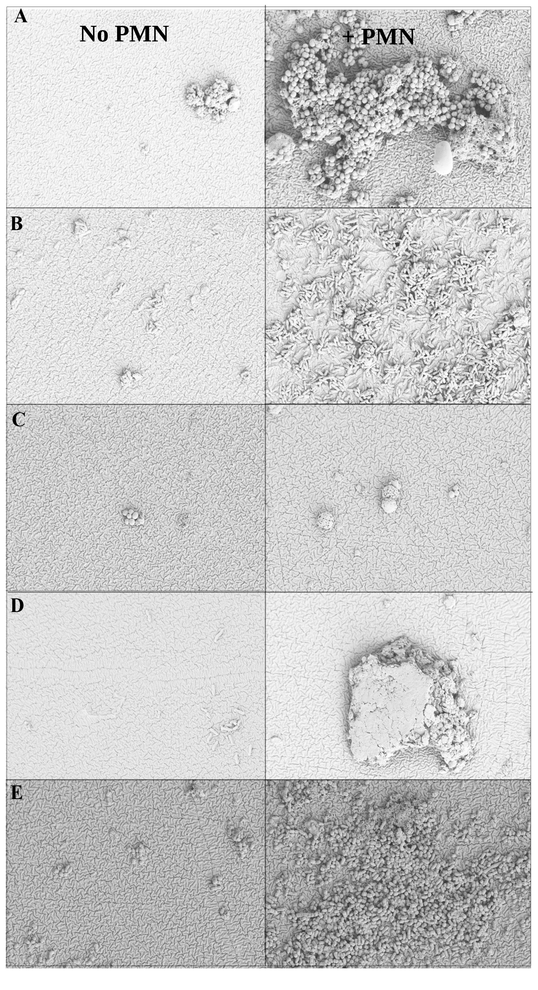


Figure 2 (left): Impact of dying PMNs on bacterial biofilm thickness using *in vitro* 3D confocal microscopy. *S. aureus (A), S. maltophilia* (B), *P. aeruginosa* (D), *S. marcescens* (E) all showed a substantial increase in thickness on CTL surfaces when incubated with PMNs compared to bacteria alone. *S. epidermidis* (C) showed little to no increase in bacterial biofilm thickness when incubated with PMNs. Scale bar: 20 µm.

- For all strains tested, BacLight staining showed small patchy regions of viable bacteria across the contact lens surface. The area and thickness of these regions was increased in the presence of dying neutrophils (Fig. 1 and 2).
- CFU analysis confirmed a significant increase in viable bacteria harvested from lenses that were co-cultured with neutrophils for 4 of the 5 strains evaluated (Fig. 3). This was not significant for *S. epidermidis*.
- Scanning electron microscopy also showed enhanced biofilm thickness and architecture on lenses incubated in the presence of PMNs for all bacterial strains with the exception of *S. epidermidis* (Fig. 4).



DISCUSSION

Contact lenses serve as a vector for the introduction of pathogens to the eye. In addition to infectious keratitis, the presence of lens-associated bioburden is also a major risk factor for the development of contact lens-related inflammatory events, which often necessitate therapeutic management and discontinuation of lens wear.[6] Our prior work has shown that invasive clinical isolates of *P. aeruginosa* can exploit the robust inflammatory response to accelerate colonization of lens surfaces.[5] In this study we demonstrate that adherence to contact lens surfaces is dramatically increased in the presence of neutrophils for certain *Staphylococcus* species, *Serratia*, *Stenotrophomonas*, and *Pseudomonas*. Importantly, these are all bacteria frequently encountered during contact lens use. Collectively, these findings suggest that, in the setting of intense inflammation under the lens, gram-negative and gram-positive pathogens possess the capacity to colonize and resist clearance by the innate immune system. Since soft lenses exhibit relatively little to no tear exchange during blinking when compared to rigid lenses, this may explain why rigid lens wear represents the safest modality of wear. Further studies are needed to correlate these findings with disease in an animal model.

REFERENCES

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Figure 4 (left): Scanning Electron Microscopy of Contact Lens Surfaces. (A-E) Representative 20x images of contact lens surfaces incubated with bacteria +/- PMNs. *S. aureus (A), S. maltophilia* (B), *S. epidermidis* (C) *P. aeruginosa* (D), and *S. marcescens* (E).

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