Chapter

Biofilm Theory for Lid Margin and Dry Eye Disease

Maria Vincent, Jose Quintero, Henry D. Perry and James M. Rynerson

Abstract

Blepharitis and dry eye disease have long been viewed as two distinct diseases with overlapping presentations and separate etiologies. Evaporative dry eye, although frequently associated with aqueous deficiency, is also considered a separate entity. We propose viewing dry eye, both evaporative and insufficiency, as the natural sequelae of chronic blepharitis induced by biofilm. We suggest describing this one chronic disease as dry eye blepharitis syndrome (DEBS). The disease process begins when normal flora bacteria colonize the lid margin beginning shortly after birth. This colonization accompanies the development of a biofilm on the lid margin. As years pass, the biofilm matures, and the increased bacterial population initiates the production of inflammatory virulence factors, such as exotoxins, cytolytic toxins, and super-antigens, which persist on the lid margin for the rest of the patient's life. These virulence factors cause early follicular inflammation and later, meibomian gland dysfunction followed by aqueous insufficiency, and finally, after many decades, loss of the dense collagen in the tarsal plate. We proposed four stages of DEBS, which correlate with the clinical manifestations of folliculitis (anterior blepharitis), meibomitis (meibomian gland dysfunction), lacrimalitis (aqueous deficiency), and lid structure damage evidenced by increased lid laxity resulting in entropion, ectropion, and floppy eyelid syndrome.

Keywords: biofilm, blepharitis, demodex, dry eye disease, eyelids, meibomian glands, quorum-sensing gene activation, tear film

1. Introduction

Blepharitis was first described by ancient Egyptian physicians in the Ebers Papyrus, which prescribed potions such as "Cream with the Milk-of-a-Woman-who-has-borne-a-Son" [1, 2]. Despite centuries of study, little progress has been made in understanding or treating this disease. The long standing dogma of multifactorial, overlapping manifestations of blepharitis and dry eye have led to the use of inaccurate terminology that creates misunderstanding among both patients and providers [3, 4]. In order to develop our understanding of DEBS, we must first establish the correct use of the word blepharitis as suggested by the origin of the word (blepharon = lid, –itis = inflammation).

The next step in understanding dry eye disease and blepharitis as a single disease process is to identify the cause of eyelid inflammation. In 1954, Thygeson first recognized that blepharitis was associated with "abnormal Staphylococcus

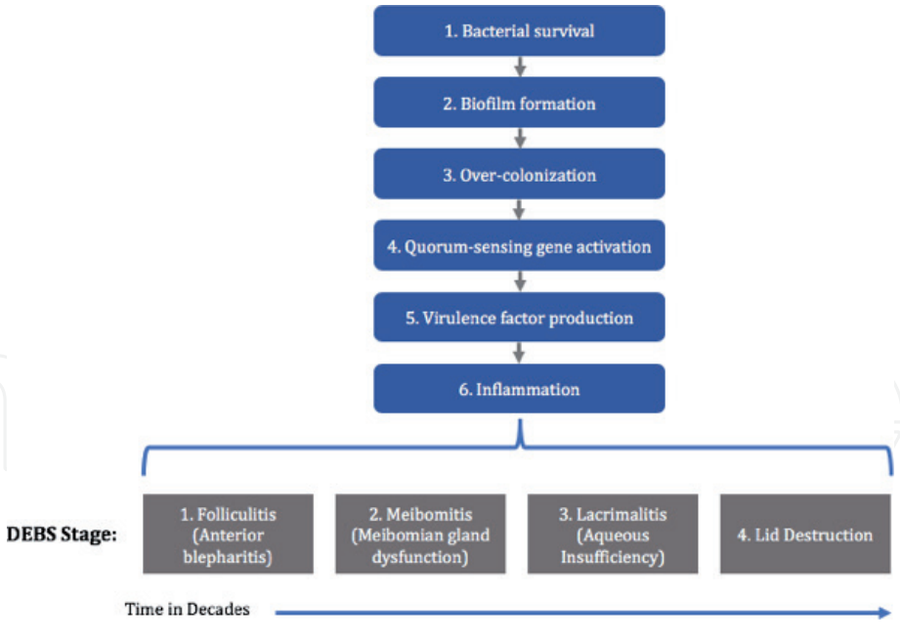

Figure 1.
Biofilm theory of dry eye disease: Schematic of six steps of bacterial biofilm development leading to the stages of DEBS.

colonization" of the eyelid margin [5]. Thygeson was describing the process by which our normal lid margin flora bacteria, primarily *Staphylococcus aureus* and *S. epidermidis*, gradually over-colonize the patient's lid margin, and over time, become pathogenic [6]. This is made possible by the bacterial biofilm, which allows the bacteria to thrive despite antimicrobials and the immune system [7]. Understanding biofilm progression links the shared underlying pathology between dry eye and blepharitis.

Biofilms are defined as groups of microbial cells enclosed in a matrix made primarily of polysaccharide material that are intimately associated with a surface. Antonie van Leeuwenhoek is credited for the first observations of biofilm, when he described the biofilm on teeth in 1684. However, further study of biofilms was limited until the development of the electron microscope in the mid-1900s. Furthermore, it was not until 1982 that the term "biofilm" was introduced, after Costerton's observation of a *S. aureus* biofilm on a cardiac pacemaker lead [8]. More recent studies have shown that cell-to-cell interactions ("quorum sensing") within the biofilm upregulate certain gene products. Further studies have implicated biofilm in many disease processes including periodontitis, endocarditis, chronic prostatitis, and medical device associated infections, as the one described by Costerton [8–10].

This chapter will explain the six steps by which our normal margin lid flora become pathogenic and cause eyelid inflammation. This inflammation, in turn, leads to the four stages of DEBS: folliculitis, meibomitis, lacrimalitis, and lid structure damage (**Figure 1**). This understanding will allow us to encompass dry eye disease, blepharitis, and meibomian gland dysfunction (MGD) in one disease process, namely, dry eye blepharitis syndrome (DEBS).

2. Biofilm

Bacteria were among the first forms of life on Earth and have survived billions of years in a myriad of different environments. While they are unicellular microorganisms, and can live in a free-floating form, the development of a biofilm provides

them a strong, virtually impenetrable defense structure [8]. Furthermore, Absalon et al. and Pickering et al. suggest that free-floating bacteria are the minority in nature by describing the biofilm as "the prevailing microbial lifestyle" [11, 12].

The biofilm helps bacteria by acting as armor against host defense responses and desiccation. It enhances survival across species by allowing bacteria to produce virulence factors, concentrate nutrients, and communicate with other bacterial species [13]. Biofilms are involved in many infections and are present in almost any environment – they form plaque on the teeth and can lead to corrosion of metal pipes. They are involved in recurrent infections from medical devices – from sutures to prostheses. Although they can also be found as floating mats submerged in or on top of liquids, they are usually sticky and adhere easily to any surface [14]. For example, *S. epidermidis* and *S. aureus* produce a protein called "adhesin", which functions as a glue, ensuring a strong adhesion between the biofilm and its host surface [15]. Once adhered, they are hard to dislodge, allowing the bacteria to remain in a desirable environment.

Biofilms are likely to grow wherever there is moisture, nutrients, and a surface [16]. These are all present at the lid margin, which has the added benefit of its inherent warmth. It is well known that the lid margin is home to normal flora bacteria consisting of mainly coagulase-negative species such as *S. epidermidis* [6]. It is also well known that species of Staphylococcus, especially *S. epidermidis*, produce biofilms [17]. In addition, a recent study by Kivanç demonstrated that 32 out of 34 isolates cultured from eyes immediately after cataract surgery were positive for being biofilm-forming species [18]. Taking all this information into account, it should come as no surprise that biofilms easily develop on the lid margin.

Furthermore, to avoid irritating our eyes with soap when we wash our face, we instinctively keep our eyes tightly shut, lid margin against lid margin, effectively blocking access to an area that needs cleaning as much as or more than any other area of the body. Therefore, the biofilm accumulates microscopically year after year, layer upon layer, without any removal. Even if home scrubs are attempted, the adhesin "glue" can prevent biofilm elimination. As patients age, the biofilm continues to accrue, leading to each of the stages of DEBS over time. This process starts much earlier in contact lens wearers, since the contact lens is itself an inert foreign body, producing a very early biofilm that allows protection for bacteria. Biofilm formation on contact lens and contact lens cases has been well documented [19]. This also helps explain why dry eye disease is more common in contact lens wearers, 50% compared to 14% in controls [20].

The biofilm forms a multi-laminar substrate that provides more surface area for bacterial replication, which in turn leads to vast over-colonization of the surface. The over-colonization within the biofilm and increase in bacterial population density is what leads to quorum-sensing gene activation [21]. The discovery of quorum-sensing gene activation by Hastings in 1999, was a groundbreaking study that lead to increased understanding of bacterial virulence [22]. Hastings demonstrated that populations of bacteria can sense when their densities achieve a certain quorum, and once that number or density is reached, dormant genes are activated [23]. The bacteria signal to each other using chemical messengers called homoserine lactones (HSLs) as well as through electric currents produced by potassium ions [24]. When enough bacteria are in close proximity to each other, the signals from these the surrounding bacteria sum to indicate a quorum [25]. These newly activated genes produce a wide array of virulence factors, many of which are extremely inflammatory. The bacteria wait to produce these factors until they have the protective biofilm in place to shield from the host immune response [26]. The inflammation from the host response to these virulence factors is the real destructive force in inflammatory lid disease, causing low-grade, chronic inflammation, beginning on

the lid surface, the structures of the lid margin such as lash follicles, meibomian glands and connective tissue, and eventually affecting the accessory lacrimal glands as it progresses.

S. epidermidis produces a small amount of a moderate cytolytic toxin, a phenol-soluble modulin, but *S. aureus* produces two groups of highly destructive and immunogenic exoproteins: exotoxins and enzymes [27–29]. Many exotoxins are super-antigens that signal T cells to secrete large amounts of cytokines, and thus, massive inflammation. Exotoxins are responsible for toxic shock syndrome, food poisoning and scalded skin syndrome (toxic shock syndrome toxin, staphylococcal enterotoxins A-E and G-I, and exfoliative toxins A and B respectively) [30, 31]. The enzymes produced consist of nucleases, proteases, lipases, hyaluronidase, and collagenase, all capable of destroying host tissue [32]. Cytolytic toxins, including hemolysins and leukocidins, further contribute to the inflammatory cascade by destroying or damaging cells [33].

These toxins and enzymes permeate the biofilm and its surroundings, creating the same massive inflammation that leads to acute, severe debilitating disease as in scalded skin syndrome, food poisoning, and even death, as in the case of toxic shock syndrome [34]. As the biofilm spreads, more areas reach the quorum needed to activate virulence factors. Thus, inflammation spreads from the lid margin, to within the lash follicles, meibomian glands, accessory lacrimal glands, possibly to the main lacrimal gland and eventually to nerve endings and even the connective tissue of the eyelid, which can affect the structural integrity of the eyelids [35]. Decades of this toxicity, and the resulting inflammation, leads to nonselective damage [36]. While the body manages to ward off some of the effects of this toxic environment until later in life, eventually no part of the lid is immune to this chronic, progressive inflammation [37].

3. The four stages of DEBS

As we have now established that inflammatory lid disease is due to the inflammatory response to virulence factors produced by a mature biofilm, we can proceed to understanding the various clinical manifestations of DEBS. The important factors to consider are lid anatomy, duration of biofilm presence and associated virulence factors along the lid margin. False descriptions such as anterior, posterior, staphylococcal or seborrheic do not accurately describe the stage of blepharitis, and merely serve as distractors. Instead, it is important to understand that inflammation is an inevitable consequence of virulence factor production, and it does not discriminate among structures of the lid. It simply takes some structures longer to be affected than others because of anatomy. Due to sticky proteins such as adhesin, as well as the biofilm's innate defense against antimicrobials and the immune system, the biofilm usually remains in place for most of the patient's life [38, 39]. This allows the inflammation to eventually affect all structures within the eyelid.

The biofilm is likely formed early in the patient's life, around the toddler stage. This early biofilm does not cause pathology in most cases because the densities of bacteria within the biofilm have not reached the quorum required to activate virulence factors. There are certainly exceptions, where children present with severe blepharitis [40]. These children likely have two particularly virulent strains of bacteria colonizing their eyelids simultaneously. The first is likely a hyper-virulent strain of *S. epidermidis*, which makes copious biofilm, and the second is a particularly virulent strain of *S. aureus*, whose quorum for gene activation is lower than normal [41] and whose toxins are more destructive. Further research into these

children's lid flora would help clarify the variation in pathogenesis. Other factors such as rosacea and Demodex also remain to be investigated.

In the majority of the population, the biofilm must be present for decades before enough bacteria accumulate to reach a quorum [42]. As previously mentioned, biofilms form wherever there is a "combination of moisture, nutrients and a surface" [16]. Therefore, the lid margin, which provides all three of these requirements, is a logical starting point for the development of the biofilm. The lid margin includes the lash line and extends just past the openings of the meibomian glands [43]. Other areas of the ocular surface are better defended from the development of biofilm. Specifically, the mechanical sweeping and flushing of tears protects the palpebral and bulbar conjunctiva, while antibacterial lactoferrin and lysozyme protect the tear film [44–46]. Goblet cells provide further protection to the epithelial surfaces by secreting mucus [47].

Despite its antimicrobial protein content, the majority of the protection given by mucus is due to its mechanical characteristics. In the large intestine, there are two layers of mucus: the outer one houses gut bacteria, and the inner, impermeable layer prevents the underlying epithelial cells from bacterial invasion [48, 49]. While the small intestine lacks the inner layer, it still prevents bacterial exposure by creating a diffusion gradient with a rapid turnover that bacteria must overcome to access the epithelial cells [50]. Mucus trapping bacteria also prevent antigen presentation, which limits immune response. These functions may well help protect the conjunctiva as well – limiting environmental antigen presentation and forming an impenetrable barrier. In addition, the rapid turnover combined with the sweeping action of blinking creates an unstable surface to which a biofilm cannot adhere. Therefore, maintenance of a healthy population of goblet cells is essential to prevent biofilm buildup on the conjunctiva.

We know that the mucus is permeable to other molecules such as antibiotics, steroids, other medicated eye drops, therefore it is logical to assume that at least some of the exotoxins, enzymes and cytolytic toxins can reach the epithelia. Similarly, if virulence factors behave like the molecules in eye drops, it may be possible for them to slowly penetrate into the eye and damage structures within the eye; for example, the trabecular meshwork. Perhaps the meshwork simply becomes "sticky" due to subclinical inflammation and more easily traps protein, white cells or RBCs. This could occur through either subclinical inflammation or direct damage from cytolytic toxins and enzymes. If this is the case, it may in part explain why the incidence of glaucoma increases with age: a thicker biofilm releases more toxins which can damage lid margin structures and internal eye structures over time.

The manifestations of DEBS vary depending on the stage of the disease, which progresses exceedingly slowly. These differing presentations have led to confusion as to the presentation and progression of blepharitis and dry eye disease. This confusion stems from focusing on the presenting problem and not understanding what preceded it. For instance, if a patient has meibomian gland dysfunction, the diagnosis is made of evaporative dry eye disease, without further consideration of the lash follicles [51]. Similarly, if there is inadequate tear lake, the patient is diagnosed with aqueous insufficiency, ignoring the status of the meibomian glands [52, 53]. We hope to eliminate this confusion by dividing DEBS into four stages and by making a logical argument for the order of this progression based on eyelid anatomy and histology.

3.1 Stage 1: folliculitis (anterior blepharitis)

The first stage of DEBS involves the lash follicles. The potential space between the eyelash and the follicle surrounding it is easily invaded by the biofilm

(**Figure 2**). Once quorum densities are achieved and virulence factor production begins, the small lash bulb can become inflamed relatively quickly. Inflammation leads to edema, which can be clinically identified with the "volcano" sign – swollen follicular tissue around the base of the lash (**Figure 3**). The swollen tissue may also appear pale, possibly due transudate and/or capillary compression due to edema. The biofilm that adheres to the lash will be pulled along as the lash grows, resulting in "collarettes" (**Figure 4**). These collarettes have also been called scurf, debris or lash dandruff. Because they originate from biofilm, they appear at different levels on the eyelash, depending on the lash's growth stage. Near the top of the lashes in **Figure 4**, it is possible to see collarettes just beginning to detach from the lid margin. This biofilm growth can also manifest as "cylindrical dandruff" [54]. Despite the term "dandruff," it is unlikely that sloughing layers of skin could form a cylinder around the eyelash. Thus, this term is most likely an inaccurate description of biofilm that accumulates around the lash base and sheathes the lash as it grows. Since the lash follicles are likely damaged through inflammation, the growth of the lash is slowed, which enables the biofilm accumulation to progress at the same rate as lash growth [55]. We have confirmed the presence of bacterial colonies in the scurf around lashes (**Figure 5**), and fluorescence microscopy was consistent with biofilm matrix around the lash [55].

A 2005 article by Gao et al. proposed that cylindrical dandruff was pathognomic for Demodex, which found that all their subjects who presented with cylindrical dandruff also had Demodex. While other studies had different findings, Gao et al. explained the discrepancy as "miscounting" by the other researchers [54]. However, even if the 100% incidence in this one study is completely accurate, correlation does not necessarily establish causation. A later article by Tsubota et al. found that "Demodex was detected in the cilia of 8 out of 10 (80%), and 22 cilia out of 30 (73%) with cylindrical dandruff" [56]. While these numbers certainly suggest an association, they do not imply causality. In fact, since Demodex were not detected in all of the lashes, it would suggest a lack of causality. Furthermore, Demodex does not extrude waste, instead storing it in its gut, which makes it unlikely that they secrete the dandruff [63]. It is much more likely that the eyelids accumulate an abundance of biofilm over time, which progresses along with eyelash growth,

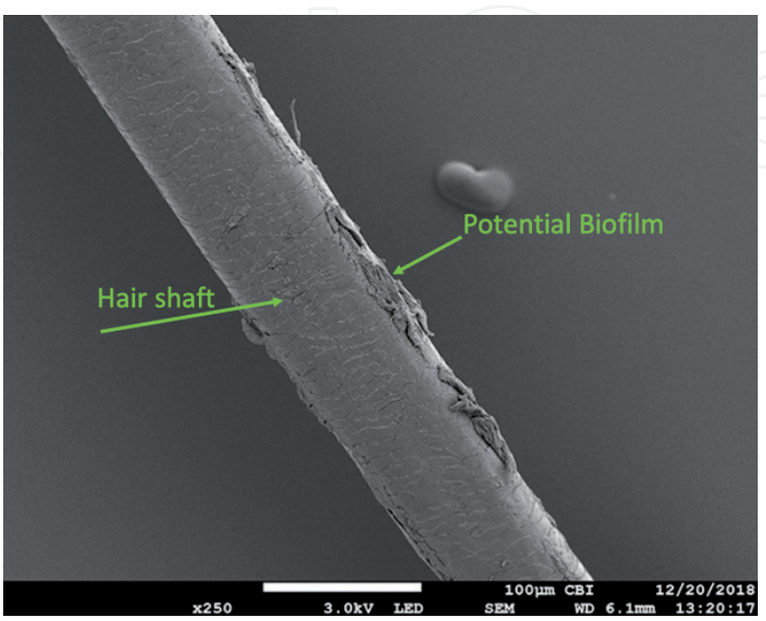

Figure 2.
Scanning electron microscopy of an eyelash hair shaft showing potential biofilm.

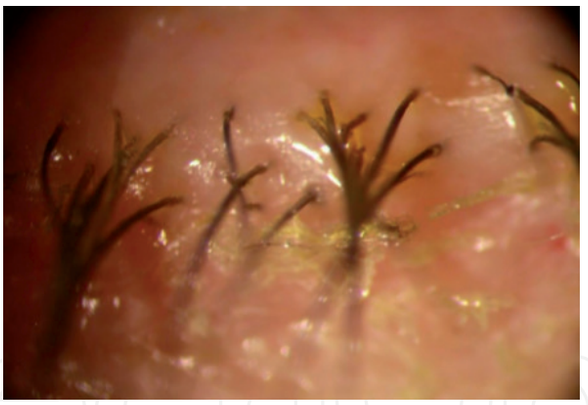

Figure 3.
The pallor around the lash follicles indicates the "volcano" sign associated with folliculitis.

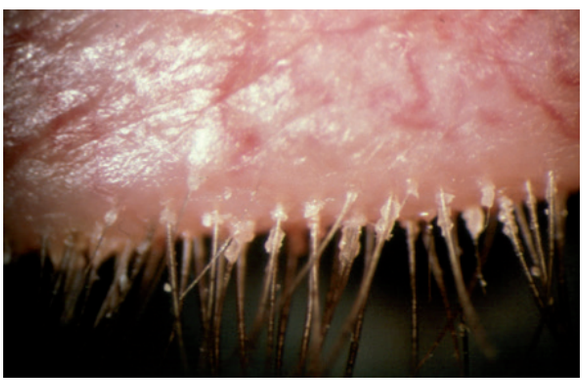

Figure 4.
Collarettes/cylindrical dandruff present on the lashes.

and that Demodex uses the polysaccharide biofilm as a rich source of nutrition. The cylindrical dandruff is likely a combination of Demodex carcasses and biofilm.

Collarettes, clumping, eye discharge, and sticky eyelids upon awakening are all evidence of bacterial biofilm along the lid margin. However, they are not required for diagnosis of blepharitis. In patients with late-stage disease, there may be significant lid inflammation without scurf. Though this may seem to be incongruous with blepharitis, the likely 40–50 years of inflammation at this stage have so badly damaged the eyelash bulb that the lashes are either barely growing or not growing at all. Therefore, there is no scurf, because there has been no/minimal lash growth to pull it away from the lid margin. Therefore, a paucity of lashes, in association with swollen lash follicles, as shown in **Figure 3**, can also indicate DEBS. In addition, these late-stage patients typically have exceedingly dry lid margins, which inhibit further biofilm production. In other words, bacteria can eventually become their own worst enemy by destroying the very moisture that is required for biofilm production.

3.2 Stage 2: meibomitis (MGD)

The next stage of DEBS involves the spread of inflammation from the lash follicles to the meibomian glands. These glands are relatively more protected than the follicles due to their narrow ductules and the constant flow of meibum out of the gland. These characteristics ensure that meibomian involvement occurs after follicular involvement. Meibomian glands are also 5–10 times larger than lash follicles, which means that inflammation takes longer to significantly hinder the working of the gland than the follicle [57]. The amount of time between Stage 1 and Stage 2

depends on the virulence and biofilm production characteristics of the patient's particular bacterial profile, but we estimate approximately 10–15 years between them.

Obvious vs. nonobvious MGD is a recent topic of discussion and can also be explained through understanding the biofilm. Obvious MGD manifests with inspissation and capping (domes over gland openings) that can be observed on exam (**Figure 6**). On the other hand, nonobvious MGD does not have these manifestations. Nonobvious MGD can be thought of as the biofilm forming layers within the gland and starting the inflammatory process [58]. As the biofilm accumulates within the gland and mixes with meibum, it eventually blocks the narrow ductule, thereby causing obstruction. This thickened mixture of biofilm and meibum takes on a "toothpaste" quality and may alter the consistency of the lipid profile of the meibum, either through the presence of abnormal lipids or decreased overall lipid secretion [59]. The mixture of meibum with biofilm has an increased melting point, which leads to thickening and obstruction. These secretions may not be expressed because the meibomian glands are large and the biofilm may not have affected a significant enough portion of them yet, therefore leading to nonobvious MGD. Once the gland is full of the thick meibum and biofilm mixture, the secretions will have

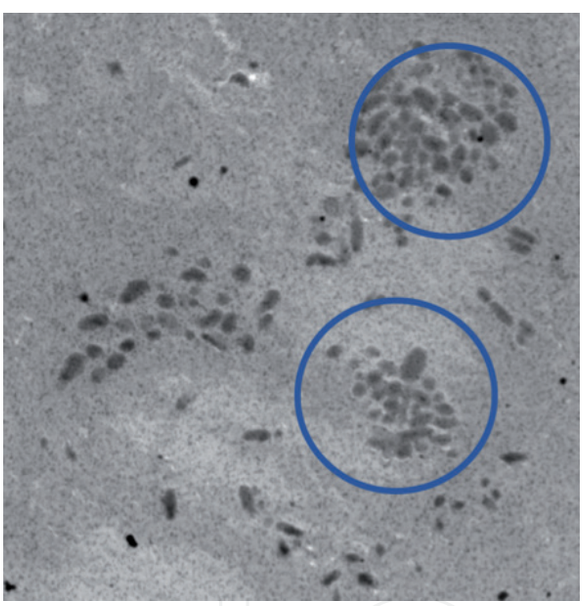

Figure 5.
Transmission electron microscopy of cylindrical dandruff showing bacterial colonies (blue circles), which suggests that the dandruff is likely biofilm.

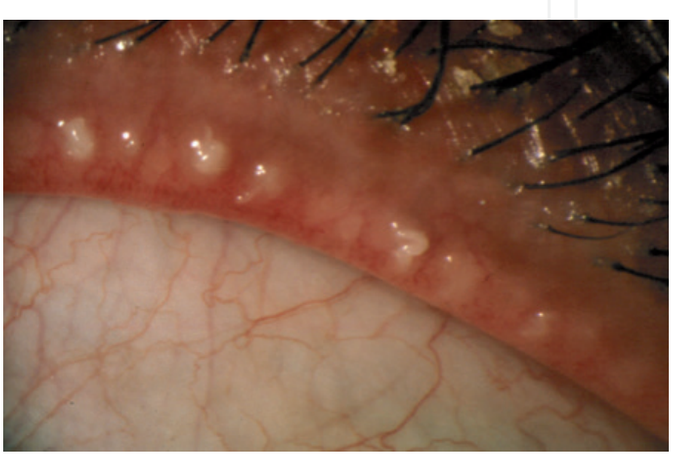

Figure 6.
Evidence of meibomian gland dysfunction showing inspissation and capping.

nowhere further to accumulate within the gland and will attempt to move up and out of the ductule. However, the original biofilm traps these secretions forming small whitish domes similar in color to what is observed within an early non-obvious occluded ductule. Expression of the glands may release copious amounts of sludge or inspissated secretions. While the composition of the peaks or caps has never been effectively studied, it is not difficult to imagine that they are composed of accumulated "altered" meibum mixed with biofilm covered by a more "pure" biofilm [60]. Therefore, the filling of the gland past its capacity is what triggers the appearance of the "domes" in obvious MGD, but it is the thickened biofilm mixture which has reached quorum-sensing that begins the early, and later obvious, signs of overt inflammation along the posterior lid margin [61]. Hence, the difference between obvious and nonobvious MGD is simply one of degree.

3.3 Stage 3: lacrimalitis (aqueous deficiency)

Stage 3 DEBS involvement of the accessory lacrimal glands of Wolfring and Krause, and probably the main lacrimal gland, leads to aqueous deficiency. There are approximately 30 lacrimal glands of Krause and about 5 glands of Wolfring on each eye. They are responsible for baseline aqueous production [62, 63]. The ducts of these glands empty along the inside lid, up near the fornices. The distance from the lid margin biofilm, the narrow ducts, and the constant flushing activity of tear production all serve to protect these glands from activity along the lid margin. However, the biofilm can spread from the lid margin by being shed into the tear film, and decades of shedding eventually leads to some biofilm infiltrating the glands of Krause and Wolfring. Alternatively, it is quite possible that a layer of biofilm, kept attenuated due to constant flushing and lid/eye movement, nevertheless eventually reaches these glands by direct extension. Because of their innately protective distance from the lid margin, these glands are the last group to become infiltrated by biofilm, and therefore, the last to succumb to the effects of inflammatory damage from biofilm virulence factors. This is supported by looking at clinical manifestations of patients with many of the symptoms of dry eye – burning, irritation, and difficulty seeing – in conjunction with excessive tearing. While exam may lead to diagnosis of evaporative dry eye with a deficient lipid layer, patients may not understand how they could have dry yet watery eyes. These patients have deficient lipid production but intact aqueous production, indicating diseased meibomian glands but still healthy accessory glands of Krause and Wolfring. On the other hand, unless there is an autoimmune disorder, it is virtually impossible to see an aqueous-deficient patient without MGD (**Figure 7**). This finding supports the conclusion that aqueous deficiency presents after evaporative dry eye due to the accessory lacrimal glands being affected after the meibomian glands.

By the time we have Stage 3 DEBS, the follicles have been subjected to chronic inflammation for many decades and are sometimes so badly damaged that lash growth is arrested, and hence, there may be little-to-no biofilm noted among the eyelashes. Lashes fall out and may not regrow or regrow very slowly. Looking closely, one will typically find significant swelling around the base of the lash along with pallor as in Stage 1.

3.4 Stage 4: lid destruction

Stage 4 DEBS is marked by the breakdown of the structural integrity of the eyelid. Lid laxity, entropion, ectropion, and floppy eyelid syndrome are often manifestations of end-stage chronic inflammatory lid disease [64]. The inflammation associated with the formation of the biofilm eventually affects connective tissue,

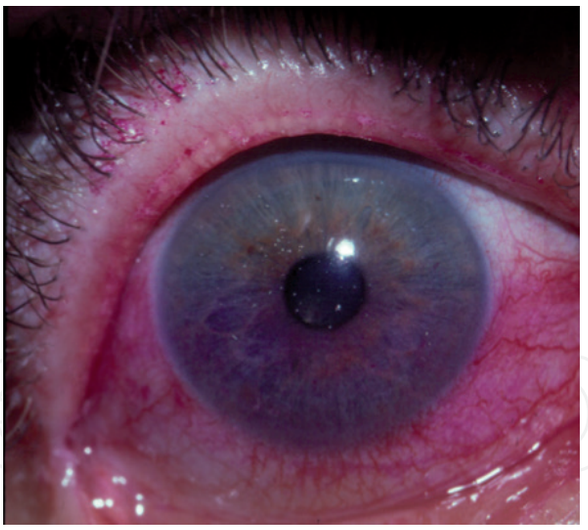

Figure 7.
Eye showing both aqueous deficiency (positive staining with rose Bengal) and meibomian gland dysfunction (pouting of glands).

muscle, and nerve endings within the lid margin, which become damaged and lose their functionality [65]. Because of the loss of the nerve endings, these patients are often asymptomatic. Also, as mentioned prior, since bacteria need moisture to grow and produce biofilms, years of dry eye may degrade their once-ideal environment to the point where they cannot sustain large colonies of bacteria, these patients often present with little to no biofilm. By this point, however, the damage to the lid and the tear glands is already done and may be irreversible.

3.5 Management

The armor provided by the polysaccharide matrix of the biofilm explains why many novel treatments proposed in the past 100 years have failed. The only treatment for chronic blepharitis universally agreed upon is lid hygiene [66]. Historically we have preferred simple salt water soaks [67]. Recently, microblepharoexfoliation (MBE) has become available [68]. This additional form of lid hygiene provides a thorough mechanical biofilm removal of the lid margin, which may have a profound impact on patient's symptoms, quality of tears, and quality of life. Therefore, we propose performing MBE of patients' lids, with electric rotary sponge cleaning, in an effort to remove the biofilm and prevent and/or slow down the progression of DEBS (**Figure 8**).

Besides the treatment of DEBS, MBE may have other potential roles in ophthalmology. It is known that endophthalmitis is most commonly associated with the presence of biofilm-forming bacteria in the patients' lid margin. The aforementioned Kivanç study demonstrated that these biofilm formers are present and can survive a Betadine wash [18]. By performing a thorough MBE of the patient's lids, we may be able to reduce the incidence of post-cataract infection. In addition, by removing the biofilm from the lid margin and meibomian glands, we should expect a better tear film and therefore more accurate pre-operative screening and, more importantly, better post-op vision. Similarly, patients undergoing refractive surgery, such as laser in situ keratomileusis, photorefractive keratectomy, and phototherapeutic keratectomy, and contact lens wearers will probably also benefit from an electromechanical debridement of their lid margin. All of these patients may benefit from the reduction or elimination of the progression of the lid biofilm with yearly electromechanical debridement.

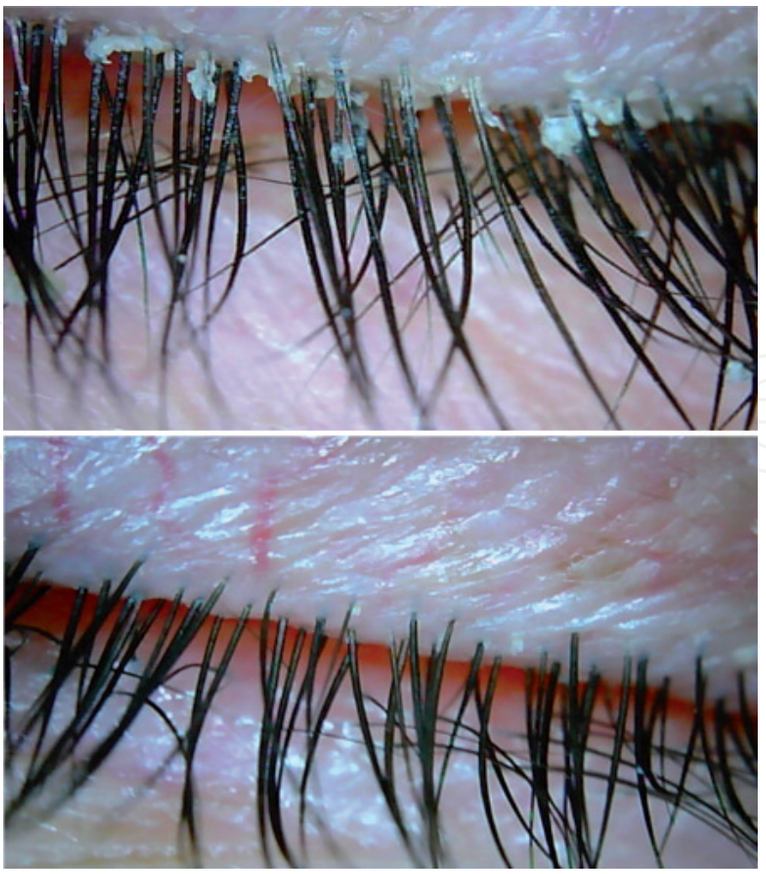

Figure 8.
Upper lid lash margin showing presence of cylindrical dandruff and "scurf". Top: Before MBE; Bottom: After MBE (Courtesy of BlephEx, Inc.).

4. Conclusions

Understanding DEBS as a singular disease process that presents in stages, over decades, throughout a person's lifetime allows us to successfully explain all clinical scenarios we encounter. DEBS explains the overlap of the so-called anterior blepharitis with posterior blepharitis, why we do not see isolated cases of aqueous deficiency and why the disease worsens with age. It also shows why some patients may become asymptomatic and why we sometimes do not see biofilm within the lash line despite severe lid disease findings. Finally, DEBS also describes chronic changes to the structural integrity of the eyelids, including lash loss.

Dentists have done a masterful job in educating patients as to the importance of routine oral hygiene. "Plaque" has become a household term for dental biofilm. While in the past years of biofilm-related inflammation caused elderly patients to require dentures, patient education is helping full dentures become obsolete. In 2006, a CDC report claimed "the baby boomer generation will be the first where the majority will maintain their natural teeth over their entire lifetime" [69].

We too can improve patient outcomes by preventing damage to the critically important meibomian glands and other eyelid structures, rather than reacting to the damage once it is already done. To prevent DEBS, we need to make routine lid hygiene akin to "brushing your teeth" and electromechanical debridement as commonplace as routine dental cleaning. This is now possible, but it must start with a new understanding of DEBS, and an active role by the ophthalmologist stressing lid hygiene and advocating for regular MBE procedures on all patients, the sooner the better, particularly on those at higher risk.

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Conflict of interest

James M Rynerson is the President and CEO of BlephEx, LLC and Henry D Perry is the senior founding partner of Ophthalmic Consultants of Long Island. The authors report no other conflicts of interest in this work.

Appendices and nomenclature

Biofilm	Groups of microbial cells enclosed in a matrix of primarily polysaccharide material that are intimately associated with a surface
Blepharitis	Inflammation of the eyelid
Cylindrical dandruff	Sleeve of material that forms around the eyelash, likely due to biofilm accumulation combined with eyelash growth
DEBS	Dry Eye Blepharitis Syndrome; a proposed unifying diagnosis that links both dry eye and blepharitis as stages of inflammation caused by progression of biofilm
Goblet cells	Specialized epithelial cells that secrete mucus, helping maintain the barrier against pathogens
Microblepharoexfoliation (MBE)	Lid margin cleaning, with electric rotary sponge, in an effort to remove accumulated biofilm
Virulence factors	Factors released by bacteria that cause inflammation, including exotoxins, enzymes, super-antigens and cytolytic toxins
Volcano sign	Swollen, follicular tissue around the base of the lash

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