Hypothesis

Crosslinking of tarsal collagen as a hypothetical therapy for dry eye disease

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ABSTRACT

Background: Dry eye disease (DED) is one of the most prevalent and distressing ocular conditions worldwide; it primarily results from alterations in the natural tear film that covers the ocular surface and is often due to enhanced evaporation of its aqueous component. This process is frequently associated with dysfunction of the meibomian glands (MGs), which are embedded within the tarsal plate of our eyelids and secrete the meibum, an oily mixture of proteins and lipids. Meibum forms the outermost layer of the tear film, playing a critical role in controlling water evaporation and stabilizing the tear film by lowering surface tension. Meibomian gland dysfunction (MGD) may result from structural abnormalities in the MGs, such as tortuosity, which impair normal delivery of meibum. Increased laxity of the eyelid is also associated with development of MGD and DED, likely due to insufficient mechanical support for the glands, and causing morphological changes.

Hypothesis: We designed and initiated the development of a noninvasive method to strengthen and stiffen the tarsal collagen containing the embedded MGs. By reducing tissue laxity, our aim is to halt further morphological deterioration of the glands and promote uniform and smooth delivery of meibum to the ocular surface. Our previous studies showed that both mechanical tensile strength and rigidity (Young's modulus) of tarsal collagen in animal and human eyelids were significantly enhanced by exposure to ultraviolet-A (UV-A) radiation with a wavelength of 365 nm in the presence of riboflavin as a photosensitizer.

Conclusions: We propose that performing this procedure at the initial manifestations of MGD and DED may prevent disease progression by restoring and preserving the normal morphology of the glands through reduced laxity, thereby ensuring proper secretion of the meibum into the tear film. The underlying principles and safety of the procedure were discussed in detail, and further pre-clinical evaluation steps were proposed and justified. Based on the proposed concept and the results of previous ex-vivo studies, in-vivo animal experiments and human clinical trials are currently in preparation.

KEYWORDS

dry eye disease; eyelid; tarsus; meibomian glands; collagen crosslinking; UV-A radiation

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INTRODUCTION

Located within the confines of our two eyelids, the meibomian glands (MGs), also known as tarsal glands (Lat. glandulae tarsales), are tubulo-acinar sebaceous glands without associated hairs, and are therefore described sometimes as "free sebaceous glands" [1]. They are classified functionally as exocrine glands with a holocrine type of secretion. Their oily secretion, the meibum, which is unique to these glands, is an inordinately complex mixture of lipids (numbering in the hundreds), proteins (about 90), and other organic compounds, and represents the main component of the superficial layer of the tear film [2–7]. This layer serves two critical functions: protection against excessive evaporation of the aqueous phase and stabilization of tear film by lowering its surface tension [2, 3, 6].

Summarizing the knowledge thus far gained [2, 8–16], each meibomian gland consists of a central duct (diameter 100–150 μm; length ~5.5 mm in the upper eyelid, ~2 mm in the lower eyelid), which is connected through ductules (diameter 30–50 μm; length ~150 μm) to circular clumps of 10–15 secretory acini (clusters of meibocytes; diameter 150–200 μm). The ductal lining consists of keratinized squamous epithelium. Reported average number of glands is 31 (range 20–40) for the upper eyelid and 26 (range 20–30) for the lower eyelid. The distal extremity of each gland is naturally closed while the other end opens at the eyelid margin through an orifice near the mucocutaneous junction. This anatomical arrangement facilitates the efficient transfer of meibum to the anterior surface of the tear film [2, 8, 9, 11, 14–16]. Figure 1 illustrates a sectioned lower eyelid margin presenting the structural components of the glands and their immediate surroundings.

In their native state, the meibomian glands are embedded inside the tarsal plate or tarsus, a tissue with combined fibrous and cartilaginous characteristics capable of providing both structural integrity to the eyelid and the stiffness needed for preserving its contour. The fibrous proteins in the tarsal plate's constitution include collagen, which imparts strength, and elastin, which provides elasticity. In brief, the tarsal tissue consists mainly of collagens types I, III, and VI, together with elastic networks of fibrillin and elastin fibers [17, 18]; this subject will be considered later in more detail.

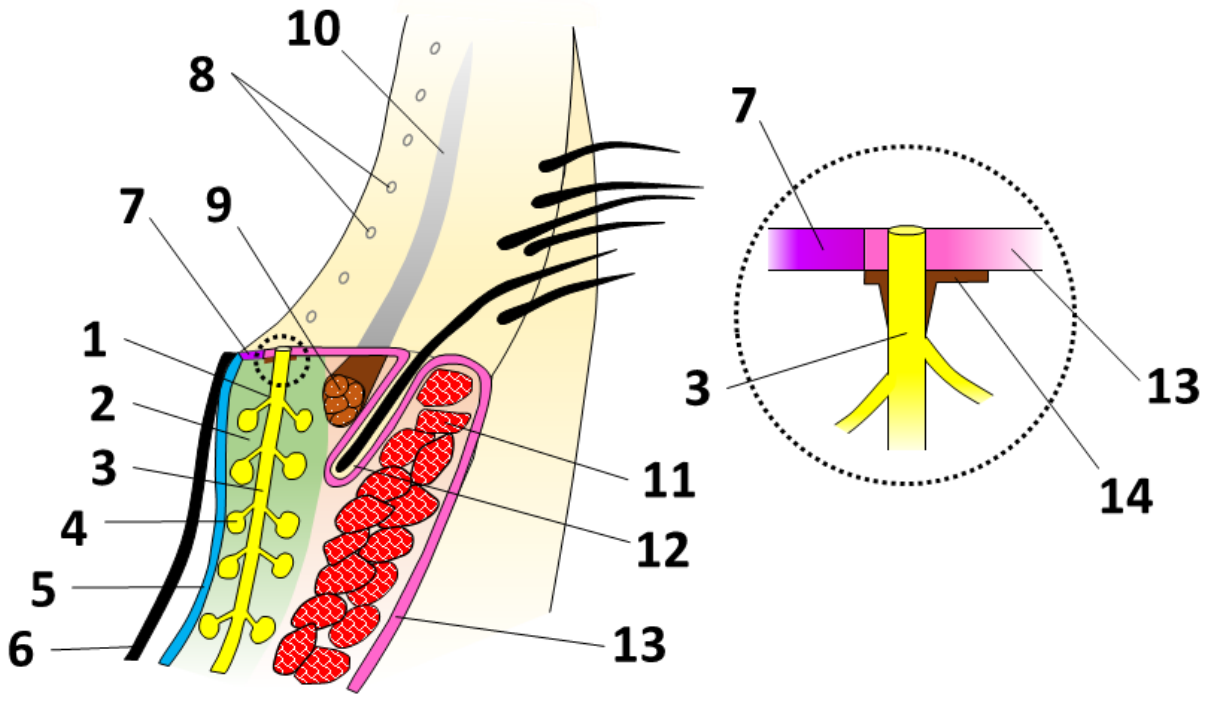

Figure 1. Schematic cross-section through a part of the lower eyelid showing the main structural components of the eyelid margin including a meibomian gland. 1–meibomian gland; 2–tarsal plate; 3–meibomian gland: main duct; 4–meibomian gland: acinus; 5–tarsal (palpebral) conjunctiva; 6–ocular surface; 7–mucocutaneous junction; 8–ductal orifices; 9–muscle of Riolan; 10–gray line; 11–palpebral orbicularis oculi muscle (pretarsal part); 12–eyelash follicle; 13–skin; 14–epidermis. This illustration is based on a compilation of information available in literature. Colors are conventional. Relative dimensions are not to scale.

Meibomian gland dysfunction (MGD) is an umbrella term for a group of disorders, both congenital and acquired, that are associated with functional anomalies of the glands and can cause altered tear film, dry eye symptoms, and ocular surface disease [12, 16, 19–21]. Acquired MGD presents in three forms: the hypersecretory form is caused by excessive secretion of lipids, the hyposecretory form leads to low delivery of lipids due to diminished gland function, and the obstructive form leads to keratinization of the ductal system [16, 19–21].

According to the Tear Film & Ocular Surface Society (TFOS), dry eye disease (DED) is currently defined [22] as "a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles". Worldwide prevalence of DED varies from 5% to 50%, and its economic burden and impact on vision, quality of life, and work productivity are considerable [23, 24]. Research activity to find effective treatments has become a never-ending pursuit [25–27].

DED and ensuing impaired vision result from disturbances in the natural tear film that bathes the ocular surface. The primary transgressor behind this disruption is an increased evaporation of the aqueous phase in the tears due to alterations in meibum secretion that are inherently related to MGD. Indeed, MGD is currently recognized as the leading cause of evaporative DED [28–32], and the vast majority of dry eye patients have indeed evaporative DED. For instance, in a clinical evaluation of a cohort of 224 patients afflicted with DED, 86% showed signs of MGD [33].

In this conceptual article, we propose a radiative method for treating early-stage DED, designed based on the mechanical response of the microenvironment surrounding MGs to ultraviolet radiation in the 315–400 nm wavelength range—this is UV-A, a non-ionizable radiation that is considered biologically safe. The photochemically induced crosslinking of tarsal collagen can lead to enhanced strength and stiffness of the surrounding connective tissue sheltering the MGs, with beneficial effects on their function.

HYPOTHESIS

Tarsus as a microenvironment for the glands

The tarsal plate of the eyelids (also known as the palpebral tarsus) is a tissue with both cartilaginous and fibrous characteristics; this has led to its occasional inclusion in the category of fibrocartilage [17, 18]. However, this classification may not be justified in the case of the palpebral tarsus—warranting the discussion below.

Cartilage is a non-vascular connective tissue found in many regions of our body, where it functionally supports a multitude of biological structures, tissues, and organs. It is commonly accepted that there are three types of cartilage: hyaline, elastic, and fibrocartilage, all consisting of extracellular matrix (ECM) and specialized cells (variably termed as chondrocytes, chondroblasts, fibrocytes, or fibrochondrocytes). Hyaline cartilage is the most common and weakest, contains mainly collagen type II, and is found in the articular regions of the body. Elastic cartilage is abundant in elastic fibers, is resilient and flexible, and is found in structures subjected to deformation (e.g., ear, larynx). These two types are enclosed within a fibrous sheath known as the perichondrium. Fibrocartilage is the strongest cartilage, does not have a perichondrium, and consists of alternating layers of hyaline cartilage reinforced with parallel bundles of collagen and interspersed with thick layers of dense collagen [34, 35]. Although cartilage itself has been identified in antiquity [36], a clear description of the fibrocartilage type was provided only much later by Toynbee [37]. Closer to our times, we witnessed notable advances in the study of fibrocartilage thanks to seminal work of Benjamin's team in Bristol [38–40], as well as to more recent activity by others [41–47].

Locations of fibrocartilage in our body are highly diverse. It is so far known to be present in about ten different locations in the body [17, 18, 42,44–49], including the meniscus, annulus fibrosus of the intervertebral disk (but not its nucleus pulposus), temporomandibular joint, triangular fibrocartilage complex of the wrist, glenoid labrum (shoulder), acetabular labrum (pelvis), entheses (places where tendons attach to bones), pubic symphysis, manubriosternal joint, and lastly—yet seldom mentioned—the eyelid's tarsal plate. Based on its functional presence in the musculoskeletal regions, it was concluded that the fibrocartilage must be found (a) where shock absorption is needed because of repetitive action; (b) where a large congruence area between tissular structures is beneficial; (c) where there is a need to increase the mechanical efficiency of the associated muscles. There are four types of fibrocartilage [46], generally determined by their location in the body and assessed by their ability to function as pads, cushions, or friction reducers, or to assist the fitting of bones into joints.

None of the above can definitely explain the presence of an isolated fibrocartilage unit serving as the tarsal plate in our eyelids, although the repetitive character of the blinking action may partly justify such occurrence. With an average of 12.5 blinks/minute [50, 51] (i.e., 12,000–15,000 blinks per waking day), this poses a significant demand on the eyelid. However, there is some reluctance in identifying the tarsus as a fibrocartilage. After a few mentions of the tarsal plate in several classic German histology handbooks (which are hard to retrieve nowadays but are all cited elsewhere [8, 17]), the first to discuss in detail the role of the tarsus and its relation to MGs and surrounding muscles was Whitnall in his monumental book on orbital anatomy [8]. As a note, a century after its first edition this book has stood the test of time and is regarded as the essential reference for surgery of orbit and eyelids, contributing considerably to the development of modern oculoplastic surgery [52, 53]. Without mentioning the term "fibrocartilage", Whitnall realized that tarsus is a distinct connective tissue composed of dense tissue and elastic fibers, and called it tarsal cartilage [8] despite a previous observation that tarsus is devoid of cartilage-specialized cells. In his viewpoint, the tarsal plate appeared functionally as a "peri-meibomian capsule", with elastic components concentrated around the glandular acini. The presence of nerves, blood vessels, smooth muscle fibers, and peripheral adipocytes is also mentioned. Whitnall believed that the role of the tarsal plate is to stiffen the eyelid and contribute to more efficient protection of the ocular surface, rather than simply be a host for MGs. However, he seemed somewhat surprised at how much space the glands occupy within the tarsal extracellular matrix (ECM) [8].

Since then, only a few studies have been published on the structure and composition of the palpebral tarsus [17, 18, 54–56]. Taking into consideration that the interactions between glandular epithelia and their surrounding ECM can influence the activity of secretory cells in diverse regions of the body, Milz et al. [17] rigorously analyzed tarsal plate specimens harvested from the upper eyelids of 14 human cadavers using immunohistochemistry. They found intense immunolabelling for collagens types I, III, and VI (type II was conspicuously absent), for glycoproteins including aggrecan, versican, tenascin, and cartilage oligomeric matrix protein (COMP), and for some glycosaminoglycans such as chondroitin-4 sulfate, chondroitin-6 sulfate, and dermatan sulfate. A few scattered fibroblasts and blood vessels were seen histologically. An amorphous and acellular layer was observed in close apposition to MGs, labelled as chondroitin 6-sulfate and dermatan sulfate and termed territorial matrix; the investigators suggested its possible role in controlling the secretory process [17]. Elastic fibers were generally distributed throughout, but congregated around the main ducts, as also noted by Whitnall [8]. It was concluded that the tarsal plate is a highly specialized tissue displaying a combination of fibrous and cartilaginous characteristics, and its stiffness may be due to a high content of aggrecan [17].

Ezra and colleagues, currently the leading research group of eyelid structure and laxity, have critically discussed [18] the validity of calling the tarsus a cartilage, given that it is discernibly a fibrous tissue devoid of chondrocytes. The group later reported a seminal study [54] on the main structural components in the human normal tarsal plate as compared to those in cases of floppy eyelid syndrome (FES), aiming at elucidating the impact that the observed changes can have on tarsal biomechanical properties. Previous studies on the laxity of tarsal ECM in FES indicated that while collagen organization remained unaltered, a reduction of elastin and elastic fibers could be routinely observed [57–59]. Rather unexpectedly, the elastin depletion is incongruous with the increased elasticity displayed by tarsal plates in FES. To elucidate such aspects, the study [54] investigated in detail the fate of elastic fibers including mature elastic fibers (MEFs), as well as oxytalan and elaunin fibers, employing specific staining techniques. Collagen types I and III were stained immunohistochemically with their respective antibodies. It was found that in FES the MEFs content decreased while oxytalan fibers content increased and elaunin content remained constant. This was interpreted as a change in elastic fiber phenotype rather than a reduction of MEFs. Based on staining intensity, the accumulation of collagens was noticeable [54]. These findings were attributed to an adaptive response of a lax tarsus to the recurring mechanical demands on the eyelid, which was confirmed in a collagen matrix model [51]. Important for our discussion here, a concentration of elastic fibers was detected in the healthy tarsal plates [50], within both the intermeibomian and perimeibomian regions, a finding confirmed histologically in ex-vivo ovine tarsal plates [60].

More recently, Gao et al. [56] reported on the structure and biomechanics of rabbit tarsus specimens. Based on results from histology and scanning electron microscopy, they proposed an intermeibomian assemblage consisting of parallel collagen lamellar network interspersed with elastin fibers, in a parallel arrangement both horizontally and sagittally, then a perimeibomian network of crossed elastin fibers.

To conclude, the absence of collagen type II, which is typical to most cartilages [61], and the presence of collagen VI, which is not found in any other cartilage [61], suggest that the eyelid's tarsal plate may not belong formally to the fibrocartilage type but is nevertheless able to fulfil its role efficiently.

Tarsus as a continuum for transmitting forces

For the validity and possible success of our concept, the palpebral tarsus has to act as a continuous medium to enable the transmission of force, i.e., the additional strength and stiffness acquired through photochemical crosslinking. In deformable continuous bodies like the tarsus, stress is the measure of the state of force transmitted inside the body. A body force is caused by action at a distance and is expressed as force per volume unit (surface traction is generated through contact between two bodies and measured as force per area unit, though this is not relevant to our discussion) [62–64]. Body forces are transmitted through a medium in the form of waves. It must be emphasized that waves transfer energy, not matter; however, the transfer is accomplished through the vibration of the medium's constituent particles (molecules, atoms). The waves transporting kinetic energy are called mechanical waves. These aspects are the subject of a discipline known as "mechanics of the continuous body" or "continuum mechanics" [62–64]. Perusal of the major textbooks in this field requires advanced knowledge of mathematical physics. Basic continuum mechanics aims at describing mechanical phenomena within a medium, disregarding its structure on a micro- or nanoscale and using mathematical formalism [62–64].

Following the crosslinking of collagen, a wave of increased strength and stiffness is generated which is supposed to be transferred to the MGs through the only available continuous medium, the tarsus itself. More precisely, this deformable medium consists of a composite network of collagen and elastic fibers intermingled with other biological entities (glycoproteins, cells, blood vessels, nerves, smooth muscle fibers). Clearly the tarsal plate is neither homogeneous nor isotropic, and is therefore far from being an ideal substrate for modelling within a mathematical framework, although certain collagenous gels and tissues as such have been modelled using a continuum mechanics framework [65]. However, the behavior of the tarsus can be regarded as identical to that of biological soft tissues, which we consider to be biopolymer hydrogels, such as collagen, fibrin, elastin, vimentin, and actin [65–72]. Herein we shall briefly mention some basic aspects of the transmission of force through three fundamental media—solid, liquid, and gel. While in a solid there is an elastic resistance against shear stress, a liquid displays zero resistance and the medium will not support a shear wave, as the pressure is transmitted uniformly in all directions. However, a gel, which is a liquid contained within a macromolecular matrix, displays certain resistance to shear stress and deforms when subjected to forces, but is incompressible and can act as an elastic continuum capable of transmitting force [73–76]. When the liquid in gels is water (like in our tissues), we call them hydrogels.

The long-range interactions between cells or between them and the ECM are mediated in our body through processes of force transfer within the ECM, which is essentially a hydrogel. Modelling studies of biopolymer hydrogels suggest that the transmission of forces, whether external or internal due to loads, body movement, or blood flow, or internal due to cellular activity, is strongly dependent on the nonlinear physical properties of the medium and on its level of tension [77–82]. Based on the above analysis, we believe that the palpebral tarsal plate can function as an elastic, deformable continuous body able to transfer forces and stress in order to support the MGs mechanically, thus contributing to maintaining their shape and size. This in turn will ensure consistent delivery of the meibum.

What happens during collagen crosslinking?

The crosslinking-induced enhancement of collagen's mechanical properties, confirmed experimentally in many publications [83–98], is also supported by theoretical treatments that have helped gain a deeper understanding of the process. The correlation between crosslink density and nanoscale/mesoscale deformation mechanisms was investigated by Markus Buehler's MIT group, who developed a series of successive molecular models [99–102] to reach some important conclusions. Thus, the essential role of crosslinks is to generate interconnectivity between collagen molecular chains within fibrils, which induces tunable strength and toughness. The absence of crosslinks brings about the commencement of intermolecular sliding that produces weak structural networks. The type of crosslinks is also consequential. At the same crosslinking density, the fibrils containing trivalent crosslinks are stronger than those containing divalent crosslinks due to the difference between the resulting levels of connectivity [99–102]. Low crosslink densities are insufficient to prevent continual slippage within fibrils. At high crosslink densities, the deformation consists of intermolecular sliding combined with molecular uncoiling, and stiffness and crosslink density are proportional. In a fully crosslinked fibril, interconnectivity is dominant and all structural networks deform synergistically, with very little sliding. The models also indicated that at a certain level of deformation, the crosslinks might start to break down. Additional crosslinking can improve the mechanical behavior of the fibrils [99–102].

Morphology and tortuosity of the meibomian glands

Both intuitively and in reality, a distorted morphology of MGs is not compatible with normal performance [12]. Nevertheless, the morphometric characteristics of MGs have been seldom contemplated as criteria to assess their dimensions, shape, or performance. Meibography techniques, sometimes combined with optical coherence tomography scans, have been the methods of choice in such investigations. A rather low number of reports are available, with varying results [103–109].

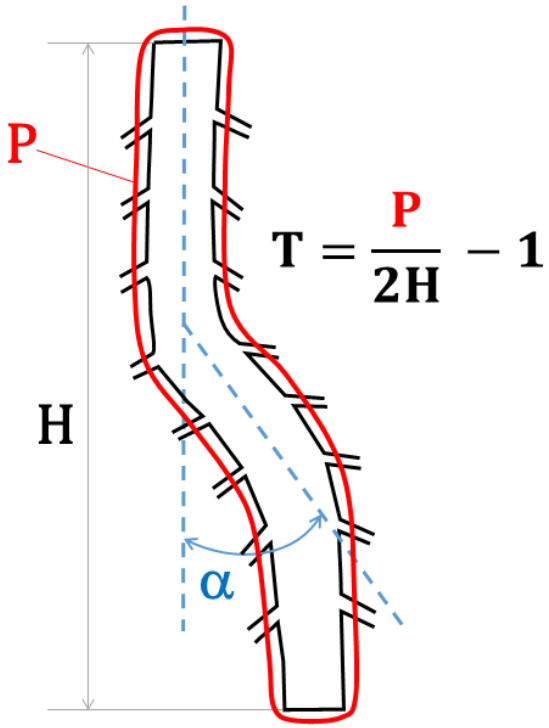

Figure 2. A simplified method for estimating the tortuosity (T) of a single meibomian gland based on data provided by meibographic image analysis. P: the perimeter enclosing the gland; H: the length of an imaginary straight line between the two ends of the gland. The bent angle (α) can also serve as an indicative criterion. Adapted from reference [109], with the approval of Dr. Q. Dai.

For instance, the morphology of MGs, including thickness and bent angle (as defined in Figure 2), has been shown to impact on dry eye symptoms—MG thickness and bent angle of the upper lid showed significant negative correlations with tear film breakup time (TBUT), suggesting that increased gland deformation is associated with reduced tear film stability [103]. Correlations of morphological characteristics of MGs associated with age and gender have been reported and recommended for evaluating the condition of the ocular surface [104], but the study did not include patients with dysfunctional MGs. Another study [105] has compared patients with MGD against patients with normal MGs and found that the glands' length and width in dysfunctional MGs were significantly lower than in the normal glands, suggesting atrophy. An enhanced distortion of MGs was found by meibography in patients with allergic conjunctivitis [106, 107], however in this instance the "meiboscores" were estimated from images of low clarity.

In an extended meibographic investigation [108] of asymptomatic children (ages 7–14) in Hangzhou, P. R. China, five different morphologies of the MGs were identified (vertical, tortuous, overriding, hooked, and U-shaped). The clinically scored distortion was not related to age. However, the MGs were more distorted in children who also had a concurrent conjunctival follicular reaction [108].

In a recent study [109], the right eyes in 60 subjects, including both patients with MGD and volunteers with normal MGs, were assessed clinically. Meibography was used to assess level of atrophy, gland density, and gland tortuosity. Tortuosity, probably a suitable measure for level of distortion, designated here by the letter T, was calculated with Equation (1), which was inspired by a previous study [110] of retinal vessel tortuosity. In the equation, P is the perimeter of a gland and H is the length of an imaginary straight line between the two ends of a gland (Figure 2) [109].

All these parameters were indirectly measured by quantifying the pixels obtained from the computer analysis of the meibographic images. It was found in both eyelids that the average tortuosity for all MGs was higher in patients with clinically diagnosed MGD, with statistically acceptable significance [109]. The study indicated that glands with a distorted morphology (i.e., more tortuous) are more likely to be associated with MGD and suggested that tortuosity could serve as an efficient tool to diagnose early-stage MGD [109].

These studies [103, 105, 108, 109] suggested a correlation between the morphology of MGs and their ability to function normally, which can play a role in the progression to DED. This may also imply that maintaining the shape of MGs in an unaltered state could be a deterrent against MGD. A quantitative correlation between stiffness of tarsal collagen and tortuosity of meibomian glands would be relevant; to our knowledge, no such study is available.

The opportune timing for applying a method to strengthen the tarsal microenvironment is associated with the pathological mechanism of MGD, where a process of keratinization may be involved. There are currently two theories regarding the mechanism. The ductal-central theory traditionally holds that MGD involves hyperkeratinization of the ductal epithelium, leading to thickening of the epithelium, obstruction of orifices, stasis of meibum secretion, cystic dilation, and acinar atrophy [16, 21, 111]. The meibocyte-centric theory is based on the observation in animal models [111–113] that obstruction of orifices can occur without the presence of ductal hyperkeratinization. It was suggested that the gland atrophy is caused by deficient differentiation and proliferation of meibocytes, from basal to mature and hypermature cells, leading to abnormal self-renewal. Since both hyperkeratinization and abnormal self-renewal of meibocytes may contribute to the development of MGD, acceptance of both theories has been proposed [111]. Keratinization of the ductal structure makes the gland material more rigid, which may require that additional strengthening be provided by the surrounding tarsal tissue in the early stages of DED manifestations: once the keratinization process has advanced, the effect of forces transmitted through the tarsal medium to the gland could be considerably reduced [16, 21, 111, 114, 115].

Regarding the timing of performing mechanical augmentation of MGs, it has been found [24, 116, 117] that the signs of MGD appear much earlier in life than the first clinical signs of DED, thus offering an opportune juncture for performing a preventive procedure. This suggests that the proposed method can be adapted to also fulfill a preventive role against the development of MGD and DED.

Delivery of meibum

We cannot overestimate the uniqueness and importance of meibum, more precisely of the tear film lipid layer (TFLL) that is observable clinically as the actual lipid film covering the tear film. Modern research has shown that it displays antimicrobial activity in addition to the traditionally accepted protective and stabilizing effects on the tear film [118]. A very recent hypothesis [119] suggested that TFLL might assist in meeting the metabolic oxygen demands of the cornea by facilitating the transfer of oxygen from gas to liquid phase. However, as highlighted in a seminal review [120], there is a need to examine more closely and redefine the roles of TFLL. That, of course, will increase our understanding of its relevance for our vision.

Smooth and uniform delivery of the secreted meibum through the ductal orifices positioned on the eyelid margins is an important factor in improving the function of MGs by reducing detrimental effects on the tear film that may contribute to the development of DED. After secretion, meibomian oil is delivered through the ductal orifices onto each lid margin close to the mucocutaneous junction, where reservoirs are created; upon blinking, these are squeezed to redistribute the oil as TFLL [2]. The process of meibum delivery is governed by the mechanics of the tissues that surround the encapsulated MGs. More precisely, the coordinated mechanical action of two anatomical portions of the orbicularis oculi muscle is essential for the secretion process: the pretarsal orbicularis oculi and its marginal fibers at the anterior eyelid margin, anatomically defined as the muscle of Riolan [9, 16, 56, 121] (see Figure 1). It is believed that during blinking the orbicularis muscle compresses the plate with the enclosed glands, thus promoting the secretion of meibum through a squeeze-driving action, similar to milking. In turn, the contraction of the muscle of Riolan compresses the terminal part of the glands and contributes to the delivery of meibum, plus prevents its outflow between blinks. MGs are embedded in the tarsal plate, where they are in immediate apposition with the fibrous elastin-collagen system. This network system plays a passive yet important role as a continuum transmitting the muscle-generated compressive forces to individual glands through an effect of homogeneous distribution of forces. In other words, the tarsal fibrous network acts as a semisolid matrix within which the forces generated by the neighboring muscles can be exerted on the glands without being in direct contact with them [9, 16, 121].

As seen above, the mechanobiology of MGs reveals the importance of a normal tarsal plate, where elastin and collagen fibers can unalterably display their mechanical properties (strength, stiffness), enabling them to assist in the meibum delivery process. Any disturbances in the structure and properties of the periglandular elastin-collagen continuum are eventually reflected in the deficient delivery of meibum and its consequences [9, 12, 16, 19, 54, 57]. However, advanced laxity and loss of tone are a characteristic of ageing ocular adnexal tissues, including the eyelids. Palpebral laxity as such is also a salient etiological aspect in a number of identifiable abnormalities of the eyelid, including lax eyelid syndromes (with floppy eyelid syndrome as the prominent example), involutional and mechanical ptosis, involutional and paralytic ectropion, involutional entropion, distension due to eyelid tumors, and blepharochalasis. Generally, eyelid laxity has been associated with a seemingly ever-expanding range of diseases and genetic conditions [122–124]. Tarsal laxity is shown to be related [54, 57, 58, 125, 126] to a decline in elastin and collagen production, alongside with alterations in their molecular organization and network characteristics, therefore weakening the collagen-elastin continuum.

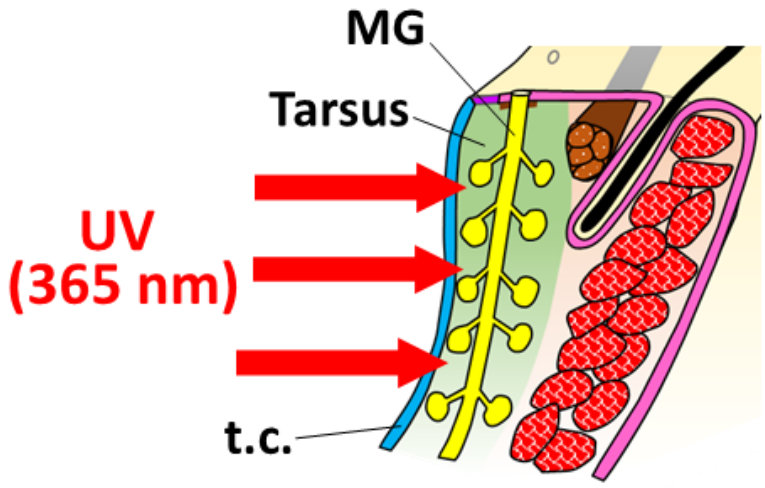

Figure 3. Principle of the procedure. Radiation penetrates across the tarsal conjunctiva (t. c.) to reach the tarsus.

An interrelation between eyelid laxity conditions (either floppy eyelid syndrome or other eyelid laxity conditions) and MGD/DED has been convincingly demonstrated [127–131]. Weakening of the fibrous elastin-collagen network in the tarsal plate compromises its roles of supporting MGs and contributing favorably to the meibum delivery process, which is actually accomplished by muscles and mediated by tarsal elastin and collagen. Lack of mechanical support provided by the territorial matrix and by the elastic fibers additionally packed within intrameibomian and perimeibomian regions, all located in direct apposition to the glands, can lead to both acinar deformity and contortion of the ductules, further compromising the normal transport and delivery of meibum. The process becomes akin to pushing a liquid through a kinked hose. Consequences include altered tear dynamics, disruption of the lid ocular surface interface, and irregular wetting. A twisted ductal system may ultimately result in the blockage of meibum circulation, which may contribute to pressure atrophy of acini and, eventually, to structural collapse of the glands [16, 127–131].

EVALUATION OF THE HYPOTHESIS

Crosslinking of tarsal plate: our preliminary experiments

Photocrosslinking of the eyelid tarsus was developed in our laboratories as a potential treatment for eyelid laxity conditions, notably FES [60, 132, 133], and patents [134, 135] have been issued for the procedure. We demonstrated [60, 132] that the exposure of ex-vivo ovine tarsal plates to UV-A radiation (365-nm wavelength, photon energy ~3.4 eV), in the presence of riboflavin (more precisely, a water-soluble derivative of riboflavin) as a photosensitizer at irradiation fluences between 5 and 20 J/cm2, induced a significant increase of both strength and Young's modulus (stiffness) in the plate material, without adverse effects to the surrounding tissues [60, 132]. Figure 3 shows the irradiation of the tarsal plate, with the delivered radiation passing through the tarsal conjunctiva.

In our subsequent study [133], tarsal plates excised from human cadavers and subjected to the same treatment displayed remarkable enhancement in their mechanical properties. For instance, an average increase of ~200% in Young's moduli was measured for a series of human tarsus specimens (n = 13; $P < 0.0002$). Histologic analysis also revealed compaction of the collagen network, which may contribute to mechanical augmentation. Following irradiation at fluences below 20 J/cm^2, there was no histologically identifiable damage to MGs and their abutting territorial matrix or to the tarsal conjunctiva [133]. Similar findings have been confirmed experimentally in other laboratories, both in *ex-vivo* human (postmortem) [136, 137] and in animal tarsal plates [138]. Compaction (packing) and improved organization of the collagen fibers upon irradiation with UV-A were also reported in the eyelid skin excised from dermatochalasis patients [139]. UV-induced collagen compaction has been reported previously in both cornea [140–142] and sclera [143–145]. It was suggested [146] that the compaction effect is due to an upgraded packing arrangement of the collagen network, possibly associated with participation of ECM proteoglycans in the crosslinking process [147]. Notably, this kind of collagen compaction is fundamentally different from collagen fiber shrinking, which is a thermally induced phase transition process [148–152].

Evaluation of the method and outcomes

Conducting human clinical trials is planned at our institution after obtaining funding and the mandatory ethical approvals. The method of treating dry eye conditions in human patients according to the proposed therapeutic strategy comprises the following steps:

(a) Conducting a comprehensive ophthalmic examination of the individual patient, including a slit-lamp examination, eyelid eversion, and assessment of the tarsal plate and palpebral conjunctiva, to establish baseline condition and suitability for the procedure.
(b) Employing meibography on patients to estimate gland tortuosity prior to treatment via the computer analysis of meibographic images and applying Equation (1).
(c) Obtaining informed consent from the patient after explaining the procedure, potential risks, benefits, and alternative treatments.
(d) Administering a local anesthetic such as oxybuprocaine (0.4%) eyedrops to the ocular surface to achieve effective anesthesia and ensure patient comfort during the procedure.
(e) Placing the patient in supine position on a flat operating table with appropriate head support to maintain stability and proper alignment during the procedure.
(f) Cleaning the periocular treatment area, including the upper and lower eyelids, using a sterile solution of chlorhexidine 0.1% or iodine 0.5% to minimize the risk of infection and ensure a sterile field.
(g) Placing a large, sterile surgical corneoscleral shield over the ocular surface to protect the cornea, conjunctiva, and sclera from potential UV radiation exposure.
(h) Everting the treatment eyelid (upper or lower) to expose the palpebral conjunctiva and the underlying tarsal plate, facilitating effective application of treatment.
(i) Applying a sterile adhesive surgical drape with a central aperture measuring 10 x 20 mm over the everted eyelid. The drape should be opaque to prevent penetration of UV radiation into non-targeted adjacent tissues and ensure precise treatment localization.
(j) Using a sterile dropper, applying the sterile photosensitizer solution to the treatment area, ensuring continuous application for 5 min and keeping the area consistently moist to facilitate optimal conditions for the crosslinking process.
(k) Delivering a beam of UV-A radiation to the treatment area for a duration that depends on the irradiance level, ensuring even and consistent distribution of radiation across the target area to achieve uniform crosslinking.
(l) After irradiation, gently rinsing the treatment area with balanced saline solution to remove any residual photosensitizer.
(m) Repositioning the everted eyelid and removing the drape.
(n) Advising the patient to use lubricating eye drops 4 times daily for one week to alleviate discomfort and support the healing process.
(o) Arranging follow-up visits at established intervals after the procedure.
(p) Assessing the dry eye symptoms during these visits, monitoring for any complications and evaluating the efficacy of the crosslinking treatment.

Evaluation of outcomes after treating dry eye conditions in human patients according to the procedure described above comprises the following steps:

(a) Scheduling and implementing regular follow-up visits at 1 week, 1 month, 3 months, and 6 months to monitor patient progress and assess treatment efficacy.
(b) Assessing treatment efficacy through standard diagnostic tests, including ocular surface disease index, TBUT, Schirmer's test, tear osmolarity testing, corneal staining, meibography and estimation of gland tortuosity, and patient-reported outcomes.
(c) Evaluating clinical outcomes at baseline and at 1 month, 3 months, and 6 months after initiation of treatment.

Statistically analyzing outcome data to determine efficacy of the therapy in improving dry eye symptoms and reducing eyelid laxity.

DISCUSSION

Risks of the procedure

The potential limitations and risks associated with the proposed method are listed below. Representative citations reflect knowledge and expertise from corneal crosslinking, on which our considerations related to safety of the procedure are based.

(a) Insufficient penetration depth of UV-A radiation, leading to incomplete crosslinking of the tarsal collagen [153].
(b) The possible need for supplemental oxygen delivery at the radiation site in order to promote a more effective crosslinking process [154, 155].
(c) Accidental radiative damage to the cornea, conjunctiva, or sclera [156, 157].
(d) Variability in outcomes: the augmentation of mechanical properties can be non-uniform due to the complexity of palpebral tissue, concentration of photosensitizer, and fluctuations in irradiance [158, 159].
(e) The possible need for debridement of the tarsal conjunctival epithelium in order to enhance radiation penetration. However, this makes the procedure more invasive, may increase intraoperative and postoperative discomfort, heighten infection risk, and delay healing [160, 161].
(f) Long-term regression and partial loss of the stiffening effect [162, 163].
(g) In case of treatment failure [28, 29], we assume that one of the reasons could be an inordinately elevated tortuosity of MGs.

Comparison with existing therapies

A considerable range of methods have been developed and are currently recommended to treat DED [164–168]. Briefly, that may include self-care procedures (e.g., regular eyelid hygiene, warm compresses, eyelid massage), physical procedures (e.g., thermal pulsation devices, intense pulsed light therapy, low-level light therapy, blepharoexfoliation, MG probing, specialized contact lenses, punctal plugs, moisture goggles), and pharmaceutical treatment (e.g., artificial tears and ocular lubricants, anti-inflammatory agents, antibiotics, topical hormonal treatments, secretagogues, mucolytics, antioxidants, amniotic membrane therapy) [164–168]. Recently, administration of deoxyribonuclease eye drops has also been proposed and is undergoing clinical trials [169]. As MGD is the leading cause of evaporative DED, specific methods have likewise been developed for this condition [170, 171], although some of them are regarded as applicable for treating DED in general. We should therefore compare our designed method with those specific to MGD. There is one such procedure that has an aspect in common with ours—exposure to electromagnetic radiation, more precisely to pulses of visible light (wavelength 400–700 nm) and a small region in near-infrared (up to 1200 nm) [170], generated by a source of noncoherent polychromatic light. Clearly, this is well outside the UV region employed in our method. The procedure, known as "intense pulsed light" (IPL), has shown potential for improving DED symptoms [170], although evidence of its efficacy remains somewhat inconsistent. A range of mechanisms have been suggested, mainly inspired from applications in dermatology. It was shown that the structure of MGs can indeed be affected by IPL [172], but the mechanism was not elucidated. A possible mechanism for the effect of IPL may be explained by the enhancement of neocollagen biosynthesis, as evidenced histologically after applying IPL to dermal modeling [173] and later demonstrated in cultures of skin fibroblasts [174]. There are no studies regarding mechanism of IPL when applied to palpebral tissue, yet it is evident that it cannot be based on the crosslinking of collagen, as the energy of radiation employed in IPL is insufficient to promote such a chemical reaction in the absence of photosensitizers.

We may also consider comparing our method with intraductal probing [170, 171], which involves mechanical insertion of a probe directly into the blocked glandular orifices in order to unblock them. Albeit relatively safe, probing is an invasive procedure that can trigger hemorrhages or trauma to the small ducts and has to be repeated in most cases [170, 171].

The strengthening of tarsal collagen as reported here is evidently based on a concept that fundamentally differentiates it from any other approaches developed for MGD and DED. Our method offers several advantages: it is not invasive, has shown a favorable safety profile in preclinical studies, and may prevent further progression of MGD when applied at the earliest onset of symptoms.

Summary

Figure 4 presents a summary of the framework underpinning the concept of the proposed technique, which is based on the following tenets:

- Meibomian gland dysfunction is a major cause of evaporative dry eye disease.
- A distorted morphology of meibomian glands contributes to their dysfunction, leading to a defective delivery of the secreted meibum.
- Eyelid laxity weakens the supporting tarsal elastin-collagen network and may lead to ductal contortion and acinar deformity.

- By crosslinking the tarsal collagen, laxity can be reduced and additional strengthening and stiffening may be provided to the glands through a natural process of force transfer.
- The tarsal plate can function as a semisolid continuum capable of transmitting augmentative stress and forces to the glands, thereby helping maintain their initial morphology and inherent functionality.
- When applied at the earliest symptoms of MGD and/or DED, the method may play a preventive role.
- The method is shown to have a favorable safety profile when applied to ocular tissues in *ex-vivo* experiments.

The proposed procedure offers several notable strengths. It is non-invasive and has the potential reduce eyelid laxity while improving meibomian gland function by preventing ductal collapse and maintaining a normal flow of glandular secretion. In turn, an optimal glandular secretion may reduce dependence on artificial lubrication and slow the progression of evaporative DED. The procedure is also readily translatable to clinical practice, as it can be performed in an office setting employing existing corneal crosslinking platforms already in use for treating keratoconus and other corneal ectatic disorders. However, certain limitations must be acknowledged. Clinical outcomes may vary due to the structural complexity and heterogeneous composition of the tarsoconjunctival tissue. In some cases, removal of epithelium may be required to achieve adequate penetration of the photosensitizer solution, such as introducing a degree of invasiveness. Excessive gland tortuosity may considerably reduce the treatment's potency. The mechanical stiffening effect may diminish over time as part of the aging process. Future work will focus on validation *in vivo* of the procedure in animal and human clinical studies, long-term assessment of changes in eyelid and ocular surface parameters, and optimization of irradiation protocols to support a safe and productive clinical translation.

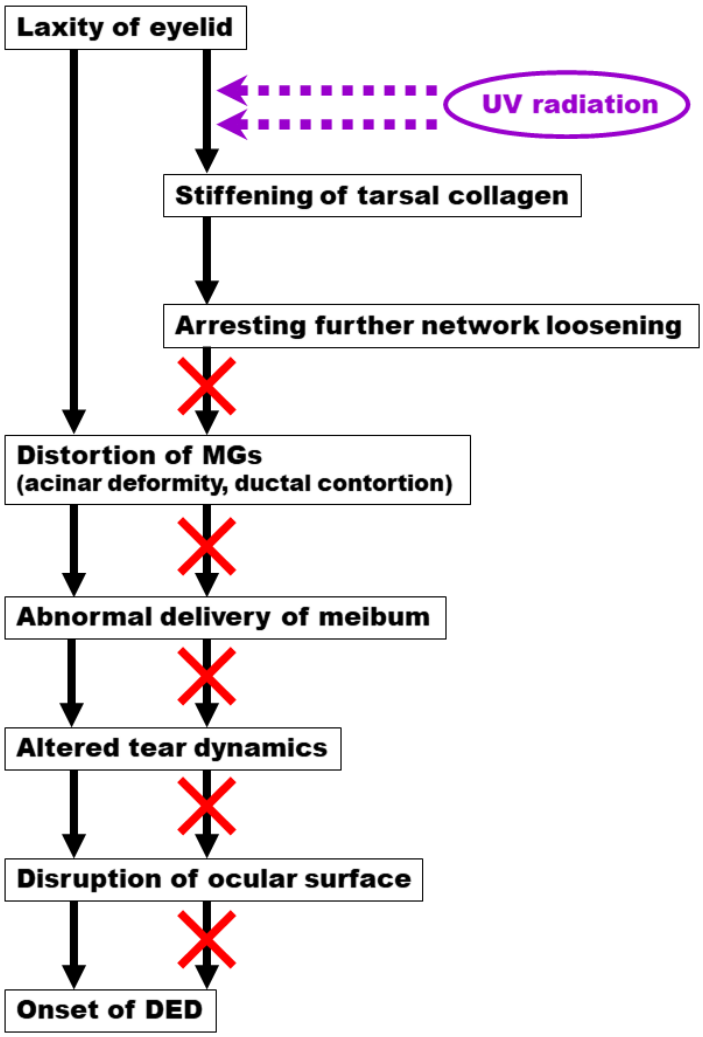

Figure 4. Effects of UV-A radiation on the putative contribution of eyelid laxity to the development of dry eye disease (DED).

CONCLUSIONS

The interplay between the tarsal elastin-collagen network and MG function appears to have an important role in the management of DED. There is a need for more advanced strategies aimed at preserving or restoring the strength and rigidity of the tarsal plate. Strengthening of the tarsal collagen network by its crosslinking may lead to a more compact embedding of MGs within the network and to the straightening of their main ducts and ductules, thereby supporting the restoration and enhancement of the glands' function and potentially offering therapeutic benefits. The authors are developing a noninvasive method based on photochemical crosslinking of tarsal collagen in order to strengthen and stiffen the eyelid and prevent progression of laxity. While requiring further clinical validation, the proposed strategy advances new therapeutic avenues to prevent or alleviate dry eye symptoms and contributes to improved ocular surface health in the ageing population.

ETHICAL DECLARATIONS

Ethical approval: No live subjects, animal or human, were involved in the preliminary experiments leading to the development of this hypothesis, as described in references [60, 132, 133]. The details of ethical approvals granted for the ex-vivo experiments are provided in the published articles [60, 132, 133]. No human clinical trials related to this study have been applied for, approved, registered, or commenced at the time of submitting this manuscript.

Conflict of interest: Australian Patent No. AU 2018201200 of 20 February 2018 and U.S. Patent No. 11,240,073 (B2) of 23 August 2022 have been granted for a method of treatment related to this research, and were assigned to Queensland Eye Institute Foundation by the authors Shuko Suzuki and Traian V. Chirila. The remaining author has no conflict of interest to declare.

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