The Surprising Power of Bacterial Biofilms

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Biofilms play an important role in a variety of infectious diseases. They pose a significant challenge to infection control because of the high level of antibiotic resistance they confer on their constituent bacteria. Advances in understanding the biology and pathophysiology of bacterial biofilms may lead to novel therapeutics and strategies for the treatment of ocular infections.

Bacteria in a microbiology laboratory are often described as “planktonic,” meaning that they are unattached and floating freely in culture medium. In nature, however, most bacteria are believed to live as part of a biofilm, an organized bacterial community that can adhere to abiotic or biotic surfaces.1 Biofilms can vary greatly in nature and complexity: the slime on a river rock and the plaque on our teeth, as different as they may seem, are both biofilms.

Biofilms are ubiquitous in nature. They form readily in aquatic environments, even extreme ones such as hot springs. For this reason bacterial biofilms often create problems in the human environment, clogging industrial pipes and forming unsightly films on kitchen sinks and bathroom showers. The clinical importance of biofilms lies in their association with acute and chronic infectious disease; and biofilms are of special concern when a medical device is involved.2,3

Unlike their planktonic counterparts, bacteria in biofilms tend to be extremely tolerant of antimicrobial treatment. If we are to prevent and treat infections from biofilm bacteria, a better understanding of this common bacterial habitat is necessary.

Structure and Ecology

Bacteria form biofilms when they establish in new locations. Given water and air, most species of bacteria can attach to an available surface and develop into a biofilm. The process begins with the attachment of planktonic bacterial cells and the formation of microcolonies (Figure 1). Quorum sensing among the bacteria controls the process by which the biofilm colony matures; and bacterial communication between bacteria within the colony regulates the making of a matrix of polymeric substances that is composed of polysaccharides, proteins,
and extracellular DNA. Once mature, the biofilm colony can release planktonic bacteria to migrate and form new biofilms at additional sites.

Within a given biofilm there may be different microenvironments that support bacterial subpopulations with different phenotypes. This diversity in the pattern of gene expression within a biofilm fosters survival of the whole community, as diversity increases the chance that at least some subpopulations of bacteria within the biofilm will survive an environmental challenge. For example, within the biofilm there are populations of bacteria that are dormant and not actively dividing; these bacterial cells may be significantly less vulnerable to antibiotics that kill by targeting replication.

The close cell-to-cell contact within a biofilm facilitates exchange not only of nutrients and signaling chemicals but also of chromosomal and extrachromosomal DNA. Horizontal gene transfer provides a mechanism for spreading beneficial attributes and promoting survival in a particular environment. For example, genetic material spread horizontally through a colony may encode for phenotypic attributes that confer antimicrobial resistance.

**A Prominent Role in Infections**

Most infections of prostheses or other non-biologic materials implanted into the body are associated with bacterial biofilms. Biofilms on catheters can lead to blood and local skin infections; those on prosthetic heart valves can cause endocarditis. Both heart and bone infections are frequently biofilm-related, and pneumonia associated with cystic fibrosis is difficult to treat in large measure because the bacteria are typically in a biofilm state.

Bacterial infections involving biofilms are often chronic and persistent. In these cases the biofilms serve as bacterial reservoirs that continuously leak bacteria into the body, leading to recurrent infections. Often these infections can’t be eradicated unless the biofilm is first physically removed. In addition to caus-

**STATEMENT OF NEED**

Ophthalmologists face numerous challenges in optimizing their competencies and clinical practices in the realm of preventing, diagnosing, and treating ocular infections and their sequelae; these challenges include:

- The widespread “off-label” use of topical antimicrobial antibiotics to prevent and treat serious and sight-threatening infections—given the reality that the most widely used topical antibiotics in ophthalmology have FDA approvals restricted to bacterial conjunctivitis.
- The escalating levels of multi-drug resistance in common ophthalmic pathogens.
- The emergence and increasing prevalence of atypical infections that may require diagnostic and treatment techniques relatively unfamiliar to comprehensive ophthalmologists.
- The introduction of new and potentially more efficacious and/or safe antimicrobial strategies.
- The introduction of new and potentially more accurate diagnostic techniques for ophthalmic infections.
- Widespread discussion over the efficacy and safety of novel or alternative delivery techniques and vehicles for prophylactic antimicrobial strategies (including but not limited to intracameral injection and topical mucosal delivery).
- Increased understanding of the inflammatory damage caused by ocular infections and the best ways to prevent/allove inflammation without fueling the growth of pathogenic organisms.

Given the continually evolving challenges described above, **Topics in Ocular Antimicrobials** aims to help ophthalmologists update outdated competencies and narrow gaps between actual and optimal clinical practices. As an ongoing resource, this series will support evidence-based and rational antimicrobial choices across a range of ophthalmic clinical situations.

**REFERENCES**


**OFF-LABEL USE STATEMENT**

This work discusses off-label uses of antimicrobial medications.

**GENERAL INFORMATION**

This CME activity is sponsored by the University of Florida College of Medicine and is supported by an unrestricted educational grant from Bausch + Lomb, Inc.

**Directions:** Select one answer to each question in the exam (questions 1–10) and in the evaluation (questions 11–16).

**DATE OF ORIGINAL RELEASE**

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**FACULTY AND DISCLOSURE STATEMENTS**

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This activity is supported by an unrestricted educational grant from Bausch + Lomb, Inc.
ing chronic inflammation at the site of infection, a biofilm may also jeopardize adjacent tissues, as inflammatory neutrophils, unable to clear the biofilm, release damaging molecules such as reactive oxygen species.

**Eye Infections**

The ocular surface does not offer an ideal environment for biofilm formation. Constant blinking and tear film are effective in removing contaminating microbes. In addition, the tear film contains an assortment of antibacterial compounds that can hamper microbial growth, including lactoferrin, lysozyme, and defensins. The picture changes radically, though, when an artificial surface, such as a contact lens, is introduced. Indeed, bacterial biofilms are frequently found on contact lenses and contact lens cases and may contribute to the development of keratitis. Planktonic bacteria released from the contact lens biofilm could, for example, adhere to and infect a mildly abraded cornea. This makes proper cleaning and disinfection crucial aspects of contact lens hygiene. Mechanical removal of potential biofilms is important, and we recommend rubbing lenses when cleaning them rather than simply letting the lenses sit passively in disinfecting solution.

Punctal plugs and ocular implants, including intraocular lenses (IOLs) and keratoprosthesis, can develop biofilms as well. In one case, a biofilm attached to an IOL was identified as a cause of recurrent endophthalmitis following uneventful cataract surgery. Biofilms can also develop in sterilizers, contaminating surgical tools and leading to postoperative infection and inflammation. In addition to causing or supporting device-related infections, biofilms may also occur during the process of infection. Biofilms have been implicated in various ocular infections including blepharitis, keratitis, postoperative endophthalmitis, and infectious crystalline keratopathy. In general, the bacterial species implicated in endophthalmitis are gram-positive organisms, such as coagulase-negative staphylococci, *Staphylococcus aureus*, and *Streptococcus* species, whereas in keratitis, gram-negative pathogens, such as *Pseudomonas aeruginosa* and *Serratia marcescens*, are more common (Figure 2).

**Diagnostic and Treatment Challenges**

Biofilm-related infections may be difficult to recognize as such. Device-related, chronic infections are often quiescent in nature. Because of low planktonic shedding rates, biofilm bacteria may be difficult to isolate or culture. Identifying an abiotic surface or device that has developed a biofilm is certainly helpful, but in many cases, a persistent and antibiotic-resistant infection may be the only hint that a biofilm is present. Biofilm bacteria are remarkably capable of tolerating both antibiotics and host immune responses. The complex architecture of a biofilm not only provides expanded surface areas for bacterial cells to grow, but also protects them against environmental challenges. Thus, biofilm bacteria can be up to 1,000-fold more resistant to antibiotics than their planktonic counterparts. Biofilm bacteria are often similarly impervious to antibody-mediated phagocytic clearance.

As previously noted, many of the bacteria in a biofilm can tolerate antibiotic treatment because they are not actively dividing. Compared to actively growing bacterial cultures, biofilms also contain a greater number of persister cells, small subpopulations of dormant bacteria that exhibit an extraordinary ability to survive an environment that would be lethal to planktonic cells. This includes environments made hostile to bacteria by high antibiotic concentrations. These persisters are believed to play an important role in biofilm resilience and recurring infections, as a few surviving persisters are enough to repopulate a biofilm.
The biofilm is also a mechanical barrier that retards drug or disinfectant penetration. Even when an agent is able to penetrate into the microenvironment of the biofilm, it may become less efficacious due to unfavorable levels of oxygen or pH.

Biofilm bacteria may also express genes that confer antimicrobial resistance. One typical example is the ndvB gene of *Pseudomonas aeruginosa* that codes for a protein that catalyzes the production of cyclic-glucans that are able to bind aminoglycosides and sequester the antimicrobial agents in the periplasm. As noted, these resistance genes could be passed from bacterium to bacterium through horizontal gene transfer. Furthermore, there is a higher natural mutation rate among biofilm bacteria that can foster development of resistance.

**Implications for Ocular Infections**

The current treatment strategy for persistent ocular infections involves going to a different class of antibiotic or adding antiinflammatory agents to reduce inflammation and tissue damage. Frequently neither is effective since antibiotic-tolerant biofilms are often at the root of such chronic infections.

Our group has demonstrated that the bioadhesive polymer used in the ophthalmic preparation of azithromycin and besifloxacin and elsewhere may in-

give the antibiotic access to the causative bacteria in an infection. Sanders and colleagues have found evidence in a rabbit model that the same bioadhesive polymer may have biofilm interference capabilities. The importance of these findings depends on what is eventually determined to be the role of biofilms in ocular infections, especially keratitis. This requires further study.

Recent advances in biofilm research have been focused on anti-biofilm agents that can disrupt the biofilm, restoring the susceptibility of its inhabitants to traditional antibiotics. Such agents could enhance an antibiotic’s bactericidal activity against biofilm bacteria, including the persisters. Silver, an antimicrobial compound in use for generations, has demonstrated such anti-biofilm ability and great potential as an antibiotic adjuvant.

In ophthalmology, anti-biofilm agents may aid in the treatment of keratitis and blepharitis, where bacteria may be sequestered in a biofilm. Anti-biofilm agents may also be used to treat ocular devices and implants to prevent bacterial attachment and biofilm formation.

**REFERENCES**

Poly microbial Ocular Infection

John D. Sheppard, MD

Knowing who is at risk for—and how to detect—poly microbial ocular infection can help avert the aggressive clinical course these infections sometimes take.

Most ocular infections are caused by a single microbe that proves capable of outgrowing other microbes and comes to dominate its milieu. However, a substantial minority of ocular infections is caused by two or more pathogens that are able to co-infect simultaneously.

Poly microbial infections are distinct from colonization by multiple organisms (which is not an infection at all) and sequential infection resulting from re-injury or reexposure. On rare occasions, however, poly microbial infections may present as a seeming sequential infection due to the difference in growth times of the pathogens. Eyecare practitioners must be watchful for poly microbial infections in their at-risk patients, as the presence of multiple pathogens can complicate diagnosis and management and lead to poorer outcomes.

Incidence

Poly microbial ocular infection is rare overall, and its incidence likely varies according to geographic region, wound type, and other factors.

Endophthalmitis following ocular surgery is fortunately rare, and poly microbial cases of postoperative endophthalmitis are very rare indeed. Studies that have examined rates of poly microbial growth among postoperative endophthalmitis cases have found rates between 0 and 17%. In the landmark Endophthalmitis Vitrectomy Study (EVS), which examined treatment options for endophthalmitis following cataract surgery, poly microbial infection was found in 9% of culture-positive endophthalmitis cases. A review at Yale-New Haven Hospital of isolates from surgical and trauma-related endophthalmitis found that 12% of culture-positive vitreous samples from 1988 through 2008 were positive for multiple pathogens. Due to the nonsterile settings in which it occurs, traumatic endophthalmitis is associated with higher rates of poly microbial infection than infectious endophthalmitis following elective surgery. Parallel microbiologic reviews at a single quaternary care center in India reported that poly microbial infections were found in post-traumatic endophthalmitis at nearly twice the frequency (20.4%) of poly microbial infections in postoperative endophthalmitis (12.5%). Interestingly, the distribution of pathogens among poly microbial infections did not mirror that of single-pathogen cases: gram-negative pathogens were proportionally more common among poly microbial infections (30%) compared to single organism infections (18%); and fungal pathogens were proportionally more often seen in bacterial coinfections (30%) than as solo infectious agents (14%). Poly microbial infection comprises a significant minority of infectious keratitis cases. Proximity to the many species of organisms that colonize ocular and periocular tissues makes the damaged cornea vulnerable to poly microbial infection. A retrospective review of all-cause, culture-positive cases of presumed infectious keratitis (N = 307) at Duke University showed that 21% were poly microbial. A separate review of infectious keratitis secondary to vegetative matter injury in India (N = 36) showed a rate of 36%. Risk Factors

As the epidemiologic data mentioned above shows, trauma-related infections, environmental, industrial, or vegetative, increase the risk for poly microbial ocular infection. These infections tend to have rapid onset and can be caused by a wide variety of organisms, depending upon the source of the trauma. Pathogen distribution in postoperative infections tends to mirror the distribution of predominantly gram-positive local flora. Staphylococcus and Streptococcus species caused 89% of postoperative cataract surgery endophthalmitis cases in the EVS, while unusual pathogens, including gram-negative bacteria and fungi, are more common among trauma-related cases. Endophthalmitis that contains gram-negative or fungal pathogens may be associated with worse outcomes.

Immunocompromised individuals comprise the second group at increased risk of poly microbial infection. Ocular
Infection due to immunodeficiency generally occurs in one of five patient classes: 1) premature and very young infants; 2) patients with hematologic malignancies such as leukemia or lymphoma; 3) patients on chemotherapy; 4) patients on aggressive immunotherapy for systemic illness such as rheumatoid arthritis or lupus; or 5) patients with acquired immunodeficiency due to HIV.13,14

A third and very common risk factor for polymicrobial keratitis is contact lens use. The good news about contact lens-related ocular surface infection is that culturing the lens case enhances the probability of identifying the pathogen(s). As contact lenses can trap bacteria and other microbes against an irritated or abraded epithelium, pathogens that might otherwise be washed away from the ocular surface can linger, adhere, penetrate, and cause significant damage. As with ocular trauma, atypical pathogens are more likely to be recovered in contact lens-associated infections than in infections that are introduced in a more controlled situation like surgery.23

**Bacteria and Acanthamoeba**

In recent years, parasites, including microsporidia and species of *Acanthamoeba*, have emerged as important pathogens in external ocular infection and are likely underreported as contributors to polymicrobial infection with fungal, bacterial, and viral copathogens.14 The relationship between amoebic and bacterial organisms is complex, with some bacteria acting as food for the amoeba, particularly many gram-negatives including *E. coli*. Others such as *Pseudomonas* are able to survive inside amoeba as endosymbionts, or so called “amoeba resistant bacteria.”15

The pairing of *Acanthamoeba* and gram-negative bacteria is especially common in contact lens-associated keratitis. The clinical course of this type of coinfection may be deceptively straightforward at first only to deteriorate after the slow-growing *Acanthamoeba* colony has begun to thrive. Patients may respond well to initial treatment with a topical antibacterial such as a fluoroquinolone or aminoglycoside. Eventually, however, these unfortunate patients will return complaining of severe pain, with radial keratodermatitis and a ring infiltrate characteristic of *Acanthamoeba* infection visible on examination. Further questioning of the patient may reveal no known trauma except contact lens wear, or exposure to a source of stagnant water, well water, sewage, or a tainted eyecare product.

*Acanthamoeba* keratitis has also been reported to occur in conjunction with infectious crystalline keratopathy (ICK) caused by gram-positive bacteria, including species of *Staphylococcus* and *Streptococcus*, in contact-lens wearers.15 *Acanthamoeba* is notoriously difficult to culture and requires specific materials and configurations in the laboratory. Some laboratories culture *Acanthamoeba* from corneal scrapings using non-nutrient media that contains *E. coli* slurries or lawns to speed its growth. This method may alleviate the need for corneal biopsy and yield positive results within 5 days.26

Alternatively, treating *Acanthamoeba* or presumed *Acanthamoeba* with lamellar keratectomy can address both diagnostic and therapeutic concerns. The first specimen is submitted for culture on special *Acanthamoeba* or *Naegleria* media. A second inoculant is then placed immediately on a slide and sent to a lab to be examined for cysts by special stain such as Wright or Giemsa stain.

**Fungus and Bacteria**

In both yeast and filamentous forms, keratomycosis has a high rate of coinfection with bacterial pathogens.4,6,17,18 Two US centers that reviewed microbiologic data associated with fungus-positive corneal infections (N = 152) found that concomitant bacterial infection was present in 20%.17 Like amoebae, fungal pathogens are slow growing and require specialized culture media and procedures; detecting them requires a high order of suspicion on the part of the physician. Coinfection with fungal pathogens has clearly been associated with increased risk for negative outcomes.2

**Neurotrophic Keratitis**

Patients with neurotrophic keratitis due to herpes simplex or varicella virus are at increased risk of secondary infection with bacteria, fungi, or protozoa due to decreased ocular surface sensation and a correspondingly reduced ability to recognize early signs of infection. Infections in patients with neurotrophic keratitis therefore usually present later in the course of their disease to the clinic with accelerated morbidity. Healing is further complicated by reduced production of neurotrophins, proteins that promote epithelial growth and healing.

**Diagnosis**

Detection of polymicrobial infection requires a high index of suspicion and a proactive approach, as some significant co-pathogens do not grow on standard bacterial media. Pearls for diagnosis include:

- Be attentive to subtleties of the patient presentation. Diagnosis is still highly dependent on the observational skills, and sometimes the intuition, of the astute clinician.
- If a fungal, protozoan, or mycobacterial pathogen is suspected, be sure to obtain cultures on specialized media.
- When culturing the conjunctiva or cornea, do so prior to use of any eye drop that contains preservatives or antimicrobial agents. If the eye must be anesthetized, use a preservative-free formulation.
- If at all feasible, obtain cultures prior to the application of topical antibiotic.
- After scraping the cornea, inoculate bacterial media first, then fungal media, then make a slide for Gram stain and other stains as indicated. This order avoids contamination from the slide surface, as well as inadvertently moving the antibiotics from fungal media onto the bacterial media and increasing the chances of a false negative bacterial culture.
- Do not delay treatment while awaiting culture results. Empiric treatment should go forward based upon the most likely pathogen or pathogen combination for the clinical scenario.
Empiric coverage for suspected fungal infection should also include coverage for bacteria, as fungi and bacteria commonly grow together in polymicrobial infections.14,16

Going Forward

Pathogen detection in polymicrobial infections will be greatly enhanced when new diagnostic technologies enter the clinical market. The convenience and speed of point-of-care testing for detection of single organisms, such as the in-office test for adenovirus (Adenoplus, RPS, distributed by NicOx), is unmatched. The development of similar tests for other pathogens such as herpes virus or Acanthamoeba would greatly facilitate diagnosis of those pathogens.

Polymerase chain reaction (PCR) is available in an increasing number of research labs around the country and is the definitive diagnostic test because it is more sensitive than traditional culture.19 Lately a switch from DNA to RNA detection technology has increased the sensitivity of this modality even further, as there are logarithmically more copies of RNA in any given pathogen than DNA.

PCR, however, is not without its limitations. One is that it is not available in every lab, and sending specimens to outside labs delays results and increases cost. Waiting for results of a test requiring even 24 hours can jeopardize the viability of an eye that is acutely infected. A second limitation of PCR is that it can detect only one organism at a time, and that target organism must be pre-selected by the clinician. PCR is not a screening strategy.

New technology that uses DNA amplification to simultaneously detect multiple pathogens is in development and already being implemented in some parts of the world. In India, Mahalingham and colleagues reported successful, rapid detection of pathogens causing polymicrobial postoperative endophthalmitis, Propionibacterium acnes and Candida in this case, using a “syndrome evaluation system” known as Xcyton.20

In the June 2013 issue of this publication, Blondeau described technology known as matrix-assisted laser desorption-ionization time of flight (MALDI-TOF) mass spectrometry that can detect multiple pathogens at once and is becoming increasingly popular in Canada and other parts of the world.21 MALDI-TOF mass spectrometry uses a laser to create a spectrum of ions or a “protein fingerprint” from whole bacterial or fungal cells which is then compared with a reference bank of spectra of known organisms to determine a match. The process takes only minutes to complete. Application of this technology would be particularly useful in eye care were inoculum size and specimen site volume are relatively extremely small.

Conclusion

Polymicrobial infections account for a subset of ocular infections and are typically found following trauma or surgery or associated with an immuno-compromised host or contact lens wear. It is important for physicians to remain suspicious of slower growing pathogens that may not be detected on initial culture, including fungal, protozoan, and mycobacterial organisms.

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REFERENCES

EXAMINATION QUESTIONS  TOPICS IN OCULAR ANTINFECTIVES, ISSUE 44

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1. Biofilm infections have been implicated in:
   A. Middle ear infections
   B. Crystalline keratopathy
   C. Heart valve-related endocarditis
   D. All of the above

2. Which of the following best describes “amoeba resistant bacteria”:
   A. Bacteria that serve as a food source to Acanthamoeba
   B. Protozoan species that are pan-resistant to antimicrobial treatment
   C. Bacteria that are used as food for protozoans in amoebic culture media
   D. Bacteria that can survive inside amoebic organisms

3. Which of the patients does not fall into a group at elevated risk for polymicrobial infection?
   A. 68-year-old male taking tamsulosin having routine cataract surgery
   B. 31-year-old female taking methotrexate and prednisone immunotherapy for lupus
   C. 59-year-old male on chemotherapy for prostate cancer
   D. 25-year-old allergic female contact lens wearer

4. All of the following are typical features of biofilms and bacteria residing within them EXCEPT:
   A. They are highly susceptible to antibiotics
   B. They can be difficult to culture
   C. The colony is tolerant of the host immune responses
   D. They can be found on biotic or abiotic surfaces

5. All of the following statements about biofilms are true, EXCEPT:
   A. Biofilms play an important role in bacterial infections in humans
   B. Biofilms can give rise to planktonic bacterial cells
   C. Bacteria within a biofilm cannot exchange genetic information
   D. Most bacterial species can form biofilms, given adequate access to water and air

6. In the Endophthalmitis Vitrectomy Study, what percentage of postoperative endophthalmitis cases were polymicrobial?
   A. 0.9%
   B. 9%
   C. 29%
   D. 69%

7. Which of the following is an unlikely site for formation of a bacterial biofilm?
   A. A rock in a swift running stream
   B. The ocular surface
   C. A shower tile
   D. An animal tooth

8. All of the following are mechanisms by which biofilm bacteria can resist antibiotics and the immune system, EXCEPT:
   A. Presence of a substantial number of persister cells
   B. Physical barrier protection of the biofilm
   C. Transfer of resistance genes among bacteria
   D. All bacteria within a biofilm become highly resistant persister cells

9. Which of the following strategies helps in detection of polymicrobial infection?
   A. Relying entirely upon bacterial and fungal culture results
   B. Obtaining routine PCR testing for relevant fungi on suspicious keratitis cases
   C. Obtaining bacterial, as well as fungal, cultures on all patients with presumed fungal keratitis
   D. All of the above

10. Which of the following clinical scenarios most likely represents a polymicrobial infection?
    A. MRSA and MRSE cultured from a nasal swab of asymptomatic patient
    B. Propionibacteria and Candida isolated from a vitreous sample of a patient who suffered ocular trauma
    C. Acanthamoeba isolated from the contact lens case of a patient with an extremely painful keratitis
    D. All of the above

EXAMINATION ANSWER SHEET  TOPICS IN OCULAR ANTINFECTIVES, ISSUE 44

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