1991

Interactions of Bacteria and Amoebae with Ocular Biomaterials

Thomas John

University of Chicago

Follow this and additional works at: http://digitalcommons.usu.edu/cellsandmaterials

Part of the Biological Engineering Commons

Recommended Citation
Available at: http://digitalcommons.usu.edu/cellsandmaterials/vol1/iss2/5

This Article is brought to you for free and open access by the Western Dairy Center at DigitalCommons@USU. It has been accepted for inclusion in Cells and Materials by an authorized administrator of DigitalCommons@USU. For more information, please contact dylan.burns@usu.edu.
INTERACTIONS OF BACTERIA AND AMOEBAE WITH OCULAR BIOMATERIALS

Thomas John*
Cornea Service and Research Unit
Department of Ophthalmology and Visual Science
University of Chicago, Pritzker School of Medicine
Chicago, IL 60637

(Received for publication April 23, 1990, and in revised form April 16, 1991)

Abstract

The use of biomaterials in periocular and intraocular sites has resulted in some ocular inflammations and infections which can result in vision-threatening ocular disease. This review addresses bacterial interactions with, and adherence to ocular biomaterials such as soft contact lenses, surgical suture materials, and intraocular lenses. In addition, adherence of Acanthamoeba to soft contact lenses is described, and the role of these lenses in the development of Acanthamoeba keratitis is discussed.

Key Words: Biomaterial, soft contact lens, suture, intraocular lens, bacteria, Acanthamoeba, adherence, P. aeruginosa, S. epidermidis, S. aureus.

Introduction

The use of various biomaterials in periocular and intraocular sites has become a part of the medical and surgical armamentarium in present-day ophthalmic care. The continued use of these materials in the clinical practice of ophthalmology has, over time, provided data on some of the inflammatory and infectious complications that can result in vision-threatening ocular disease. Hence, our interest has focused on the interactions of various microorganisms (bacterial and amoebic) with different ocular biomaterials (1, 14, 15, 17-20, 22, 23). In this review, the discussion of bacterial interactions will precede that of amoebic interactions with these biomaterials. For this discussion, "ocular biomaterial" is defined as any foreign material used in periocular, ocular, or intraocular sites in man.

Interactions of Bacteria with Ocular Biomaterials

Bacteria can interact with and adhere to ocular biomaterials such as soft contact lenses, surgical suture materials, and intraocular lenses.

Soft contact lenses (SCLs)

It has been well established that bacterial keratitis can be related to soft contact lenses (35, 37). Research on bacterial interactions with SCL has shown that bacteria can adhere to the surfaces of both new and used lenses. Duran et al. (7) showed that P. aeruginosa adhered to new SCLs after exposure in vitro of the bacteria to SCLs, and that the density of adherent bacteria on the lens surface increased with increasing exposure time. The bacteria adhering to the surface of these new SCLs showed no definite distribution pattern.

Biofilm has been shown to play a role in bacterial adherence to SCLs. Cristina et al. (10, 11) have implicated adhesive slime-producing bacteria as the causative agents in biomaterial-related infections and in osteomyelitis. Subsequently, Slusher et al. (40) showed that bacterial slime layer or biofilm (polysaccharide) can play a role in the adherence of P. aeruginosa and S. epidermidis to new, unworn extended-wear SCLs. They demonstrated ruthenium red-positive slime layer on the lens...
Bacterial adherence to SCL

**Fig. 1:** Schematic representation of the relative adherence of untreated, heat-killed, and hydrogen peroxide treated *Pseudomonas aeruginosa* to new unworn soft contact lens.

**Fig. 2:** Transmission electron micrograph of live, untreated *P. aeruginosa* adherent to the surface of an unworn, new SCL. The dense, ruthenium red-positive material is seen surrounding the bacteria and in the bacteria-contact lens interface. Bar = 0.1 µm. (From ref. 22, with permission).

**Fig. 3:** Transmission electron micrograph showing heat-killed *P. aeruginosa* adherent to an unworn SCL. The bacterial outer polysaccharide layer is mostly absent. Multiple cell membrane breaks and peripheral clumping of electron-dense material giving it a cartwheel appearance. Bar = 0.1 µm. (From ref. 22, with permission).

When a new SCL is placed on the cornea, it rapidly gets coated with mucus-like material and with other tear components. Coating of SCLs with mucin facilitates adherence of *Pseudomonas* to SCLs (42). John *et al.* (22) have shown that bacterial adherence to new SCLs appears to be an active process, because heat-killed or altered *P. aeruginosa* adhered less to new SCLs than did live *P. aeruginosa* (Figs. 1-3). Experiments (1) comparing the relative adherence of *P. aeruginosa* and *S. aureus* to new and used SCLs showed that *P. aeruginosa* adheres more avidly to new and used extended-wear SCLs than does *S. aureus* after in vitro exposure of these lenses to the bacteria (Fig. 4). This may partially explain the relative predominance of *Pseudomonas*
Interactions with Ocular Biomaterials

among bacteria causing SCL-related bacterial keratitis.

Recently we reported (1) on the preferential adherence of *P. aeruginosa* to large surface deposits on worn SCL (Figs. 5-7). It was shown (1) that such adherence is aided by pili-like appendages on the bacteria (Fig. 8). Hence, worn lenses with large deposits may serve as potential sites for bacterial adherence and possible bacterial colonization. SCL contaminated with adherent bacteria may serve as a vehicle for the introduction of bacteria to the ocular environment when such a lens is placed on the surface of the eye. Shedding of bacteria from the SCL to the tear film bathing the eye can occur and may result in bacterial keratitis and/or corneal ulceration. When the susceptibility of monkey corneas to *P. aeruginosa* infection after extended wear of SCLs with and without *Pseudomonas* contamination was studied, corneal surface changes were noted after SCL wear. However, no break in the corneal epithelium occurred, and none of the corneas developed bacterial keratitis or ulceration (23). When the stress on the cornea was increased by complete tarsorrhaphy in a rabbit model, 79% of the eyes that received *P. aeruginosa*-contaminated SCL developed bacterial keratitis (26). Corneal epithelial breaks appear to be a prerequisite for the onset of bacterial keratitis associated with extended-wear SCLs. When SCLs contaminated with *P. aeruginosa*
were placed on monkey corneas, shedding of the bacteria resulted in bacterial colonization of the conjunctiva (23).

Changes in the corneal epithelium that result from the use of extended-wear SCLs, including epithelial thinning and microcyst formation, have been reported (12). When the corneal epithelium breaks down, probably secondary to the use of contact lenses, either due to mechanical factors such as minor trauma, eye rubbing and, or other factors such as hypoxia during sleep, poor tear exchange beneath a SCL, bacteria can adhere to the edges of the abraded epithelium, which is protected by the overlying contact lens from the sweeping action of the eyelids. Such bacterial adherence can advance to keratitis and/or ulceration of the cornea.

**Surgical Suture Materials**

Surgical sutures can be the initial nidus of postoperative ocular infection; however, very little information is available in the ophthalmic literature on the interaction of bacteria with surgical sutures.

Exposed, loose, and broken sutures have been implicated in postoperative microbial keratitis. Forty-five to fifty percent of cases of corneal graft infection (microbial keratitis and/or corneal ulceration) have been reported to be suture-related (8, 43). Ocular infections, including endophthalmitis, have resulted from cutting of surgical sutures after cataract surgery (9).

John *et al.* recently reported (14) their findings on the initial interaction with and adherence of *Staphylococcus epidermidis* to various surgical suture materials *in vitro* (Figs. 9-11). The absorbable sutures that were tested included PDS (polydioxanone), coated Vicryl®, chromic gut, and plain gut. The non-absorbable sutures included Prolene® (polypropylene), Mersilene® (braided polyester), Ethibond® (braided polyester coated with polybutylate), silk, Nurolon® (braided nylon), Ethilon® (monofilament nylon), and steel. Sutures were exposed...
to *S. epidermidis* from a human endophthalmitis isolate, at 3.75 x 10⁸ colony-forming units (CFU) per milliliter of sterile phosphate-buffered saline (PBS), pH 7.2, stirred (100 rpm) in an incubator shaker at 37°C for various exposure times, rinsed in PBS (pH 7.2), and processed for scanning electron microscopy (SEM). In addition, each suture material was exposed to sterile PBS for the same time as the experimental sample and was processed for SEM as controls. The number of adherent *S. epidermidis* per square millimeter of the sutures was determined.

At 0 minute of exposure of suture segments to *S. epidermidis*, all eleven types of absorbable and non-absorbable sutures had bacteria adherent to their surface. At 45 minutes, of the absorbable sutures, chromic gut showed a higher density of bacteria than did Vicryl®, PDS, and plain gut. At 90 minutes, of the non-absorbable sutures, Ethilon® had the greatest number of adherent bacteria. Braided sutures did not show any greater bacterial adherence than did non-braided sutures.

This study by John et al. (14) showed that *S. epidermidis* can adhere to a wide range of surgical suture materials. This adherence occurred almost immediately in each of the eleven types of sutures tested. There was a time-dependent increase in the number of bacteria adhering to these sutures during the first 45 minutes of their exposure to bacteria. The size, chemical composition, and surface coating of the sutures all appeared to influence bacterial adherence. Adherence of bacteria to surgical sutures thus may play a role in suture-related infection of surgical wounds.

**Intraocular Lenses**

Implantation of intraocular lenses (IOLs) has become a part of cataract surgery, mostly because of significant improvements in biomaterials, operating microscopes, microsurgical instruments, and surgical techniques over the past few decades. Postoperative anterior segment inflammation, which usually follows cataract surgery with IOL implantation, usually resolves within the first month following surgery without causing any significant ocular damage. It has been suggested that the postoperative inflammation may be secondary to excessive intraoperative manipulation, with resultant damage to intraocular tissues, lens irritation of the iris and ciliary body (25), low-grade infections (39), and possible leaching out of chemical irritants from the IOL surface (32). A more serious, less common complication of cataract surgery with IOL implantation is intraocular infection such as endophthalmitis. In a series of 51

**Fig. 9:** SEM showing many *S. epidermidis* adherent to the surface of monofilament nylon suture (Ethilon®). Bar = 5 µm.

**Fig. 10:** *S. epidermidis* adherent to the surface of braided nylon suture (Nurolon®). Bar = 5 µm.

**Fig. 11:** Clusters of *S. epidermidis* are seen adherent to steel suture. Bar = 5 µm.
cases of culture-proved endophthalmitis, 33% were associated with cataract surgery (intracapsular or extracapsular cataract extraction) with IOL implantation (3). Seymour et al. (38) studied the reasons for IOL removal and found that one of the most frequent reasons was suspected bacterial endophthalmitis. Bacteria thought to be *Staphylococcus* have been demonstrated on IOLs that had been removed from human eyes because of recurrent episodes of intraocular inflammation (6).

Recently *Propionibacterium acnes* a gram-positive anaerobic pleomorphic rod has been implicated in a chronic and indolent form of intraocular inflammation and endophthalmitis following extracapsular cataract extraction and posterior chamber intraocular lens implantation (13, 31, 36). *P. acnes* is an ubiquitous organism and has been recovered from 10-40% of eyes subjected to routine anaerobic cultures of the lids and conjunctiva (30, 34). It is possible that IOL contaminated by conjunctival flora including *P. acnes*, prior to implantation of posterior chamber IOL within the eye may play a role in the postoperative intraocular inflammation and endophthalmitis that have been described following cataract surgery.

Vafidis et al. (44) placed 100 new, sterile IOLs on the conjunctival flap and the corneoscleral or corneal section of 50 patients for 5 seconds during cataract surgery and demonstrated a bacterial contamination rate of 26%. Thus, contaminated IOLs may serve as a vehicle for the intraocular introduction of bacteria during cataract surgery with IOL implantation.

We recently reported (17) our findings on the interaction with and adherence of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* to new anterior (AC) and posterior-chamber (PC) intraocular lenses (Figs. 12-14). We studied the adherence of *S. epidermidis* and *P. aeruginosa* to three types of IOLs: new PC IOL (polymethylmethacrylate optic, with or without positioning holes, and polypropylene haptic), and new AC IOL (polymethylmethacrylate optic and haptic). A human endophthalmitis isolate of *S. epidermidis* and a corneal-ulcer isolate of *P. aeruginosa* were grown overnight in tryptic soy broth at 37°C to 3 x 10^8 CFU/ml and 4.5 x 10^6 CFU/ml of PBS (pH 7.2), respectively. Intraocular lenses were placed in *S. epidermidis* or *P. aeruginosa* suspension, shaken (at 60 rpm) for various exposure times and rinsed for 30 seconds in sterile PBS prior to quantitation of the number of adherent bacteria on the IOL surface. For the most part, in all IOL types, fewer *S. epidermidis* than *P. aeruginosa* adhered per square millimeter of IOL (both optic and haptic). Similarly,

---

**Fig. 12:** Surface characteristics of an anterior chamber IOL as seen under a SEM. Bar = 0.5 mm.

**Fig. 13:** Numerous *S. epidermidis* are seen adherent to the optic surface of the PC IOL. Bar = 5 µm.

**Fig. 14:** Many *P. aeruginosa* are seen adherent to the optic surface of a PC IOL. Bar = 5 µm.
fewer S. epidermidis than P. aeruginosa adhered to all IOL haptics. At 60 minutes, S. epidermidis did not show any significant difference in the number of adherent bacteria between the optic and haptic parts of AC IOL. At 60 minutes, S. epidermidis showed more adherence to the optic than to the haptic part of PC IOL.

This study shows that S. epidermidis and P. aeruginosa can adhere to new AC and PC IOL. Such adherence to IOLs before implantation during cataract surgery may play a role in IOL-related inflammation and/or endophthalmitis.

**Adherence of Acanthamoeba to unworn SCLs**

Infection of the cornea with *Acanthamoeba* is serious, vision-threatening, and debilitating. Between 1973 and 1988, more than 200 cases of *Acanthamoeba* keratitis were identified in the United States (41). The association of *Acanthamoeba* keratitis with contact lens wear was known for some time (2, 4, 33, 45), but only recently has contact lens wear been identified as the predominant risk factor for this disease (41). The use of non-sterile, homemade solutions and cold disinfection methods for SCLs can increase the risk of development of *Acanthamoeba* keratitis (29). In cases of contact lens-related *Acanthamoeba* keratitis, amoebae have been cultured from the lenses (4, 28), from solutions in the soft contact lens case (33, 45), from distilled-water bottles (24), and from homemade saline solution (28).

John et al. (17) recently reported their findings on the adherence of *Acanthamoeba castellanii* cysts and trophozoites to new, unworn, extended-wear SCL. A human corneal isolate of *A. castellanii* in normal saline (cysts = $6.3 \times 10^5$/ml, trophozoites = $3.6 \times 10^5$/ml) was used. Segments of unused hydrogel polymacon contact lenses were exposed to AC cysts or trophozoites with or without stirring. Following exposure times ranging from 0-7 hours, SCLs were or were not washed and the adherence of *A. castellanii* was determined by a standardized light-microscopic technique. Both cysts and trophozoites adhered to unworn SCLs (Figs. 15-16). The trophozoites demonstrated acanthopodia, lobopodia, and filopodia. This study indicated that both cysts and trophozoites of *A. castellanii* can firmly adhere to unworn SCL. John et al. (19) have also shown that *A. castellanii* cysts and trophozoites adheres to new unworn daily wear, extended wear and disposable SCLs. They have also demonstrated the adherence of *A. castellanii* cysts and trophozoites to human-worn hydrogel contact lenses (16).

Based on the findings of our studies and those of previous studies we proposed (18) a sequence of events that may lead to SCL-related *Acanthamoeba* keratitis (Fig. 17). When a SCL is exposed to solutions containing *Acanthamoeba*, cysts and trophozoites adhere to the surface of the lens (Fig. 17). Suboptimal cleaning and disinfection of the lens may allow amoebic cysts and trophozoites to remain adherent to the surface of the
Fig. 17: Proposed sequence of events that may lead to SCL related Acanthamoeba keratitis. (Reprinted from Am J Ophthalmol 108: 658-664, 1989. Published with permission from the American Journal of Ophthalmology. Copyright by the Ophthalmic Publishing Company.)
lens. When such a lens is placed on the human cornea, it introduces *Acanthamoeba* to the ocular environment. The contact lens acts as a vector for the introduction of the *Acanthamoeba* to the ocular surface. These amoebic cysts and trophozoites may subsequently invade the human cornea either through an intact corneal epithelium or through breaks in the corneal epithelium which may be related to SCL-wear (Fig. 17). Once the amoebic trophozoites invade the human cornea this can lead to *Acanthamoeba* keratitis. John et. al. (5, 21, 27) have also established an animal model of *Acanthamoeba* keratitis and this model has been used to study therapeutic effects of some of the medications used in the treatment of *Acanthamoeba* keratitis.

**Conclusions**

It is evident from this review that microorganisms such as bacteria can adhere to soft contact lenses, surgical sutures, and intraocular lenses, even when the exposure time is very brief. *Acanthamoeba*, both as cysts and trophozoites, can also adhere to SCL. Ocular biomaterials currently in use can act as carriers or vehicles for the introduction of these microorganisms to the ocular surface or to the interior of the human eye and can result in potentially vision-threatening ocular infections.

**References**


Discussion with Reviewers

Reviewer 1: How were the samples prepared for scanning electron microscopy?

Author: Samples were fixed in half-strength Karnovsky’s fixative (2% paraformaldehyde, 2.5% glutaraldehyde) and dehydrated in alcohol at increasing concentrations (15%, 30%, 50%, 70%, 80%, 95%, and 100%). Specimens were critical point dried using CO2, mounted on aluminum stubs, sputter-coated with gold, and examined at 20 kV at a working distance of 12 mm.

K. Okada: The author states "Postoperative anterior segment inflammation, which usually follows cataract surgery with IOL implantation, usually resolves within the first month following surgery without causing any significant ocular damage". IOL’s are not inert in the inner eye but render a postoperative cellular reaction on the surface of implanted intraocular lenses. This cellular reaction is accepted as a foreign body reaction of the host to the implants [see e.g., Wolter JR (1984): Pathology of intraocular lenses; in: Cataract and Intraocular Lens Surgery, Ginsberg SP (ed.), Aesculapius Publishing Company, Alabama, vol. 2, pp. 652-670; Wolter JR (1985) Cytopathology of intraocular lens implantation. Ophthalmolology 92:135-142; Kappelhof JP et al. (1986) The proteinaceous coating and cytology of implant lenses in rabbits. Am J Ophthalmol 102: 750-758] and it is categorized into a type of chronic granulomatous inflammation. Actually, its time course in living human eyes has been directly observed by specular microscopy [Okada K, Sagawa H (1989) Newton rings on the surface of implanted intraocular lenses. Ophthalmic Surgery 20: 33-37] and in most cases it persists at least one month after intraocular lens implantation. Indeed, the effect of this cellular reaction on ocular tissues is still under investigation (see also Okada et al., this issue).

Author: Thank you for your comments.
M.S. MacSai: It would be interesting if the author could address the various presentations of *Acanthamoeba* keratitis with his different theories of pathogenesis which may relate to these presentations, for example, epithelial irregularity, radial neuritis, ring corneal infiltrate, and frank corneal ulceration.

Author: Corneal ring infiltrate, radial keratoneuritis, and pseudodendrites are important clinical signs of *Acanthamoeba* keratitis. Although these signs are different, the theory of pathogenesis of *Acanthamoeba* keratitis is as described in the text. *Acanthamoeba* keratitis occurs only after the entry of amoebic trophozoite(s) into the cornea. Radial keratoneuritis is thought to be related to amoebic localization in the region of the corneal nerves.

K. Okada: Dilly and Sellors (text reference 6) reported that adherent bacteria (*Staphylococcus epidermidis*) were found to be more numerous on the polypropylene haptic than on the polymethylmethacrylate optic both *in vitro* and *in vivo* cases. Do you have any discussion and/or comment on the difference of the results between your report and that by Dilly and Sellors?

Author: Dilly and Sellors' *in vivo* study included two IOLs that were explanted from the human eye and studied under SEM. These two IOLs had cells covering the haptic and not the optic part of the IOLs. This may explain why there were more bacteria localized to the haptic than the optic part of the explanted IOLs. Obviously the explanted IOLs have different surface characteristics and cannot be compared to our study.

In Dilly and Sellors' *in vitro* study they used six new IOLs which were first placed in thioglycolate broth for a total of 21 days at 37°C. During the last seven days, the broth also contained *S. epidermidis*. It is clear that their *in vitro* study is markedly different from ours reported in the text and therefore it is not appropriate to compare these studies to come to any meaningful conclusions.