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Photocrosslinking of Enzymatically Degraded Tarsal Plate in *ex Vivo* Ovine Eyelids: Biomechanical and Histological Analysis

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Abstract

The crosslinking of tarsal collagen induced by controlled exposure to UVA radiation (365 nm) was developed in our laboratories as a potential treatment for eyelid laxity. It is known that the pathophysiologic mechanism of palpebral laxity involves the enzymatic degradation and ensuing disorganization of the network consisting of elastin and collagen fibres. In this study, tarsal specimens excised from sheep eyelids were subjected *in vitro* to elastolysis or collagenolysis for variable durations. The degraded samples were then crosslinked by irradiation with UVA light at an irradiance of 75 mW/cm^2 for 3 min, corresponding to a radiant exposure of 13.5 J/cm^2, and their mechanical properties were measured before and after enzymolysis. The samples exposed to elastase for the shortest time (30 min) displayed enhanced stiffness when compared to non-degraded samples, both before and after further crosslinking, probably due to an initial major depletion of elastin associated with a transient predominance of collagen that was not yet degraded. However, at longer durations of digestion, the measured values for stiffness reached a plateau that remained constant. Following crosslinking, the degraded samples recovered

mechanically, although a net increase was not supported statistically. Collagenolysis of tarsal specimens led to values that plateaued at the very beginning of digestion. The stiffness was restored by subsequent crosslinking, without a statistically definite increase. The study showed that the UVA-induced crosslinking can reverse the effect of enzymolytic degradation of the tarsus.

Keywords

Eyelid Laxity, Tarsal Collagen, Ultraviolet A Radiation, Photochemical Crosslinking, Elastolysis, Collagenolysis, Mechanical Properties, Histological Analysis

1. Introduction

The eyelid is a constituent of the ocular adnexa that fulfills foremost tasks such as maintaining the tissular integrity, motion, and lubrication of the eye. The tarsal plate (or tarsus) is a compact connective tissue component of the eyelid that is responsible for its mechanical stability, contour, and stiffness, and for housing the meibomian glands (MGs). The latter are of crucial significance in retaining a normal ocular surface and visual function by controlling the quality of the tear film through secretion of the uppermost lipid layer of the tears, the meibum.

Aging or pathological circumstances can cause laxity of the eyelids (upper, lower, or both), also known as palpebral laxity, which is involved in a variety of conditions such as floppy eyelid syndrome (FES), ectropion, entropion, ptosis, blepharochalasis, facial dysmorphisms, and others. The tarsal plate plays a major etiologic role in palpebral laxity due to its structure and properties. Whitnall was the first to suggest that the tarsus is a cartilaginous tissue [1], despite lacking cartilage-specialized cells. Subsequent reports on the anatomy, structure, and composition of the palpebral tarsus have been rather scanty [2]-[13]. Its description as a fibrocartilage is questionable, considering that it does not contain collagen type II but contains collagen type VI (along with types I and III, elastic fibers, other matrix proteins, glycoproteins, and glycosaminoglycans) [2], while typical cartilages contain collagen type II [14], but not type VI. The presence in the tarsus of other components has been revealed histologically [1]-[4], such as blood vessels, nerve fibers, smooth muscle fibers, scattered fibroblasts, and peripheral adipocytes. Ultimately, the tarsus should be regarded as a specialized fibrous connective tissue.

The concurrent existence of two fibrous networks, based respectively on fibrillary collagen and both elastic and elastic-related fibres, is a crucial feature of the eyelid's tarsal plate. This dual network system is ultimately responsible for the mechanical properties of the eyelid. While collagen provides strength and stiffness, elastin generates extensibility, compliance, and stretchiness that include recoil capacity. Furthermore, due to its structure, the tarsal plate can function as a deformable semi-solid, hydrogel-like continuum able to transmit forces generated by the action of palpebral muscles that control blinking and the delivery of meibum,

or by externally applied mechanical reinforcement procedures. In principle, these forces can impinge on the meibomian glands in a contact-free mode and may contribute to maintaining their normal morphology and alignment, a hypothetical premise that could serve as the basis for a potential procedure we have suggested [15] for treating dry eye conditions.

The fact that fibrillary collagen and elastin present in our connective tissues are stabilized by natural covalent crosslinking has been recognized for decades [16]-[18]. These *endogenous* processes, which can be either enzyme-driven or non-enzymatic, are not under our control.

The *exogenous* crosslinking *in vitro* of collagen and elastin involves artificial structural modifications of non-living protein materials, isolated from animal connective tissues, and is aimed at producing biomaterials to be used as implants, scaffolds, or devices in tissue engineering and regenerative medicine. Such applications *in vitro* (or *ex vivo*) are based on crosslink-induced enhancement of both mechanical properties and the capacity to withstand enzymolysis. The exogenous crosslinking of collagen and elastin material substrates using chemical, photochemical, enzymatic, or thermal methods has become a noteworthy source for improved engineered biomaterials [19]-[30].

The *exogenous* crosslinking *in vivo* involves the direct exposure of selected connective tissue locations in the body to agents able to trigger and accomplish crosslinking reactions *in situ*; it is under our control and is aimed at inducing specific therapeutic effects. Although collagen macromolecules are commonly considered the main target of the chemical process, elastin can also undergo crosslinking under the same conditions. Among the crosslinking agents in use, irradiation with region A of ultraviolet (UV) radiation (wavelength from 315 to 400 nm), associated with suitable photosensitizers, has proven to be an effective procedure without harmful effects on tissues. For instance, by using UVA radiation and riboflavin (as a photosensitizer) to achieve the photochemical crosslinking of corneal stromal collagen, a successful treatment for keratoconus has been established and has become a routine procedure able to arrest the progression of this visually debilitating disorder [31]-[36]. In our laboratories, we have extended the application of the UVA/riboflavin system to the treatment of eyelid laxity through tarsal collagen crosslinking. The significant enhancement of strength and stiffness revealed in both cadaveric animal [37] [38] and human tarsal plates [39] can retard or partially reverse eyelid laxity. Subsequent investigations of the method in animal models have shown that the potential radiation damage to non-target tissues is negligible [40], and the thermal effects associated with irradiation did not result in temperatures able to induce protein denaturation [41].

As discussed later in this report, the pathophysiologic mechanism of palpebral laxity is based, at least in some measure, on an interplay between fibrillary collagen and elastin before and after their partial digestion by proteolytic enzymes such as elastases and collagenases. Here, we have investigated the effect of UVA radiation on animal (ovine) tarso-conjunctival excised specimens after being subjected *in*

vitro to elastolysis or collagenolysis and involving specimens prior to and after UVA-induced photochemical crosslinking.

2. Materials and Methods

2.1. Materials

Riboflavin 5'-phosphate monosodium salt (RF-5P) was supplied by Cayman Chemicals (Ann Arbor, MI, USA). A solution (0.1% w/v) in phosphate-buffered saline (PBS) was used as a photosensitizer for the UVA-induced collagen crosslinking. Elastase from porcine pancreas (Lot No. U1122327529-1), doubly crystallized, with an activity of 81 U/mg, was purchased from MP Biomedicals, LLC (Solon, OH, USA). Collagenase Type I isolated from *Clostridium histolyticum* (Product No. 17100017), with an activity of 315 U/mg, was supplied by ThermoFisher Scientific (Waltham, MA, USA). All other substances and agents (stains for histology, buffers, etc.) were supplied by MilliporeSigma (Burlington, MA, USA) through various distributors. Water of high purity (MilliQ or equivalent) was used in the experiments.

2.2. Tissue Preparation

Upper eyelids were excised on site from the cadavers of sheep that were sacrificed for commercial purposes at the abattoir unit (Brisbane Valley Meats Pty Ltd, Esk, Queensland, Australia) and immediately transported on ice to the laboratory. Nineteen cadaveric sheep (Merino breed, 1 - 2 years old) were used in this study, providing a total of 38 eyelids. The eyelids were individually dissected into tarsoconjunctival strips with a mean thickness of 1.5 ± 0.4 mm, according to a procedure reported in detail elsewhere [39], and subsequently distributed over 6 groups that included 6 specimens each for tensile testing. Two strips were used for histology, treated and untreated. All experiments were performed within 24 h of their excision.

2.3. Enzymatic Treatment

An elastase solution containing 10 U/mL was prepared by diluting the original enzyme solution with an aqueous solution made from TRIS-buffered saline (TBS) at pH 7.8 (100 mM) and containing calcium chloride (1 mM) and sodium azide (0.02% w/v). Aliquots (5 mL) of this solution were added to the wells of 6-well plates to cover the tarsal strips. The plates were then shaken at 160 rpm for 0.5, 1, and 4 h at 37˚C (*i.e.*, three sets of 6 specimens each). Following elastolysis, the samples were rinsed three times with cold PBS. Prior to mechanical testing, all specimens were kept in PBS at room temperature.

One set of tarsal strips was subjected to collagenolysis. A solution containing 10 U/mL collagenase was prepared in TBS (50 mM, pH 7.4), which contained calcium chloride (10 mM) and sodium azide (0.02% w/v). Into each well of the 6-well plate, 5 mL of this solution was added to cover the tissue specimens. The plate was shaken at 160 rpm for 0.5 h at 37˚C. The residual samples were rinsed in cold

PBS as described above.

For histological analysis, two tarso-conjunctival strips were sectioned into 6 segments, each 5 - 6 mm in length, and treated with enzyme solutions (1 mL for each) in wells of a 24-well plate as described above.

2.4. Mechanical Testing

The Instron® Materials Testing System Model #5943 (Instron, Norwood, MA, USA) was employed to obtain the stress-strain plots. The machine was equipped with a 50-N load cell, and the measurements were performed in uniaxial mode. The tarsal strips were loaded using pneumatic grips, set to a gauge distance of 15 mm, and submerged in PBS buffer (pre-heated to 37˚C ± 2˚C) in a BioPuls™ unit for the duration of each measurement. A tensile preload of 100 mN was applied at an elongation rate of 0.01 mm/s. Fifteen repeated cyclic tensile loading/unloading tests were performed at 10% displacement (strain) at a rate of 1%/s. The last cycle (#15) was used to calculate Young's modulus (M_Y) from the final region of the stress–strain plot. The specimens were then removed from the grips, exposed to irradiation to achieve crosslinking, and re-evaluated in the machine after soaking in PBS for at least 30 min.

2.5. Irradiation

The tarsal strips were soaked in RF-5P solution (0.1% w/v) for 30 min at room temperature. Each specimen was then irradiated with UVA radiation of 365-nm wavelength generated by the UV Curing System OmniCure™ 1500 (Excelitas Technologies Corp., Waltham, MA, USA). Irradiation was performed on the tarsal side (*i.e.*, not through the conjunctiva). The irradiance at the exposure site was monitored with the radiometer Dymax ACCU-CAL 50 (Dymax Corp., Torrington, CT, USA). The required irradiance was achieved by adjusting the distance between the radiation source and the target. The tarsal side of each specimen was exposed to an irradiance of 75 mW/cm^2 for 3 min, corresponding to a radiant exposure (fluence) of 13.5 J/cm^2.

2.6. Histology

The tarso-conjunctival specimens reserved for histological analysis, with or without enzymatic treatment, were fixed in 10% neutral buffered formaldehyde solution for 24 h, washed three times, and stored in PBS. The specimens were then placed in plastic tissue processing cassettes and processed for a duration of 9.5 h in the Leica ASP300S (Leica Biosystems, Nussloch, Germany). The processed samples were embedded in paraffin with the dissected region facing down. Sections (3 µm thick) were cut with a Leica RM2245 microtome (Leica Biosystems) and collected onto labelled SuperFrost™ Plus slides (Menzel-Gläser, Braunschweig, Germany). Slides were stained individually with the Verhoeff-Van Gieson (VVG) staining technique, and the procedure was carried out manually. Following deparaffinization, the slides were stained with Verhoeff agent. They were then differen-

tiated with 2% ferric chloride, washed, and exposed to a solution of sodium thiosulfate. The slides were finally counterstained with Van Gieson agent and dehydrated.

The slides were scanned with a 40× objective using a Pannoramic® Scan II Slide scanner (3DHISTECH Ltd, Budapest, Hungary). The data were visualized using the 3DHISTECH CaseViewer software (version 2.4).

2.7. Statistical Analysis

The results of mechanical testing were expressed as mean values ± s.e.m. of 6 specimens. For their statistical comparisons, the GraphPad® Prism software (version 6.0) was used to carry out the paired 2-tailed *t*-test for each set of specimens before and after irradiation, or the Mann-Whitney *U* nonparametric 2-tailed test for comparison between unpaired groups.

3. Results

Figure 1 summarizes the measured M_Y following 30-min, 1-h, and 4-h elastolysis, and for 30-min collagenolysis, all compared to samples that were not subjected to enzymolytic degradation, prior to and after UVA-induced crosslinking of the tarsus. The measured tensile strength values followed a very similar pattern and are not presented here. A further increase in the digestion duration with either elastase or collagenase did not significantly affect the measured values of stiffness or strength.

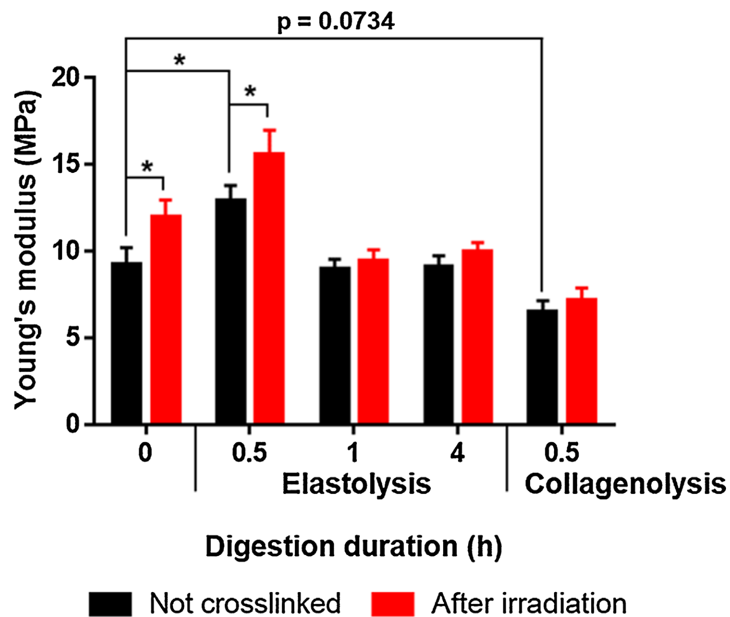

Figure 1. Comparative bar graphs of Young's modulus (stiffness) in ovine tarsus specimens subjected *in vitro* to elastolysis or collagenolysis, prior to and after exposure to UVA (365 nm) radiation in the presence of riboflavin as a photosensitizer. Irradiation parameters include an irradiance of 75 mW/cm^2 applied for 3 minutes, corresponding to a radiant exposure (fluence) of 13.5 J/cm^2. Data points (±s.e.m.) are the result of 6 measurements for each specimen. The asterisk denotes statistical significance ($p < 0.05$).

In Figure 2, representative histologic photomicrographs are shown for tarsal specimens exposed *in vitro* to elastolysis (panels (B) and (C)) and collagenolysis (panel (D)), and are compared to non-digested tissue (panel (A)).

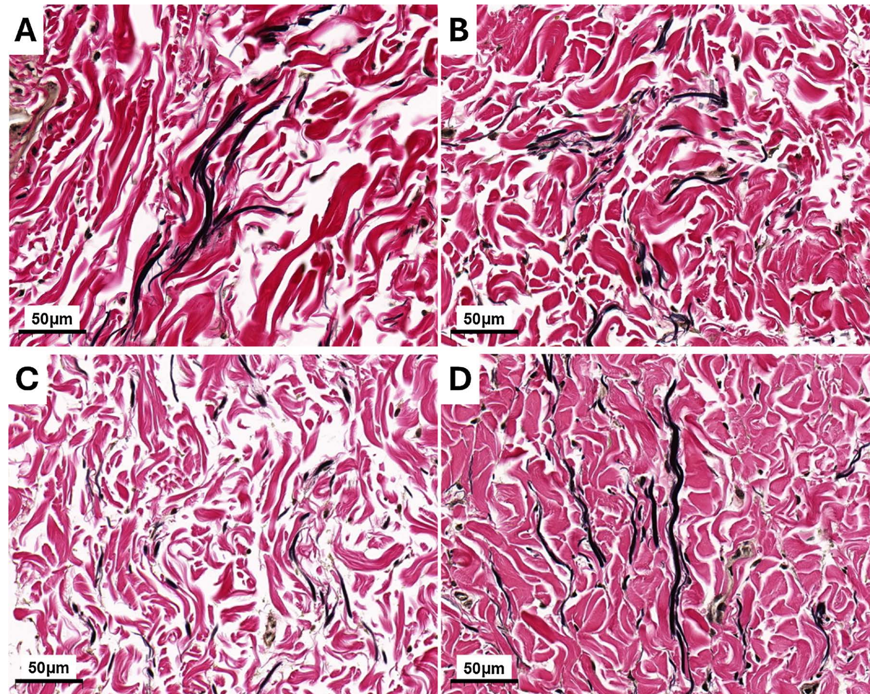

Figure 2. Histology of ovine tarsus specimens. Elastic fibers (black) and collagen (red) were stained using the VVG staining technique. (A) Not exposed to enzymolytic digestion; (B) elastolysis for 30 min; (C) elastolysis for 4 h; (D) collagenolysis for 30 min.

4. Discussion

The pathophysiologic mechanism of palpebral laxity has been investigated mainly in regard to FES, with certain conclusions extendible to other laxity-related eyelid conditions. While a general mechanism for eyelid laxity has yet to be set forth, it is accepted that a combination of background causal etiologic factors is involved, including the aging process, trauma, oxidative stress caused by intermittent ischemia/reperfusion, associations with systemic disorders, and genetic predisposition [42]-[44]. By studying the fate of elastin and collagen fibres in FES [3] [4] [7], valuable additional information regarding the innermost mechanism of laxity has been gained. Thus, upregulation of elastolytic enzymes was observed together with a significant reduction of elastin and elastic fibres. It was also found [7] that the mature elastic fibres almost vanished, while two types of elastic-related fibres (oxytalan and elaunin) became more abundant, suggesting the possible involvement of phenotypic plasticity. However, the enhanced elasticity of eyelids, as a salient clinical feature of laxity, is at variance with the quantitative reduction of elastic fibres. Such discordance can be reconciled by admitting the accumulation of collagen [7] as part of an active connective tissue repair process able to counterbalance the loss of tarsal elastic components. Similar processes have been observed

in arteries due to hypertension [45], or in the abdominal aorta due to aneurysmal dilatation [46]. The fact that collagen can also be digested by certain elastolytic enzymes [47]-[51] may lessen such compensatory processes.

To our knowledge, only one previous study has reported on the effect of a UVA/riboflavin irradiation system on enzymatically degraded tarsal plates [52], which assessed the variation in stiffness (M_Y). As expected, the specimens subjected to crosslinking were stiffer than the control samples (not crosslinked, not digested), which in turn were stiffer than samples subjected to *in-vitro* elastolysis. Following crosslinking, the degraded samples became stiffer than the controls, but without exceeding the stiffness of the non-degraded crosslinked specimens. These authors' findings were supported by valid statistical significance ($p < 0.05$).

Before comparing the mentioned results [52] with the results of the present work, we shall inspect the differences between the experimental conditions in the two studies, which include the following: 1) human tissue [52] versus animal tissue; 2) frozen tissue that had to be thawed [52] versus tissue samples never subjected to temperatures lower than ~0˚C; 3) only one duration for elastolysis (2 h) [52] versus our use of a series of increasing durations; 4) UVA irradiation parameters comprised an irradiance of 6 mW/cm^2 and a duration of 18 min (leading to a fluence of 6.48 J/cm^2) [52] versus our corresponding values of 75 mW/cm^2, 3 min, and 13.5 J/cm^2, respectively; 5) the photosensitizer was delivered as a drop every 30 s throughout the entire duration of irradiation [52], while in our experiments we soaked the whole samples in the photosensitizer solution for 30 min prior to irradiation; 6) different brands of mechanical testers.

In the present study, we found that UVA-induced crosslinking led to a stiffening of the tarsal plate, as indicated by the measured M_Y (**Figure 1**), confirming both our previous findings [37]-[41] and subsequent reports [52]-[55]. Somewhat surprisingly, the tarsal specimens subjected *in vitro* to 30-minute elastolysis were stiffer than the control samples ($p < 0.05$). After crosslinking, their stiffness was still higher than that of the crosslinked controls. We suggest that upon initial digestion, elastin is depleted and collagen becomes transiently prevalent, leading to additional stiffening. However, elastolysis performed at increasing durations (1 h, 4 h, or longer) resulted in no significant differences between the stiffness values measured before and after crosslinking (**Figure 1**). There was no statistically significant difference between the stiffness of control specimens and that of samples subjected to elastolysis at durations of 1 h and longer. It appeared that elastase reached a plateau in its action upon elastin and collagen. When the samples were subjected *in vitro* only to collagenolysis, the resulting reduction of stiffness prior to crosslinking was evident (**Figure 1**) but still not significant after 30 minutes ($p = 0.0734$); crosslinking indeed led to enhanced stiffness, however, it was not statistically significant. In general, following collagenolysis for longer durations, the drop in stiffness and its partial recovery due to irradiation were similar, but the associated increases were not statistically significant. We may therefore admit the existence of a plateau in the crosslinking

process that limits its further strengthening effects. Palpebral elasticity is maintained or even increased, likely because elastin was no longer being digested, while collagen fibres would have been further affected. The situation may become even more convoluted if other extracellular matrix proteins are involved. For instance, it was found [56] that during UVA/riboflavin-induced corneal crosslinking, proteoglycan molecules can be crosslinked between themselves or with collagen.

Figure 2 shows selected histologic photomicrographs of the tarsus, all stained with VVG stain, where elastic fibers and elastin appear in black, and collagen in red. Post-elastolysis fragmentation and depletion of elastic fibers (**Figure 2(B)**) are obvious when compared to non-degraded tarsus (**Figure 2(A)**). The pattern shown after longer digestion (e.g., after 4 h, **Figure 2(C)**) was maintained almost the same at longer digestion durations. We noticed that the elastic fibers mostly disappeared after elastolysis for 24 h. Histology of a tarsal specimen subjected to collagenolysis for 30 min is illustrated in **Figure 2(D)**. When compared to non-degraded tarsus (**Figure 2(A)**), the collagen network appears disrupted, while the elastic fibers remained unaffected. It must be mentioned that for all subsequent longer durations of collagenolysis, the same pattern was maintained with no statistically significant changes.

It remains conjectural to what extent the differences between experimental details could have led to the noted discrepancy between our results here and those previously reported [52]. Nevertheless, the obvious prevailing conclusion in both studies is that the exogenous crosslinking of the tarsus is able to reverse the decline in mechanical properties caused by enzymatic degradation. Due to the complexity of the pathophysiological mechanism of palpebral laxity, it is rather speculative to interpret these results as a corollary to the mechanism itself.

5. Conclusion

Degradation of the ovine tarsus *in vitro*, due to treatment with either elastase or collagenase, decreases its mechanical properties. Exposure to irradiation with UVA light reverses this outcome partially, and further mechanical enhancement is evident, although not statistically significant.

Ethics Approval and Consent to Participate

Not applicable. No experiments on live animals or trials on human subjects were involved in this study.

Author Contributions

T.V.C. initiated the project, conducted the literature search, and wrote the first draft. S.S. designed the study and coordinated the experimental work. All authors were involved in the experiments and interpretation of results, contributed to the final draft, reviewed it, and approved its submission. The authors assume full responsibility for the results reported in this article.

Availability of Data

All relevant data supporting this study are included in this published article. Additional information is available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Whitnall, S.E. (1932) The Anatomy of the Human Orbit and Accessory Organs of Vision. 2nd Edition, Oxford University Press, 155-159.

[2] Milz, S., Neufang, J., Higashiyama, I., Putz, R. and Benjamin, M. (2005) An Immunohistochemical Study of the Extracellular Matrix of the Tarsal Plate in the Upper Eyelid in Human Beings. *Journal of Anatomy*, **206**, 37-45. https://doi.org/10.1111/j.0021-8782.2005.00363.x

[3] Netland, P.A., Sugrue, S.P., Albert, D.M. and Shore, J.W. (1994) Histopathologic Features of the Floppy Eyelid Syndrome. *Ophthalmology*, **101**, 174-181. https://doi.org/10.1016/s0161-6420(94)31368-6

[4] Schlotzerschrehardt, U., Stojkovic, M., Hofmannrummelt, C., Cursiefen, C., Kruse, F. and Holbach, L. (2005) The Pathogenesis of Floppy Eyelid Syndromeinvolvement of Matrix Metalloproteinases in Elastic Fiber Degradation. *Ophthalmology*, **112**, 694-704. https://doi.org/10.1016/j.ophtha.2004.11.031

[5] Ezra, D.G., Beaconsfield, M. and Collin, R. (2009) Surgical Anatomy of the Upper Eyelid: Old Controversies, New Concepts. *Expert Review of Ophthalmology*, **4**, 47-57. https://doi.org/10.1586/17469899.4.1.47

[6] Ezra, D.G., Ellis, J.S., Beaconsfield, M., Collin, R. and Bailly, M. (2010) Changes in Fibroblast Mechanostat Set Point and Mechanosensitivity: An Adaptive Response to Mechanical Stress in Floppy Eyelid Syndrome. *Investigative Opthalmology & Visual Science*, **51**, 3853-3863. https://doi.org/10.1167/iovs.09-4724

[7] Ezra, D.G., Ellis, J.S., Gaughan, C., Beaconsfield, M., Collin, R., Bunce, C., *et al.* (2011) Changes in Tarsal Plate Fibrillar Collagens and Elastic Fibre Phenotype in Floppy Eyelid Syndrome. *Clinical & Experimental Ophthalmology*, **39**, 564-571. https://doi.org/10.1111/j.1442-9071.2011.02506.x

[8] Sun, M.T., Pham, D.T., O'Connor, A.J., Wood, J., Casson, R., Selva, D., *et al.* (2015) The Biomechanics of Eyelid Tarsus Tissue. *Journal of Biomechanics*, **48**, 3455-3459. https://doi.org/10.1016/j.jbiomech.2015.05.037

[9] Gao, Q., Xu, P., Hu, S. and Ye, J. (2022) The Micro-Structure and Biomechanics of Eyelid Tarsus. *Journal of Biomechanics*, **133**, Article ID: 110911. https://doi.org/10.1016/j.jbiomech.2021.110911

[10] Goold, L.A., Casson, R.J., Selva, D. and Kakizaki, H. (2009) Tarsal Height. *Ophthalmology*, **116**, 1831-1831.e2. https://doi.org/10.1016/j.ophtha.2009.05.035

[11] Kim, Y.S. and Hwang, K. (2016) Shape and Height of Tarsal Plates. *Journal of Craniofacial Surgery*, **27**, 496-497. https://doi.org/10.1097/scs.0000000000002369

[12] Coban, I., Sirinturk, S., Unat, F., Pinar, Y. and Govsa, F. (2018) Anatomical Description of the Upper Tarsal Plate for Reconstruction. *Surgical and Radiologic Anatomy*, **40**, 1105-1110. https://doi.org/10.1007/s00276-018-2064-7

[13] Kakizaki, H., AlSheikh, O., Edward, D.P., Takahashi, Y. and Morishige, N. (2025)

Tarsal Thickness Comparison between Upper and Lower Eyelids. *Journal of Cosmetic Medicine*, **9**, 7-10. https://doi.org/10.25056/jcm.2025.9.1.7

[14] Bielajew, B.J., Hu, J.C. and Athanasiou, K.A. (2020) Collagen: Quantification, Biomechanics and Role of Minor Subtypes in Cartilage. *Nature Reviews Materials*, **5**, 730-747. https://doi.org/10.1038/s41578-020-0213-1

[15] Manta, A.I., Suzuki, S. and Chirila, T.V. (2025) Crosslinking of Tarsal Collagen as a Hypothetic Therapy for Dry Eye Disease. *Medical hypothesis discovery and innovation in ophthalmology*, **14**, 233-250. https://doi.org/10.51329/ophthal1535

[16] Eyre, D.R., Paz, M.A. and Gallop, P.M. (1984) Cross-Linking in Collagen and Elastin. *Annual Review of Biochemistry*, **53**, 717-748. https://doi.org/10.1146/annurev.bi.53.070184.003441

[17] Tinker, D. and Rucker, R.B. (1985) Role of Selected Nutrients in Synthesis, Accumulation, and Chemical Modification of Connective Tissue Proteins. *Physiological Reviews*, **65**, 607-657. https://doi.org/10.1152/physrev.1985.65.3.607

[18] Reiser, K., McCormick, R.J. and Rucker, R.B. (1992) Enzymatic and Nonenzymatic Cross-Linking of Collagen and Elastin. *The FASEB Journal*, **6**, 2439-2449. https://doi.org/10.1096/fasebj.6.7.1348714

[19] Paul, R.G. and Bailey, A.J. (2003) Chemical Stabilisation of Collagen as a Biomimetic. *The Scientific World Journal*, **3**, 138-155. https://doi.org/10.1100/tsw.2003.13

[20] Parenteau-Bareil, R., Gauvin, R. and Berthod, F. (2010) Collagen-Based Biomaterials for Tissue Engineering Applications. *Materials*, **3**, 1863-1887. https://doi.org/10.3390/ma3031863

[21] Gu, L., Shan, T., Ma, Y., Tay, F.R. and Niu, L. (2019) Novel Biomedical Applications of Crosslinked Collagen. *Trends in Biotechnology*, **37**, 464-491. https://doi.org/10.1016/j.tibtech.2018.10.007

[22] Meyer, M. (2019) Processing of Collagen Based Biomaterials and the Resulting Materials Properties. *BioMedical Engineering OnLine*, **18**, Article No. 24. https://doi.org/10.1186/s12938-019-0647-0

[23] Nair, M., Best, S.M. and Cameron, R.E. (2020) Crosslinking Collagen Constructs: Achieving Cellular Selectivity through Modifications of Physical and Chemical Properties. *Applied Sciences*, **10**, Article 6911. https://doi.org/10.3390/app10196911

[24] Zheng, M., Wang, X., Chen, Y., Yue, O., Bai, Z., Cui, B., *et al.* (2022) A Review of Recent Progress on Collagen-based Biomaterials. *Advanced Healthcare Materials*, **12**, Article ID: 2202042. https://doi.org/10.1002/adhm.202202042

[25] Zhu, L., Yu, Z., Li, S., Xu, C., Hou, Y., Liao, L., *et al.* (2024) Recent Advances on Collagen Biomaterial: From Extraction, Cross-Linking to Tissue Regeneration. *Polymer Reviews*, **64**, 1031-1059. https://doi.org/10.1080/15583724.2024.2382128

[26] He, Y., Wang, X., Niu, Y., Wang, J., Yang, X. and Zhang, Y. (2025) Novel Crosslinked Recombinant Human Collagen Scaffolds with Excellent Cytocompatibility and Wound Healing Effect. *RSC Advances*, **15**, 35368-35378. https://doi.org/10.1039/d5ra04354a

[27] Leach, J., Wolinsky, J., Stone, P. and Wong, J. (2005) Crosslinked A-Elastin Biomaterials: Towards a Processable Elastin Mimetic Scaffold. *Acta Biomaterialia*, **1**, 155-164. https://doi.org/10.1016/j.actbio.2004.12.001

[28] Daamen, W.F., Veerkamp, J.H., van Hest, J.C.M. and van Kuppevelt, T.H. (2007) Elastin as a Biomaterial for Tissue Engineering. *Biomaterials*, **28**, 4378-4398. https://doi.org/10.1016/j.biomaterials.2007.06.025

[29] Yeo, G.C., Aghaei-Ghareh-Bolagh, B., Brackenreg, E.P., Hiob, M.A., Lee, P. and Weiss, A.S. (2015) Fabricated Elastin. *Advanced Healthcare Materials*, **4**, 2530-2556.

https://doi.org/10.1002/adhm.201400781

[30] Zhang, Y., Avery, R.K., Vallmajo-Martin, Q., Assmann, A., Vegh, A., Memic, A., *et al.* (2015) A Highly Elastic and Rapidly Crosslinkable Elastin-Like Polypeptide-Based Hydrogel for Biomedical Applications. *Advanced Functional Materials*, **25**, 4814-4826. https://doi.org/10.1002/adfm.201501489

[31] Sorkin, N. and Varssano, D. (2014) Corneal Collagen Crosslinking: A Systematic Review. *Ophthalmologica*, **232**, 10-27. https://doi.org/10.1159/000357979

[32] Sachdev, G.S. and Sachdev, M. (2017) Recent Advances in Corneal Collagen Crosslinking. *Indian Journal of Ophthalmology*, **65**, 787-796. https://doi.org/10.4103/ijo.ijo_648_17

[33] Santhiago, M.R. and Randleman, J.B. (2021) The Biology of Corneal Cross-Linking Derived from Ultraviolet Light and Riboflavin. *Experimental Eye Research*, **202**, Article ID: 108355. https://doi.org/10.1016/j.exer.2020.108355

[34] Wu, D., Lim, D.K., Lim, B.X.H., Wong, N., Hafezi, F., Manotosh, R., *et al.* (2021) Corneal Cross-Linking: The Evolution of Treatment for Corneal Diseases. *Frontiers in Pharmacology*, **12**, Article 686630. https://doi.org/10.3389/fphar.2021.686630

[35] Papachristoforou, N., Ueno, A., Ledwos, K., Bartuś, J., Nowińska, A. and Karska-Basta, I. (2025) A Review of Keratoconus Cross-Linking Treatment Methods. *Journal of Clinical Medicine*, **14**, Article 1702. https://doi.org/10.3390/jcm14051702

[36] Hafezi, F., Kling, S., Hafezi, N.L., Aydemir, M.E., Lu, N., Hillen, M., *et al.* (2025) Corneal Cross-linking. *Progress in Retinal and Eye Research*, **104**, Article ID: 101322. https://doi.org/10.1016/j.preteyeres.2024.101322

[37] Smith, T.M., Suzuki, S., Cronin, B.G., Haghighatpanah, M., Petcu, E.B., Philippa, C.J., *et al.* (2018) Photochemically Induced Crosslinking of Tarsal Collagen as a Treatment for Eyelid Laxity: Assessing Potentiality in Animal Tissue. *Ophthalmic Plastic & Reconstructive Surgery*, **34**, 477-482. https://doi.org/10.1097/iop.0000000000001063

[38] Smith, T.M., Suzuki, S., Sabat, N., Rayner, C.L., Harkin, D.G. and Chirila, T.V. (2019) Further Investigations on the Crosslinking of Tarsal Collagen as a Treatment for Eyelid Laxity: Optimizing the Procedure in Animal Tissue. *Ophthalmic Plastic & Reconstructive Surgery*, **35**, 600-603. https://doi.org/10.1097/iop.0000000000001413

[39] Manta, A.I., Pop, N.E., Tripon, R.G., Vultur, F., Suzuki, S., Cordos, B.A., *et al.* (2024) Photochemical Crosslinking of Tarsal Collagen as a Treatment for Eyelid Laxity: Evaluation in Ex Vivo Human Tissue. *Ophthalmic Plastic & Reconstructive Surgery*, **41**, 28-35. https://doi.org/10.1097/iop.0000000000002709

[40] Pop, N.E., Suzuki, S., Tripon, R.G., Manta, A.I., Lee, K.M., Parisot, M., *et al.* (2026) Photochemical Crosslinking of Eyelid Tissue by Trans-Conjunctival Exposure to UV-A Radiation: Implications for Biological Safety. *Journal of Biophotonics*, **19**, e202500458. https://doi.org/10.1002/jbio.202500458

[41] Suzuki, S., Pop, N.E., Manta, A.I. and Chirila, T.V. (2026) *Ex Vivo* Thermographic Analysis of the Eyelid Conjunctival Surface during Ultraviolet A-Mediated Photochemical Crosslinking of Tarsal Collagen: A Step in Translational Safety Evaluation. *Global Translational Medicine*, **5**, 87-94. https://doi.org/10.36922/gtm025410078

[42] Shah-Desai, S., Sandy, C. and Collin, R. (2004) Lax Eyelid Syndrome or 'Progeria' of Eyelid Tissues. *Orbit*, **23**, 3-12. https://doi.org/10.1076/orbi.23.1.3.28990

[43] Damasceno, R.W., Avgitidou, G., Belfort Jr., R., Dantas, P.E.C., Holbach, L.M. and Heindl, L.M. (2015) Eyelid Aging: Pathophysiology and Clinical Management. *Arquivos Brasileiros de Oftalmologia*, **78**, 328-331. https://doi.org/10.5935/0004-2749.20150087

[44] Scarabosio, A., Surico, P.L., Patanè, L., Tambasco, D., Kahale, F., Zeppieri, M., *et al.* (2024) The Overlooked Floppy Eyelid Syndrome: From Diagnosis to Medical and Surgical Management. *Diagnostics*, **14**, Article 1828. https://doi.org/10.3390/diagnostics14161828

[45] Wagenseil, J.E. and Mecham, R.P. (2012) Elastin in Large Artery Stiffness and Hypertension. *Journal of Cardiovascular Translational Research*, **5**, 264-273. https://doi.org/10.1007/s12265-012-9349-8

[46] Thompson, R.W., Geraghty, P.J. and Lee, J.K. (2002) Abdominal Aortic Aneurysms: Basic Mechanisms and Clinical Implications. *Current Problems in Surgery*, **39**, 110-230. https://doi.org/10.1067/msg.2002.121421

[47] Mainardi, C.L., Hasty, D.L., Seyer, J.M. and Kang, A.H. (1980) Specific Cleavage of Human Type III Collagen by Human Polymorphonuclear Leukocyte Elastase. *Journal of Biological Chemistry*, **255**, 12006-12010. https://doi.org/10.1016/s0021-9258(19)70234-8

[48] Heck, L.W., Morihara, K., McRae, W.B. and Miller, E.J. (1986) Specific Cleavage of Human Type III and IV Collagens by Pseudomonas Aeruginosa Elastase. *Infection and Immunity*, **51**, 115-118. https://doi.org/10.1128/iai.51.1.115-118.1986

[49] Watanabe, H., Hattori, S., Katsuda, S., Nakanishi, I. and Nagai, Y. (1990) Human Neutrophil Elastase: Degradation of Basement Membrane Components and Immunolocalization in the Tissue. *The Journal of Biochemistry*, **108**, 753-759. https://doi.org/10.1093/oxfordjournals.jbchem.a123277

[50] Nagano, T., Hao, J.L., Nakamura, M., Kumagai, N., Abe, M., Nakazawa, T. and Nishida, T. (2001) Stimulatory Effect of Pseudomonal Elastase on Collagen Degradation by Cultured Keratocytes. *Investigative Ophthalmology & Visual Science*, **4**2, 1247-1253.

[51] Zhu, Y.K., Liu, X.D., Sköld, C.M., Umino, T., Wang, H.J., Spurzem, J.R., *et al.* (2001) Synergistic Neutrophil Elastase-Cytokine Interaction Degrades Collagen in Three-Dimensional Culture. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **281**, L868-L878. https://doi.org/10.1152/ajplung.2001.281.4.l868

[52] Ugradar, S., Karlin, J., Le, A., Park, J. and Goldberg, R.A. (2020) Photochemical Collagen Cross-Linking Reverses Elastase-Induced Mechanical Degradation of Upper Eyelid Tarsus. *Ophthalmic Plastic & Reconstructive Surgery*, **36**, 562-565. https://doi.org/10.1097/iop.0000000000001635

[53] Ugradar, S., Le, A., Lesgart, M., Goldberg, R.A., Rootman, D. and Demer, J.L. (2019) Biomechanical and Morphologic Effects of Collagen Cross-Linking in Human Tarsus. *Translational Vision Science & Technology*, **8**, Article 25. https://doi.org/10.1167/tvst.8.6.25

[54] Akella, S.S., Liu, J., Miao, Y., Chuck, R.S., Barmettler, A. and Zhang, C. (2021) Collagen Structural Changes in Rat Tarsus after Crosslinking. *Translational Vision Science & Technology*, **10**, Article 3. https://doi.org/10.1167/tvst.10.5.3

[55] Kocer, A.M., Sen, E.M., Caydere, M., Yenigun, S. and Hucumenoglu, S. (2022) The Histopathological Findings in Excised Upper Eyelids of Patients with Dermatochalasis Following Collagen Cross-Linking Treatment. *Graefe's Archive for Clinical and Experimental Ophthalmology*, **260**, 2737-2743. https://doi.org/10.1007/s00417-022-05629-2

[56] Zhang, Y., Conrad, A.H. and Conrad, G.W. (2011) Effects of Ultraviolet-A and Riboflavin on the Interaction of Collagen and Proteoglycans during Corneal Cross-Linking. *Journal of Biological Chemistry*, **286**, 13011-13022. https://doi.org/10.1074/jbc.m110.169813

Symbols and Abbreviations

FES	floppy eyelid syndrome
MG	meibomian gland
M_Y	Young's modulus
PBS	phosphate-buffered saline
RF-5P	riboflavin 5'-phosphate monosodium salt
s.e.m.	standard error of the mean
TBS	TRIS-buffered saline
UV	ultraviolet radiation
UVA	region A of ultraviolet radiation (wavelength range from 315 to 400 nm)