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Complexity of the tear film: Importance in homeostasis and dysfunction during disease

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The tear film is a unique thin fluid layer of approximately 3μm thick and 3μl in volume that covers the outer mucosal surfaces of the eye. As such it is the interface of the ocular surface with the environment. This film is transparent and has an aqueous/mucin phase, decreasing in mucin concentration towards a distinct superficial lipid layer. These layers or regions contain distinct biochemistries which underlie distinct functions. The lipid layer of the tear film is thin, in the order of 50 to 100nm, yet contains many different lipid species including non-polar lipids such as cholesterol and wax esters which make up it bulk, and polar lipids such as (O-acyl)-ω-hydroxy fatty acids and phospholipids which interact with the aqueous layer. The majority of these lipids, with the possible exception of the phospholipids, are secreted from meibomian glands located at the lid margin. The most significant role of the lipid layer is in retarding evaporation of tears from the ocular surface. The aqueous/mucin layer forms the bulk of the tears. Most of the aqueous fluid is secreted from the lacrimal glands, which also secretes a specific variety of proteins, electrolytes, and water. The conjunctival epithelium is a second source of electrolytes and water in the tears. The aqueous phase provides oxygen and nutrients to the underlying avascular corneal tissue and flushes away epithelial debris, toxins and foreign bodies. Many of the mucins are secreted by specialised goblet cells in the conjunctival epithelium, and some transmembrane mucins are released into the tear film from corneal and conjunctival epithelial cells. When anchored into the epithelial cells, these transmembrane mucins project into the aqueous phase and help stabilise the tear film.

The production of tears is regulated. The lacrimal and meibomian glands receive both parasympathetic and sympathetic innervation, and goblet cells are also believed to be under neurogenic control via parasympathetic innervation. The ocular surface is richly innervated by trigeminal afferent nerves. The cornea and lid margins are particularly densely innervated. Inputs and outputs from these nerves form the basis of a reflex arc which adjusts tear secretion to meet daily demands. The menisci, lying at the interface between the lid

margins and the surface of the globe, permit the tears to move towards the lacrimal puncta and canaliculi, from whence they drain into the nasolacrimal system. Tears lost by evaporation and drainage are replaced by tear secretion. Tear turnover rate is 1–3μl/minute under normal circumstances but can increase greatly upon stimulation by irritants or in response to emotion. Indeed, three different types of tears have been described; basal tears that are normally present on the ocular surface, reflex tears produced upon stimulation and closed-eye tears which bathe the eye during sleep. Each of these tear types contains it own unique biochemistry. For example, the levels of secretory immunoglobulin-A (sIgA) decreases in concentration from closed-eye, to basal to reflex tears whereas other tear proteins such as lactoferrin, lipocalin-1 and lysozyme do not appreciably change their concentration in the different tear types.

One of the major functions of the tear film is to protect the ocular surface from potentially pathogenic microbes, and it appears to be extremely effective in this function. In a healthy eye the apparent density of microbiota of the ocular surface is very low and the types of microbes are very restricted. When compared to the microbiome of another mucosal surface such as the oral cavity or intestinal tract, the ocular surface is hardly colonised by microbes at all. This is due to several things including the wiping action of the lid during the blink (which also helps to replenish the tears), sloughing of the epithelial cells, and the many varied and redundant antimicrobials in tears. Mucins can have an antimicrobial function by acting a decoy receptors for microbes preventing them adhering to the underlying tissues. Many of the major tear film proteins such as lysozyme, lactoferrin and sIgA are either directly toxic to bacteria (lysozyme which is an enzyme that hydrolyses the peptidoglycan cell walls of bacteria) or reduce nutrients needed for microbial growth (lactoferrin chelates iron). Secretory IgA prevents adhesion of microbes to the epithelia and enhances phagocytosis (sIgA) by neutrophils that enter the tear film during sleep to patrol and protect the enclosed globe. These can act in synergy to enhance their antimicrobial actions. In addition, tears contain a rich assortment of antimicrobial peptides. These peptides, such as the defensins, destabilise microbial cell membranes to such an extent that normal membrane function is lost and membranes may even burst.

The dynamic nature of the tear film perhaps allows it to tolerate introduction of contact lenses. These are a relatively safe form of vision correction worn by some 140 million people in the world. However introduction of contact lenses can disrupt the stability of the tear film and a normal layered tear film probably never forms for any length of time on the surface of contact lenses as they are today. Contact lenses increase the evaporation of the tear film by approximately 2 fold, and the tear film breaks up on a contact lens about twice as fast as on the surface of the cornea. Even with these disruptions, multiple challenges or factors are needed cause ocular surface disease.

When disease does occur the major forms of disease are either dry-eye, ocular allergy or those associated with contact lens wear. Dry-eye disease affects ≥20% of the population. The aetiology of this disease is believed to involve inflammation of the ocular surface and/or the lacrimal gland and excessive evaporation of tears. The evaporation increases tear film osmolarity that can lead to inflammation and a vicious cycle that may need anti-inflammatory therapy to disrupt it and alleviate symptoms. Ocular allergy is a spectrum of

clinical conditions, ranging from the common conditions of seasonal allergic conjunctivitis to the clinically more severe and chronic diseases of vernal or atopic keratoconjunctivitis. Each form of disease involves different cellular and molecular pathways. Conjunctival epithelial cells are probably the first cells to encounter air-borne allergens and respond to them with release of cytokines. Recent evidence implicates goblet cells in to the pathology of allergic conjunctivitis via histamine and leukotrienes. The mast cells in the conjunctiva can recognise allergens and release histamine and a variety of other inflammatory mediators.

It is the ability of the ocular surface to respond to disease and the dynamic nature of the tear film that has encouraged people to search for biomarkers in tears for a variety of diseases. Changes to the tear film proteome in response to local diseases such as dry-eye have been shown. But the biochemistry of tears also appears to change in response to diseases of the eye such as retinopathy associated with diabetes. Perhaps more surprisingly, biomarkers have been found in tears that seem to show good specificity and sensitivity in the diagnosis of remote diseases such as breast cancer.

The tear film has yet to be completely characterised in terms of composition, structure and function. But recent advances in many of these areas are highlighted in the manuscripts in this Special Edition of Experimental Eye Research.

Butovich examined the published data on the tear lipidome. This important outer layer of tears is most likely involved in preventing evaporation of the tear film and perhaps in ensuring stability of the overall tear film. New analytical methods and instrumentation (most notably those based around mass spectrometry) have resulted in high sensitivity and selectivity that have produced a dramatic increase in our knowledge of the biochemistry of lipids from the Meibomium glands and those of the tear film. Meibum is composed mostly of wax- and cholesterol-esters, making up to 70% of the whole. As these wax- and cholesterol-esters are highly hydrophobic, tears contain polar lipids including (O-acyl)-ω-hydroxy fatty acids and phospholipids. Whilst these polar lipids are in lower amounts (<5% of the total), they are important in spreading the lipid layer over the aqueous tear film.

Sweeney, Millar and Raju reviewed the current knowledge on tear film stability. Their review highlighted a range of both clinical and laboratory techniques that have been used to examine the stability of the tear film. Tear film stability is likely to be important in many conditions including dry-eye and during contact lens wear. Tear film break up times after instillation of fluorescein can be measured using slit-lamp biomicroscopy, although the repeatability of this measure is poor. Attempts to increase repeatability have included using non-invasive techniques which often involve the observation of an illuminated grid pattern reflected from the anterior tear surface, or corneal topography systems. Novel techniques that allow simultaneous examination of tear film break-up and lipid layer and its detail during tear thinning are being developed. The evaporation rate of the tear film have often been a centre point of many studies into tear film stability. Evaporation rate is controlled by the tear film lipid layer. The formation of liquid crystals of tear film lipids oriented at right angles to the surface may brace each other and resist collapse of the lipid layer and hence prevent evaporation. The authors also review the factors such as contact lens wear, the environment and ethnicity that can affect tear film stability.

Karnati, Laurie, and Laurie focused on tear proteins, especially lacritin and lipocalin-1, as potential biomarkers, drug targets, and perhaps biotherapeutics for ocular surface disease. The ocular surface epithelia and ocular adnexa, specifically the lacrimal gland, secrete a wide variety of proteins into the tear film. Little research has focused on identifying the myriad of tear proteins and determining their function on the ocular surface. Tear proteins as natural biotherapeutics could be effective in treating ocular surface disease, especially dry eye, as they could alleviate specific symptoms rather than have a palliative effect. Lacritin and lipocalin-1, both secreted by the lacrimal gland, are highlighted. These two proteins are multifunctional and their various roles in the tear film are described.

McDermott reviewed the antimicrobial compounds in tears. There are many proteins in tears with antimicrobial activity such as lysozyme, lactoferrin, secretory immunoglobulin A as well as other less well known compounds such as secretory phospholipase A2 and small cationic proteins such as β-defensins. At the present time definitive proof of antimicrobial activity of all of these in tears is lacking, but there are examples of synergy between antimicrobial proteins in tears that may facilitate actions of many of these even when they are in low concentrations. Research on the effect of factors, such as contact lens wear, that are associated with increased microbial infection on levels of antimicrobial proteins is an area identified for further research. The high concentration and types of antimicrobials in tears perhaps helps explain the sparse numbers of cultivable microbes that can be isolated from the ocular surface.

Hodges and Dartt explored the mucin layer of the tear film. The over twenty mucins identified to date are divided into small secretory, large gel-forming, and membrane-spanning. Although most of the tissues that produce tears also secrete mucins, the most important mucins for ocular surface protection are the large gel-forming mucin MUC5AC secreted by the conjunctival goblet cells and the membrane-spanning mucins MUC 1, MUC4, and MUC 16 produced by the stratified squamous cells of the cornea and conjunctiva. These mucins form the tear film layer that is closest to the surface of the corneal and conjunctival epithelium and thus play critical protective and homeostatic roles. Tear film mucin production can be affected by ocular surface disease and thus can contribute to disease progression. Mucins are produced by all the wet mucosal surfaces of the body, but those produced by the airways and gastrointestinal tract are best studied. The differences in mucin type, function, regulation of production, and role in disease by the cornea/conjunctiva, airway epithelia, and gastrointestinal tract epithelia were discussed.

Meng and Kurose examined the mechanisms by which corneal afferent sensory nerves regulate tear production. Corneal sensory nerves form a rapidly responding, protective system that regulates tear secretion. In spite of the importance of these nerves, they have been woefully under investigated. Stimulation of corneal sensory nerves can cause pain or lacrimation. The complex wiring for this neural reflex originates in the ocular surface, connects through several levels in the brain and then targets the secretory organs. The present review described the state of current knowledge on the multiple levels of this complicated neural reflex and suggests a potential role of the dysfunction of the neural reflex or the tear secreting glands in dry eye disease.

Mann and Tighe highlighted the interactions of contact lenses with the tear film. Whilst contact lens wear can change most components of the tears including lipids, proteins, mucins and electrolytes, the authors argue that changes to the tear film lipids and deposition of these on contact lenses might be of primary importance since lipid immobilisation increases their susceptibility to autoxidative degradation which may in turn liberate reactive species that cause discomfort. The authors show that proteins can deposit differentially on the anterior (e.g. kinin) and posterior (e.g. vitronectin) surface of contact lenses and this may also be a factor in contact lens discomfort. Research on the effects of contact lens wear on mucins has been inconclusive to date. The authors conclude that we need to develop a greater interest in the detailed response of the individual to contact lens wear. With the advent of more sensitive biochemical and biophysical analytical techniques this type of personalised analysis may be coming of age.

Willcox examined the concept of a normal ocular microbiota. The level of cultivable microbes from the lids, conjunctiva and tears are low, typically <100 colony forming units (cfu) from a swab of the mucosa or <100cfu/μl from tears. This contrasts starkly to the numbers that can be isolated from the mucosal surfaces or saliva of the oral cavity; 100 cfu/one tongue mucosal cell and 10^7–10^8 cfu/μL of saliva. Coagulase-negative staphylococci, *Propionibacterium* sp., and *Corynebacterium* sp. are the most common bacteria grown from the conjunctiva, lids or tears. Contact lens wear may change the ocular microbiota but the effect seems to depend on the length of lens wear, type of polymer or use of lenses on particular wear schedule (e.g. daily versus extended wear). With the advent of the human microbiome project, use of microbial identification techniques that are not based on culture is being applied to the ocular surface. Currently only a few examples of this have been published, but this is an area of research growth.

Leonardi surveyed the inflammatory mediators of the tear fluid that are produced in ocular allergy. Although there are clinical metrics for diagnosing allergic conjunctivitis, there are no specific markers for monitoring disease progression or treatment effectiveness. As the mediators produced during disease are complex and numerous, new technologies that can evaluate multiple compounds simultaneously could be helpful. It is important to identify the inflammatory mediators that could function as biomarkers for diagnosing or measuring disease progression. In addition these mediators could contribute to understanding the immune mechanisms at play in ocular allergy and could serve as potential targets for therapeutic intervention. Development of laboratory tests for diagnosis and monitoring of allergic conjunctivitis is a high priority.

Pflugfleder and de Paiva reviewed the role of innate inflammatory pathways in dry eye disease. Current dry eye therapies are mostly palliative. To develop treatments that target the innate inflammatory products could have potential for treating the ocular surface disease component of dry eye. This article focuses on the cytokines produced by the infiltrating T cells and how they alter the normal cytokine balance. The Th2 cytokine IL-13, the Th1 cytokine IFNγ, and the Th17 cytokine IL-17 are the cytokines highlighted. Therapies that target these three cytokines have potential for treating the ocular surface disease component of dry eye.

Hohenstein-Blaul, Funke and Grus examined the current state-of-play of tears as a source for biomarkers for various diseases including dry eye disease (including Sjögren's syndrome), glaucoma, diabetic retinopathy and cancer. Biomarkers have been discovered using a variety of proteomic techniques, many of which are ideally suited to use with tears as they require only small sample volumes yet generate information on many different proteins. There is good evidence for elevated levels of cytokines and damage-associated molecular pattern proteins such as members of the S100 proteins, and others, in dry-eye disease. Combinations of biomarkers have been shown to be useful in diagnosing dry-eye disease. Biomarkers in tears for breast cancer also include S100A8 along with complement C1q and others.

Jalbert reviewed the effect of nutrition on the tear film. This is an area of research that has been somewhat neglected. Nutrition is known to be important for ocular health (e.g. vitamin A), but effect of diet on the tear film has only recently received renewed attention. Changes in diet have been noted in Sjögren's patients, but controversy remains on cause and effect. It has been argued that the severe dry mouth symptoms experienced by Sjögren's patients may impact their dietary habits. There has been epidemiological evidence for a role of omega-3 fatty acid intake and prevention of dry-eye, and whilst anti-inflammatory effects are likely the biochemical basis of this is not known. Jalbert makes note of the fact that many clinical trials of nutrient supplementation have not been optimally designed, often lacking a placebo control arm and also being underpowered.