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Review

Integrative Mechanisms and Emerging Therapies for Lacrimal Gland Aging

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ABSTRACT: To synthesize current evidence on structural, cellular, metabolic, and neuro-immune mechanisms of lacrimal gland (LG) aging in humans and animal models, and to map translational strategies for age-related aqueous-deficient dry eye (ADDE). Narrative synthesis of clinical tear-function studies; human histopathology/biobank data; and mechanistic experiments in mouse, rabbit, and non-human primates, with emphasis on single-cell and spatial omics, autonomic regulation, and immune remodeling. LG aging is characterized by acinar attrition, ductal–stromal fibrosis, mitochondrial fragmentation with lipofuscin accumulation; and diminished parasympathetic and sympathetic innervation. Convergent mechanisms—oxidative stress, mitochondrial insufficiency, cellular senescence, immunosenescence with low-grade inflammation, and disrupted cholinergic/catecholaminergic signaling—jointly depress tear volume and alter composition. Cross-species comparisons reveal conserved pathways alongside species-specific susceptibilities that inform model selection. Therapeutic avenues under investigation include anti-inflammatory and metabolic modulators, senolytics, hormone/secretagogue approaches, and regenerative platforms leveraging stem cells, lacrimal organoids, and bioengineered scaffolds. Emerging organoid and ex vivo secretion assays hold potential for mechanism-based screening and phenotyping that bridge preclinical and clinical endpoints. LG aging is a tractable, multifactorial driver of ADDE. Priorities for translation include standardizing functional readouts, aligning cross-species endpoints, and testing mechanism-selected cohorts in early-phase trials with objective measures of tear secretion and ocular-surface integrity. Integrating multi-omics with advanced models and regeneration-focused strategies offers a path from symptomatic relief to durable restoration of gland function.

Keywords: lacrimal gland, aging, aqueous-deficient dry eye, cellular senescence, neuro-immune regulation, autonomic nervous system

1. Introduction

Age-related decline in lacrimal gland (LG) function is a central driver of aqueous-deficient dry eye disease (ADDE), a disorder characterized by insufficient tear production and ocular surface instability [1-3]. Converging clinical and epidemiological evidence indicates that basal tear secretion and tear film integrity progressively diminish with advancing age, with older individuals exhibiting significantly reduced tear flow, lower tear meniscus height, and a thinner tear film lipid layer compared to younger cohorts [3-5]. Moreover, the global increase in longevity has further amplified the prevalence and impact of age-related ocular surface disorders such as dry eye disease [6, 7].

Despite the clinical significance of age-related LG dysfunction, the underlying biological mechanisms remain only partially elucidated. Emerging evidence suggests that LG aging involves a multifactorial interplay of tissue remodeling, stem/progenitor cell exhaustion, chronic low-grade inflammation, and neuroendocrine dysregulation [8]. Advances in human histopathological

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analysis and in vivo animal models—particularly in rodents and rabbits—have yielded key mechanistic insights into the trajectory of LG aging and its contribution to ocular surface pathophysiology [8, 9]. In addition, single-cell RNA sequencing (scRNA-seq) has revealed an unexpected degree of LG cellular heterogeneity, identifying previously unrecognized cell subpopulations (e.g., Car6^+ hybrid acinar–ductal cells) and delineating age-associated changes in the immune microenvironment [10-12]. Furthermore, high-dimensional flow cytometry has delineated immune cell subsets in the aging LG—such as tissue-resident memory T cells and innate lymphoid cells (ILCs)—that accumulate with age and potentially drive chronic inflammation and functional decline [13, 14].

This review consolidates current findings on age-related morphological and functional changes in the LG, with emphasis on epithelial atrophy, ductal and immune abnormalities, stem cell niche dynamics, and declines in autonomic innervation. It also examines species-specific patterns of LG aging, evaluates the translational value and limitations of various animal models, and highlights emerging therapeutic approaches ranging from anti-inflammatory and hormonal treatments to regenerative and gene-based strategies. Notably, human lacrimal gland organoids—derived either from adult tissue or from pluripotent stem cells—have demonstrated native-like secretory function and neural responsiveness in vitro, and have achieved functional rescue of tear production in animal transplantation models, underscoring their translational potential [15, 16]. In parallel, mesenchymal stem cell (MSC)-based interventions have advanced into early-phase clinical trials, with initial results indicating improvements in tear production and reductions in inflammatory markers [17]. Furthermore, integrative multi-omics analyses implicate metabolic dysfunction and oxidative stress in LG aging, revealing new potential therapeutic targets [8, 18].

By integrating anatomical, cellular, and molecular perspectives—augmented by recent single-cell and flow cytometry insights—this review aims to establish a comprehensive framework for understanding the biology of LG aging and to inform the development of targeted strategies to manage age-related tear dysfunction. Notably, age-related hormonal changes—such as the postmenopausal decline in androgens—predispose women to an accelerated decline in tear production, which partly explains the higher prevalence of dry eye disease among elderly women [19-21]. Collectively, these integrated insights provide a foundation for translating mechanistic understanding into therapies designed to prevent or reverse the functional deterioration associated with LG aging.

2. Structure of the Lacrimal Gland and Mechanism of Tear Secretion

2.1 Anatomy and Structure

The lacrimal gland is a paired tubuloacinar exocrine organ that produces the aqueous phase of the tear film [9, 22]. In humans, it occupies the superotemporal orbit within the lacrimal fossa of the frontal bone and comprises a larger orbital lobe and a smaller palpebral lobe, separated by the aponeurosis of the levator palpebrae superioris (Fig. 1). Histologically, the gland consists of multiple lobules containing secretory acini lined by acinar epithelial cells and invested by basal myoepithelial cells [9, 23]. The acini drain into a hierarchical system of ductules and larger excretory ducts that coalesce and empty into the superior conjunctival fornix [24].

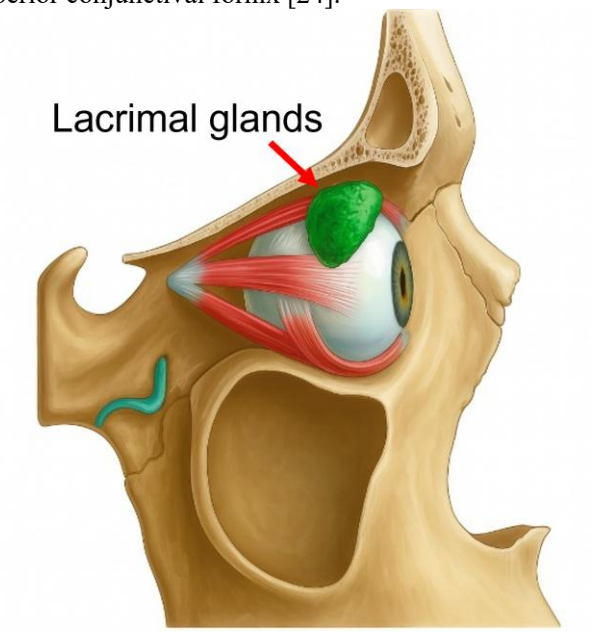

Figure 1. Anatomical location of lacrimal gland.

Tear fluid is a complex secretion composed of water, electrolytes, and abundant proteins—including lysozyme, lactoferrin, and secretory IgA—that nourish and protect the cornea and conjunctiva [25-27]. Myoepithelial cells form a contractile basket around acini and facilitate expulsion of secretory contents in response to neural stimulation [25-27]. The gland is densely vascularized and richly innervated, supporting its metabolic demand and precise regulation of secretion [9, 23, 26].

Building upon the classical lobular-ductal architecture [22, 23, 26, 27], recent single-cell and histologic studies refine compartment-specific function. In murine and human lacrimal glands, scRNA-seq delineates major cell classes, including acinar and ductal epithelia, myoepithelial cells, stromal fibroblasts,

macrophages, B/plasma cells, innate lymphoid cells (ILCs), and tissue-resident T cells [12]. Acinar epithelial cells, enriched for AQP5 and MIST1, generate the protein-rich primary fluid and contribute antimicrobial factors such as lipocalin-1, PIP, and the polymeric immunoglobulin receptor that mediates dimeric IgA transcytosis. Luminal ductal cells, characterized by high lactoferrin (LTF) and NKCC1 expression, fine-tune electrolyte composition and reinforce antimicrobial defense. A discrete Car6$^+$ hybrid acinar–ductal population expressing carbonic anhydrase VI (CA VI) localizes to the acinus–duct junction and is implicated in luminal pH buffering and ductal homeostasis [28, 29].

Surrounding acini, ACTA2$^+$ myoepithelial cells not only drive contractile expulsion but also likely release paracrine factors that influence epithelial turnover and repair. Within the stroma, plasma cells supplying dimeric IgA and macrophages engaged in immune surveillance are readily identified at single-cell resolution [30]. Table 1 summarizes principal lacrimal gland cell types and their key secretions, illustrating how coordinated cellular specialization governs tear volume regulation, antimicrobial defense, and ocular-surface homeostasis.

Table 1. Major Lacrimal Gland Cell Types and Their Principal Secretions.

Cell type	Representative markers	Main secreted products*	Key functional roles
Acinar epithelial cells	AQP5, MIST1/BHLHA15	Lactoferrin, lysozyme, DNase-1, lactoperoxidase, prolactin-induced protein (PIP), mucin 7 (MUC7) /PIP, polymeric-Ig receptor (PIGR)	Generate the bulk of protein-rich primary tear fluid; transcytose dimeric IgA via PIGR; supply multiple antimicrobial & homeostatic proteins [10, 11, 12]
Car6$^+$ hybrid acinar–ductal cells	Carbonic anhydrase VI (Car6)	Soluble carbonic-anhydrase VI	Provide local pH buffering at the acinus–duct junction and in downstream ducts [10, 11,12]
Luminal duct cells	Lactoferrin (LTF), NKCC1/SLC12A2	Supplemental lactoferrin; electrolytes & water	Fine-tune ionic/osmotic composition of tears; reinforce antimicrobial defense with extra lactoferrin [10, 11,12]
Myoepithelial cells	ACTA2 (α-SMA), CK5	Epidermal growth factor (EGF), platelet-derived growth factor-A (PDGFA) and related trophic factors	Contract to expel acinar contents; secrete growth factors that support epithelial maintenance, repair & regeneration [10, 11,12]
Fibroblasts (stromal)	PDGFRA, COL1A1/2, vimentin (VIM)	Collagens I/III, fibronectin, matrix metalloproteinases (MMPs) ± TIMPs, fibroblast growth factor 10 (FGF10), hepatocyte growth factor (HGF), cytokines (e.g., IL-6)	Build & remodel the extracellular matrix; provide structural support; release paracrine factors that influence epithelial growth and immune modulation [10, 11,12]

* Only molecules that have been demonstrated or are strongly inferred to either reach the tear film or act locally on neighboring lacrimal gland/ocular surface cells are listed.

2.2 Secretory Mechanism and Neural Regulation

Lacrimal gland secretion is under stringent regulatory control and requires coordinated interactions between epithelial and neural components. Acinar cells account for the majority of tear fluid and protein secretion, accomplished via two primary mechanisms: electrolyte and water transport mediated by ion channels and transporters, and protein release through exocytosis of secretory vesicles [27]. Tight junctions between acinar cells ensure polarized transport of fluid into the acinar lumen, from which fluid flows into the ductal system [12, 27]. Ductal epithelial cells, constituting approximately 10% of glandular cells, further modify this primary fluid by reabsorbing or secreting ions and water and adding select proteins, thereby fine-tuning the final tear composition [11, 27].

Lacrimal secretion is predominantly controlled by the autonomic nervous system. Sensory nerves from the ocular surface (trigeminal afferents) detect external stimuli and relay signals to brainstem nuclei involved in lacrimation, including the superior salivatory nucleus. Parasympathetic efferent fibers originating in the superior salivatory nucleus travel via the facial nerve, greater petrosal nerve, and pterygopalatine ganglion to provide the primary secretomotor drive to the gland [23, 31]. Parasympathetic stimulation—mediated chiefly by acetylcholine acting on M_3 muscarinic receptors and by vasoactive intestinal peptide (VIP)—activates intracellular second-messenger cascades in acinar cells, leading to protein exocytosis and ion flux that collectively generate fluid secretion [27].

Sympathetic innervation from the superior cervical ganglion reaches the gland via perivascular pathways and also modulates secretion. While norepinephrine acting on

α1-adrenergic receptors contributes to basal tear regulation, recent evidence suggests its role is context-dependent: under physiological conditions, it may support baseline secretion, but during sustained stress, α1-adrenergic signaling activates mitochondrial uncoupling pathways (e.g., Ucp2) that suppress excessive fluid secretion [23, 31-33]. Parasympathetic input remains the dominant driver of reflex tearing [23, 31]. This dual autonomic innervation enables rapid and adaptive adjustments in tear output in response to environmental or emotional stimuli, thereby maintaining tear film stability under diverse conditions [27, 34].

Recent experimental evidence has delineated how aging alters autonomic regulation of the lacrimal gland. In BALB/c mice, advanced age is associated with a significant reduction in both parasympathetic nerve density and acetylcholine (ACh) release capacity, resulting in diminished secretory responses to cholinergic stimulation. Sympathetic nerve fiber density likewise declines with age, accompanied by a parallel drop in norepinephrine (NE) release that impairs α1-adrenergic signaling efficacy [35].

Additionally, chronic age-related inflammation, characterized by elevated levels of proinflammatory cytokines (e.g., IL-1β, TNF-α), may further impair neurotransmitter release, exacerbating the imbalance between sympathetic and parasympathetic inputs and accelerating lacrimal gland dysfunction [36]. Taken together, these rodent data indicate that aging perturbs neurotransmitter signaling and autonomic homeostasis, ultimately leading to functional impairment of tear secretion.

Finally, an intact corneal sensory innervation is crucial for normal lacrimal gland function. Mouse models of corneal nerve injury demonstrated disrupted lacrimal secretory rhythms and reduced tear volumes, confirming the essential role of afferent signals from the ocular surface to the central lacrimal pathway [37].

Overall, the evidence underscores that intact neural circuits—encompassing ocular surface sensory nerves, brainstem nuclei, and autonomic efferents—are indispensable for maintaining normal lacrimal gland function and tear film stability. Disruptions at any point along this reflex arc, whether precipitated by aging, nerve injury, or chronic inflammation, significantly compromise tear secretion and contribute to the pathogenesis of aqueous-deficient dry eye disease.

2.3 Immune Microenvironment

Aging drives substantial remodeling of the lacrimal gland immune microenvironment in both mice and humans [4, 38, 39]. Aged glands develop robust leukocytic infiltrates with marked shifts in cellular composition, including expansion of lymphocyte and innate compartments. In murine models, B cells frequently organize with T cells into ectopic germinal centers (tertiary lymphoid structures), although human data primarily show diffuse lymphocytic aggregates without definitive germinal center formation [14, 39].

Concomitantly, CD4+ T cells—particularly T follicular helper cells that potentiate B-cell responses—increase in abundance, alongside accumulations of long-lived plasma cells and innate immune subsets (e.g., mast cells and group 1–2 innate lymphoid cells). Antigen-presenting cells, including macrophages and dendritic cells, are likewise enriched [18, 39, 40]. Transcriptomic analyses corroborate a chronically activated, proinflammatory milieu in aged glands, with upregulation of gene programs associated with B/T-cell activation, chemokine signaling, and local immunoglobulin production [14, 18, 41]. In parallel, immune regulation is compromised: regulatory T cells persist but exhibit reduced suppressive capacity with age, as evidenced by impaired ability to constrain pathogenic T-cell activity in the lacrimal gland [8, 13, 42].

Aging also reprograms epithelial–immune crosstalk. In vitro studies suggest IFN-γ can induce MHC class II expression, potentially enabling non-classical antigen presentation and contributing to local T-cell activation in inflammatory contexts [43]. Conversely, infiltrating innate lymphocytes provide epithelial trophic cues—for example, type 2 innate lymphoid cells secrete amphiregulin, an EGF-family growth factor—highlighting bidirectional disruption of epithelial–immune homeostasis [30].

Collectively, these compositional and functional changes establish a chronically inflamed tissue environment with diminished glandular secretory performance, providing a mechanistic link between advanced age, persistent ocular surface inflammation, and aqueous-deficient dry eye disease [8, 44].

3. Impact of Aging on Lacrimal Gland Structure and Function

3.1 Histological and Ultrastructural Changes in the Lacrimal Gland Induced by Aging

With advancing age, the LG exhibits pronounced histological and ultrastructural degeneration that underpins functional decline [45]. In humans and animal models, the dominant histological features are progressive acinar atrophy and increasing fibrosis, often beginning in midlife [46]. These changes manifest as reduced acinar number, loss of parenchymal volume, and expansion of fibrous stroma, with augmented collagen deposition around acini and within interlobular septa [39]. Grossly,

the gland appears paler and denser, with thickened collagen bundles and reduced weight [47, 48]. Ductal architecture is likewise perturbed: age-related dilation, tortuosity, and cystic transformation—particularly in interlobular and hilar regions—correlate with periductal fibrosis and chronic low-grade inflammation [39]. As fibrosis advances, secretory outflow is impeded, further promoting disuse atrophy of residual acinar units [45, 49]. Fatty infiltration is a frequent age-related change, observed in a substantial proportion of elderly individuals, particularly in women [39, 45]. By contrast, young LGs contain densely packed acini, patent ducts, and minimal adipose tissue.

At the ultrastructural level, aging disrupts organelle integrity. Electron microscopy reveals swollen mitochondria with disorganized or collapsed cristae in acinar cells, indicative of impaired bioenergetics. Lipofuscin ("age pigment") accumulates within the cytoplasm, reflecting aggregates of oxidatively modified proteins and lipids; although rare in young glands, it appears as yellow-brown granules on histology and is a canonical marker of cellular aging [35, 47, 50]. The endoplasmic reticulum becomes distended and irregular, secretory granules are disarrayed, and acinar nuclei may display pyknosis or fragmentation, consistent with increased apoptosis [51]. Collectively, mitochondrial dysfunction, lipofuscin accumulation, and defective autophagy are associated with reduced lacrimal secretory capacity and contribute to dry eye pathogenesis [8].

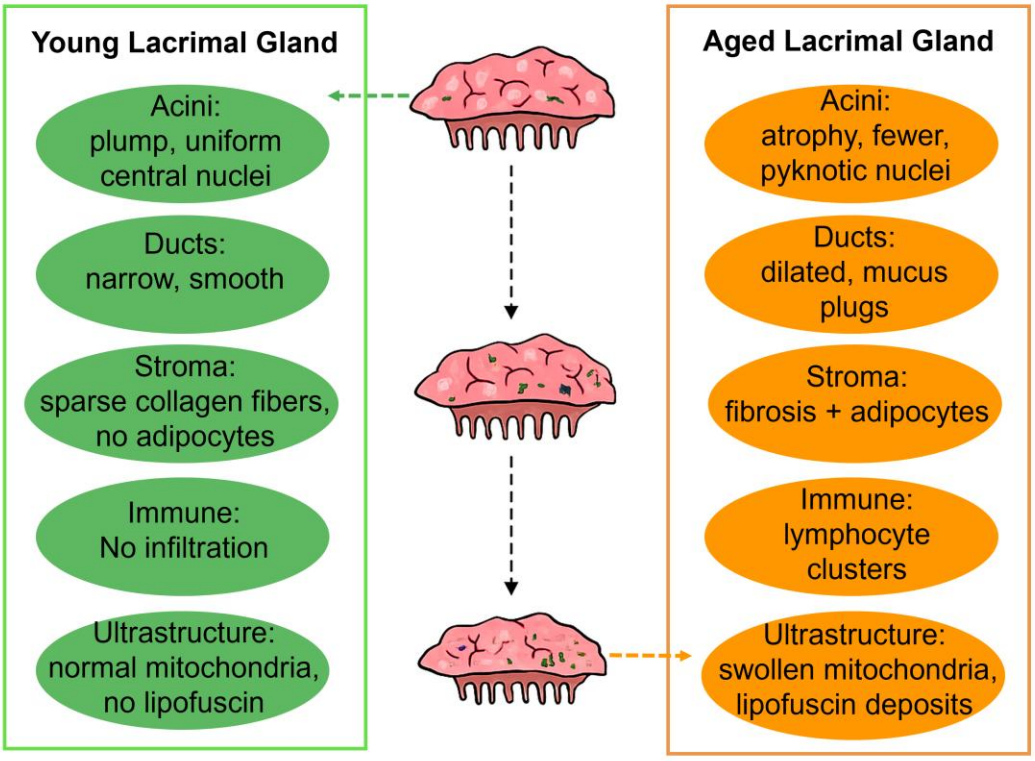

Figure 2. Comparative schematic overview of structural features in young versus aged human lacrimal glands. This figure illustrates the histological and ultrastructural differences between young and aged lacrimal glands. The left panel (green) depicts the youthful gland, characterized by plump and uniform acini with centrally located nuclei, narrow and smooth ducts, sparse extracellular matrix without fat infiltration, absence of immune cell infiltration, and intact ultrastructure including healthy mitochondria without lipofuscin accumulation. In contrast, the right panel (orange) shows the aged gland, marked by acinar atrophy, ductal dilation with mucus plugs, fibrosis and adipocyte infiltration in the stroma, prominent lymphocytic clusters, and cellular degeneration typified by swollen mitochondria and lipofuscin deposits. These features reflect the multi-level degenerative changes underlying age-related decline in tear secretion function.

Aged acinar cells also exhibit classical markers of cellular senescence. Proliferative activity declines (fewer Ki-67–positive cells), while expression of cell-cycle inhibitors p16^INK4a and p21^Cip1 increases [8, 52, 53]. Immunohistochemistry demonstrates elevated p16 in periacinar regions, signaling irreversible growth arrest [54]. Senescent acinar cells in experimental models acquire a senescence-associated secretory phenotype (SASP), characterized by constitutive release of proinflammatory cytokines (e.g., IL-6, IL-1β, CXCL10)

and matrix-degrading enzymes (e.g., MMP-1, MMP-3, MMP-9) [55, 56]. These mediators act in a paracrine manner to amplify inflammation and extracellular-matrix remodeling, thereby accelerating functional decline of the LG [39, 55, 57].

In summary, aging imposes multilayered injury on the LG—from macroscopic atrophy, fibrosis, and fatty infiltration to mitochondrial damage, lipofuscin accumulation, and cellular senescence **(**Fig. 2). This composite structural and molecular pathology provides the substrate for diminished tear secretion and the emergence of age-related dry eye disease.

3.2 Autophagy, Mitophagy, and Mitochondrial-Reactive Oxygen Species Imbalance

Aging elevates mitochondrial reactive oxygen species (ROS), induces cellular senescence, and impairs autophagic capacity, creating a self-reinforcing loop that accelerates lacrimal gland decline. Zhao et al. (2024) reported that rapamycin treatment in middle-aged mice activated PINK1/Parkin-regulated mitophagy, coinciding with reduced ROS, preserved mitochondrial ultrastructure, decreased acinar apoptosis, and improved tear secretion; conversely, pharmacologic inhibition of mitophagy with Mdivi-1 exacerbated mitochondrial injury, oxidative stress, fibrosis, and functional deterioration [8].

Broader reviews indicate that defective autophagic flux—evidenced by reduced LC3B, Beclin-1, or ATG5 with concomitant mTOR hyperactivation—permits accumulation of dysfunctional organelles and inflammatory mediators, potentially sustaining a ROS-driven inflammatory cycle in dry eye conditions [58, 59].

In experimental dry eye models, evodiamine restores autophagic activity via p53/mTOR modulation, enhances tear secretion, and attenuates oxidative and inflammatory injury [60]. Similarly, in Sjögren's syndrome models, MSC treatment likewise modulates autophagy markers and suppresses inflammatory gene expression in lacrimal tissue, paralleling functional improvement [61], though their efficacy in age-specific contexts requires further validation.

3.3 Decline in Tear Secretion Function with Aging

3.3.1 Age-Related Changes in Tear Secretion Function Indicators

Population-based studies demonstrate that lacrimal secretory capacity progressively declines with advancing age. Clinically, this manifests as significant reductions in both tear volume and stability. Clinical assessments reveal a significant age-related decline in Schirmer I test values. Measurements obtained under standard unanesthetized protocols show a reduction from the 15–20 mm range typically observed in younger adults (<40 years) to approximately 10 mm in elderly populations (>65 years) [36, 62, 63]. This reduction in wetting length underscores profound functional impairment. Clinical assessments confirm a pronounced age-related shortening of tear film breakup time (TBUT), a critical measure of ocular surface integrity. Tear film stability significantly decreases with age: healthy young adults (<40 years) exhibit TBUT values substantially exceeding 10 seconds (e.g., mean TBUT in the 20-29 age group: 35.2 ± 7.95 seconds for males, 36.3 ± 7.39 seconds for females), whereas healthy elderly individuals (>65 years) maintain TBUT values above the 6–8 second diagnostic cutoff (e.g., >75 age group: 13.1 ± 3.18 seconds for males, 15.9 ± 4.35 seconds for females). Only elderly cohorts with clinically confirmed dry eye symptoms commonly exhibit TBUT values approaching or below the diagnostic cutoff (e.g., median non-invasive tear breakup time (NIBUT) = 8–9 seconds in symptomatic groups) [63, 64]. Age-related declines in antimicrobial proteins (e.g., lysozyme, lactoferrin) are documented clinically, though quantitative thresholds remain unstandardized. Critically, tear osmolarity significantly increases with age ($r = 0.59$, $P < 0.05$), reflecting diminished aqueous volume and evaporative concentration [36, 65]. This clinically significant reduction reflects progressive tear film destabilization with aging. These critical metrics, summarized in Table 2, provide diagnostic anchors for age-related dry eye.

Collectively, these quantified deficits—reduced secretion, accelerated breakup, and altered composition—establish aging as a primary driver of aqueous-deficient dry eye. The consistent numerical trends across studies necessitate age-adapted diagnostic thresholds.

3.3.2 Mechanisms of Basal and Reflex Tear Secretion and Their Age-Related Alterations

Tear secretion operates through two partially distinct modes: basal and reflex pathways. Basal secretion denotes the continuous, low-level output that sustains corneal hydration under resting conditions and is governed by constitutive activity in the main and accessory lacrimal glands, low-grade autonomic tone, hormonal influences, and mucosal immune inputs. Reflex secretion, by contrast, is a rapid, high-amplitude response of the main lacrimal gland to external stimuli—e.g., corneal or conjunctival nerve activation—mediated by a trigeminal sensory afferent–parasympathetic efferent reflex arc, with acetylcholine as the principal neurotransmitter.

Table 2. Comparative Analysis of Tear Film Parameters in Young versus Aged Subjects.

Parameter	Young Adults	Aged Subjects	Physiological Significance	Representative Sources
Schirmer I test (5 min)	15 – 20 mm wetting	≈ 10 mm wetting	Decline in basal + reflex tear output reflects lacrimal hypofunction	[63, 213]
Tear break-up time (TBUT)	means 25-45 s	means 13-19 s	Faster evaporative thinning, unstable lipid layer	[63, 64]
Tear Proteins				
Total protein concentration	~6 mg/mL	Markedly decreased	Global loss of protective proteins	[72, 214]
Lysozyme	~ 1.0 mg/mL	~ 0.7 mg/mL	Antibacterial muramidase, declines with age	[72, 215]
Lactoferrin	~ 1.3 mg/mL	~ 0.9 mg/mL	Iron-chelation & antimicrobial defense; drop signals lacrimal dysfunction	[216, 217]
Inflammatory mediators				
IL-6	~ 13 pg/mL	markedly elevated	Drives ocular-surface inflammation	[49, 218, 219]
TNF-α	<0.5 pg/mL	markedly elevated	Disrupts epithelial barrier, amplifies cytokine cascade	[101, 220]
Protective/trophic factors				
IGF-1	~ 2 ng/ml	≈ 50 % of young level	Maintains epithelial vitality; lower levels correlate with dry-eye severity	[5]
MUC5AC	IQR based on Schirmer's strip: 2.86 (2.54–3.21)	Decreased total amount but increased concentration	Goblet-cell mucin critical for lubrication	[68, 69, 221]
Immunoglobulins				
IgA	Predominant (~186 μg/mL)	Markedly decreased	First-line mucosal immunity	[72, 222]
IgG	Trace (< 10 μg/mL)	Elevated (>20 μg/mL)	Indicates barrier leak & inflammation	[72]

Aging differentially affects these modes. When reflex tearing is pharmacologically suppressed with topical anesthesia, basal output shows minimal functional impairment relative to reflex secretion, though still exhibiting a statistically significant age-related decline [36, 66]. In contrast, reflex tearing is markedly compromised with age, primarily due to reduced corneal sensitivity and afferent nerve function, with possible contributions from diminished lacrimal gland responsiveness [26, 36, 65]. Consequently, unanesthetized (total) tear secretion—which incorporates the reflex component—declines substantially in older adults [62]. Figure 3 provides a mechanistic overview of basal and reflex secretory pathways in the lacrimal gland. In aggregate, aging primarily disrupts the sensory–autonomic reflex arc, attenuating reflex tearing, whereas basal secretion is comparatively preserved. Clinically, this pattern yields reduced but often subclinically sufficient lubrication at rest in healthy elders, though hovering near pathological thresholds in vulnerable subgroups, coupled with a diminished capacity to mount stimulus-evoked secretion—conditions that exacerbate tear-film instability and heighten symptom burden in older adults. Notably, basal tearing can also be suppressed by autonomic neuropathy (e.g., diabetes), corneal nerve injury, and anticholinergic or sedative medications; clinical interpretation should incorporate comorbidities and medication exposure.

3.3.3 Changes in Tear Composition in the Elderly and Their Physiological Significance

Tear fluid is a complex mixture of proteins, mucins, and soluble mediators that sustain ocular-surface health. Aging drives broad compositional shifts, notably an increase in inflammatory mediators, loss of protective factors, and alterations in immunoglobulin profiles.

(1) Inflammatory Mediators: A persistent, low-grade inflammatory state ("inflammaging") is detectable in the tears of older adults [26]. Cytokines and chemokines—including IL-6, IL-8, TNF-α, and IL-1β—are significantly elevated relative to younger individuals, even in the absence of clinically diagnosed dry eye [26]. These mediators perpetuate ocular-surface inflammation, impair lacrimal and goblet cell function, and destabilize the tear film. Increased MMP-9 further compromises epithelial tight junctions, promoting evaporative loss and sustaining inflammation [1].

(2) Growth Factors and Mucins: Tear concentrations of EGF, NGF, and IGF-1 decline with age, potentially slowing corneal epithelial renewal and wound repair [6, 26]. Mucin 5AC (MUC5AC), secreted by conjunctival goblet cells and critical for tear-film stability,

is reduced in elderly tears potentially linked to chronic inflammation; contributions from altered vitamin A metabolism remain hypothetical [67-69]. Even modest mucin deficiency diminishes lubrication and heightens dryness risk. Together, these deficits weaken the tear film's capacity to counter inflammatory stress, increasing susceptibility to dry eye [70, 71].

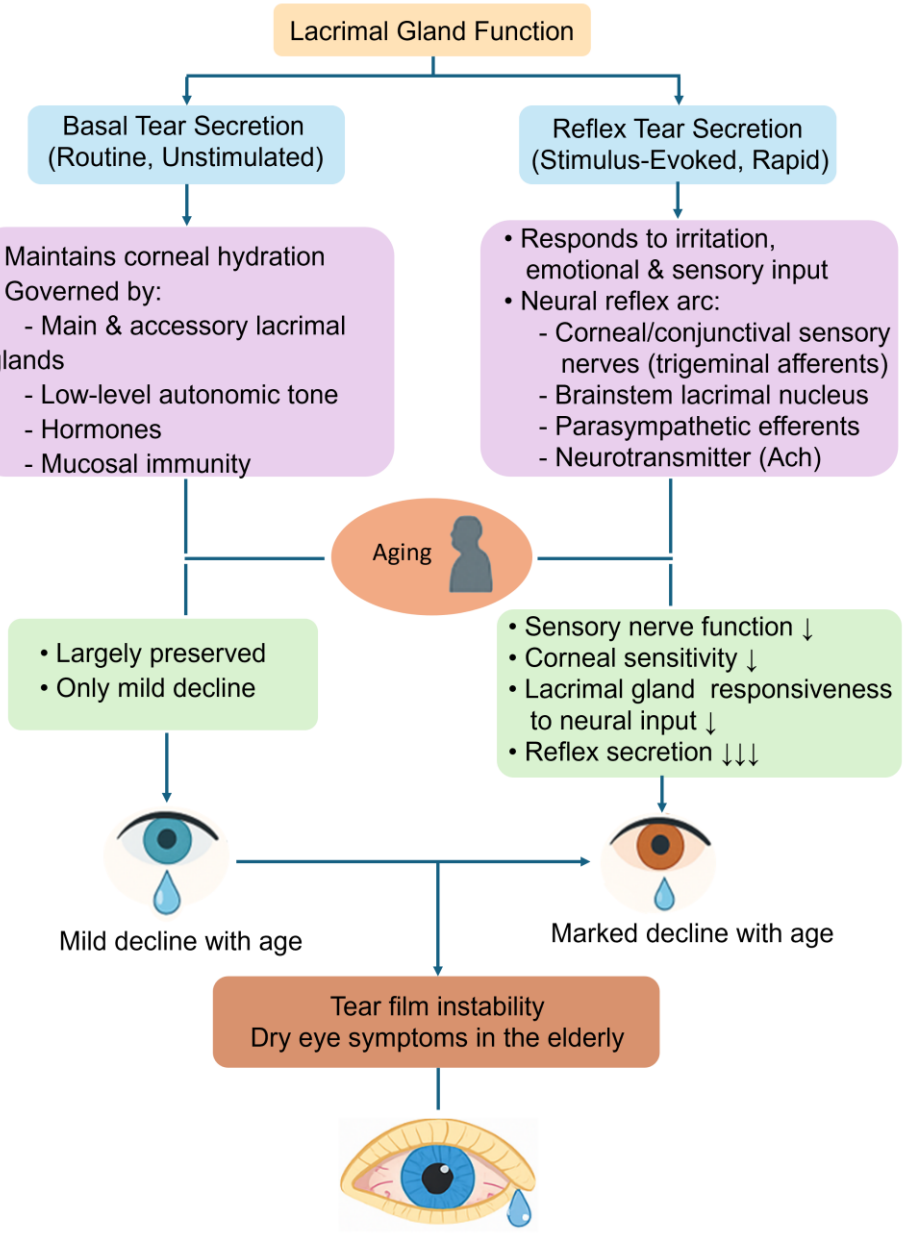

Figure 3. Mechanistic overview of basal and reflex tear secretion pathways in the lacrimal gland and their age-related changes Basal tear secretion provides routine corneal lubrication and is governed by constitutive activity of the main and accessory lacrimal glands, low-level autonomic tone, endocrine cues, and mucosal immune homeostasis. Across aging, baseline output shows heterogeneous trajectories across populations and measurement methods (e.g., anesthetized vs. unanesthetized Schirmer testing, phenol-red thread), whereas reflex tearing declines more consistently due to neurosensory pathway impairments involving trigeminal afferents, brainstem lacrimal nuclei, and parasympathetic efferents, together with reduced lacrimal gland responsiveness. The diminished capacity to rapidly augment tear production in response to stimuli—rather than basal hyposecretion per se—likely contributes to tear-film instability and symptoms in older adults. When reporting age effects, studies should specify the method, stimulus condition, and time window to distinguish basal from reflex components.

(3) Immunoglobulins: Aging also reshapes the immunoglobulin repertoire. Secretory IgA (sIgA)—the predominant immunoglobulin in healthy tears and a cornerstone of mucosal defense—declines significantly with age, reducing resistance to ocular-surface infection [71, 72]. By contrast, IgG levels rise, potentially reflecting inflammation-driven vascular leakage or local antibody production, though mechanistic studies are limited [72]. IgM may also decline in animal models, although age-related changes in human tears are less consistent [49, 73].

Overall, tears from older adults contain more proinflammatory mediators, fewer protective growth factors and mucins, and a shift toward IgG dominance. This imbalance correlates with tear film instability and ocular surface damage and is associated with increased dry eye risk in aging populations. To link functional readouts to tissue pathology, we next synthesize murine studies that jointly report tear function (Schirmer/PRT, TBUT) and ocular-surface staining alongside lacrimal histopathology.

3.3.4 Structural–Functional Correlations in Aged Murine Models

To directly couple physiology with pathology, we synthesized murine studies reporting Schirmer (or phenol red thread, PRT) values and tear-film metrics alongside corneal fluorescein staining (CFS) and lacrimal histopathology across aging cohorts. Across independent datasets, reductions in tear output and higher CFS scores are associated with acinar attrition, periductal/interacinar lymphocytic aggregates, fibrosis, oxidative injury, and reduced cholinergic/adrenergic innervation [35, 36, 38, 40, 41, 52, 74].

In naturally aged C57BL/6 mice, Schirmer/PRT declines from midlife onward while CFS increases, paralleling the emergence of periductal lymphoid aggregates and stromal fibrosis; molecular signatures include upregulated IFN-γ/TNF-α/CXCL13 and markers of senescence and oxidative stress [36, 38, 40, 41]. Function–structure concordance is also evident in stress-accelerated models: $Sod1^{-/-}$ mice exhibit significantly lower tear production with higher CFS, alongside 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (4-HNE) accumulation, acinar loss, and stromal remodeling [74]. Conversely, interventions that attenuate oxidative or inflammatory injury (e.g., caloric restriction, mitochondrial protection) yield functional improvement with reduced fibrosis [44, 51].

Functional–tissue–mechanism chain: Integration suggests a potential pathological cascade: diminished Schirmer/PRT may relate to impaired neurotransmission, while concurrent acinar loss and fibrosis further compromise secretion. In aggregate, these alterations contribute to deficient fluid secretion and tear film instability [8, 35, 36, 38, 40, 41, 74]. Where reported, decreases in tear proteins (e.g., lactoferrin, lysozyme) correlate with acinar compromise [36, 72]. Table 3 summarizes key murine reports with side-by-side functional and structural readouts to standardize cross-study interpretation.

Limitations: Functional assays and scoring methods vary (Schirmer vs. PRT; anesthetized vs. unanesthetized; staining scales), most datasets are cross-sectional, and murine TBUT data remain sparse—factors that collectively limit meta-analytic precision [35, 36, 38].

3.4 Commonalities and Differences in Lacrimal Gland Aging and Degeneration Across Species

Commonalities: Across species—including humans, mice, rats, and rabbits—lacrimal gland aging exhibits convergent degenerative features: acinar reduction, ductal pathology, and chronic inflammation. Histological surveys indicate that lymphocytic infiltration, acinar atrophy, and fibrosis are commonly observed in middle-aged and elderly humans [75, 76]. Mouse models recapitulate these hallmarks, with acinar loss, stromal fibrosis, and ductal dilation evident by ~12 months and progressing with advanced age [35, 39]. Aged rats similarly show decreased gland weight, increased collagen deposition, and reduced tear secretion [49]. Immune infiltration—predominantly periductal and interacinar lymphoid aggregates—is a conserved feature across these species [36, 49, 75, 76]. This "age-related lymphocytic infiltration," reflecting chronic low-grade inflammation ("inflammaging"), occurs even without overt autoimmunity [77]. Accumulating T lymphocytes may drive progressive glandular damage [45, 75].

Differences and Specificities: Despite shared patterns, species-specific distinctions are apparent. (1) Acinar phenotypes: Human lacrimal glands are predominantly serous. In rodents, aging associates with increased mucous granules in acini, though functional significance remains unclear; comparable changes have not been systematically documented in humans [39, 49, 78]. (2) Structural differences: Rabbits possess mixed serous–mucous glands, and their acini and ducts differ in size and organization relative to rodents and humans [45, 79]. Although systematic aging studies in rabbits are limited, available data suggest similar trends of acinar decline and fibrosis, with species-specific kinetics and magnitude. (3) Sex differences: In humans, acinar atrophy is more pronounced in postmenopausal women, with some studies reporting lower acinar density in older females [45, 79]. In rodents, ovariectomized females

exhibit accelerated immune-mediated atrophy, suggesting potential contributions from estrogen loss [80].

Table 3. Structural–Functional Concordance in Aged Mice.

Study (strain/sex)	Age (mo)/n	Tear metric (method)	Schirmer/PRT change vs young	CFS/staining	Lacrimal histology/ultrastructure	Key mechanism notes
Ríos 2005 (C57BL/6, M) [35]	3 vs 24–30 / NR	Evoked secretion (ex vivo); nerve markers	↓ Evoked protein/fluid (ACh/α_1)	NR	Mild acinar change at mid-age; ↓VIP/ChAT fibers	Early neurosecretory deficit precedes gross structural loss
Rocha 2008 (review of aged mice) [36]	≥12 / –	Schirmer/PRT (various)	↓ (method-dependent)	↑	Acinar atrophy, periductal fibrosis, lymphocytes	Integrates structure-function decline across cohorts
McClellan 2014 (C57BL/6, M/F) [38]	24 / NR	Tear/PRT (selected reports)	↓ (where reported)	↑ CFS	Dacryoadenitis, epithelial injury	Aged ocular surface disease with LG inflammation
Kojima 2012 (Sod1$^{-/-}$, M) [74]	50 / NR	Schirmer/PRT	≈ 50% ↓ vs WT	↑	8-OHdG/4-HNE, acinar loss/fibrosis	Oxidative stress → function drop + pathology
Yoon 2020 (C57BL/6, M) [52]	12 & 24 vs 2 / NR	Tear volume (normalized BW)	≈ 30–40% ↓	NR	Age-typical acinar changes	Quantified output decline with aging
Elbasiony 2023 (C57BL/6J, F) [40]	24 / NR	Clinical signs (surface)	NR	↑ Epitheliopathy	↑ Mast cells, lipid dysregulation	Immune–lipid axis linked to surface damage
Liu 2023 (C57BL/6, M) [41]	6→24 / cohort	–	–	–	Temporal transcriptome: ↑IFN-γ/TNF, senescence	Molecular aging programs mapping to function
Bakeeva 2016 (rat, SkQ1) [51]	24 / NR	–	–	–	Mitochondrial preservation	Mito-protection aligns with structural rescue
Mauduit 2025 (CR, C57BL/6) [44]	Adult / cohort	Schirmer/PRT	↑ vs ad lib	↓ CFS	↓ Fibrosis/oxidative markers	Calorie restriction improves both function & structure

PRT, phenol-red thread; CFS, corneal fluorescein staining; NR, not reported. Where only evoked secretion was measured (Ríos 2005), we list the directionality of functional response rather than basal tearing.

In summary, while the fundamental degenerative features of lacrimal gland aging—parenchymal loss, structural disorganization, and inflammation—are conserved, each model exhibits distinct cellular, architectural, and sex-dependent nuances. Recognizing these commonalities and differences is essential for interpreting animal studies and translating findings to human aging.

3.5. Animal Models and Translational Relevance

Most studies of lacrimal gland aging have employed murine models (e.g., aged C57BL/6 mice), which enable mechanistic dissection and genetic or pharmacologic manipulation. However, mice do not recapitulate human endocrine aging trajectories, including declines in estrogen and androgens, and exhibit distinct anatomical and immune features, limiting direct translatability to humans [52].

Rabbit models (e.g., lacrimal gland resection) offer advantages for surgical studies and drug delivery, but are often used for acute injury studies; their utility for modeling gradual senescence requires further validation [81].

By contrast, nonhuman primate (NHP) models, particularly desiccating stress-exposed macaques, display clinical features aligning with human disease (e.g., shortened TBUT, corneal damage) [82]. While these concordances support translational relevance, systematic therapeutic validation—including corticosteroid responses—is pending. High costs and low throughput constrain NHP use.

Table 4 summarizes the translational strengths and constraints of available animal models of lacrimal gland aging. No single model reproduces the full spectrum of human pathology. A multi-model translational strategy is therefore warranted: murine systems for mechanistic and target validation, rabbit models for dosing and delivery optimization, and NHP models for safety assessment and human-like pharmacodynamics.

4. Cellular and Molecular Mechanisms of Lacrimal Gland Aging

4.1 Oxidative Stress and Cellular Senescence

4.1.1 The Key Role of Oxidative Stress in Lacrimal Gland Aging

Accumulating evidence from murine models indicates that oxidative stress is a significant contributor to age-related LG dysfunction. Studies consistently report increased levels of ROS and markers of oxidative damage (e.g., 8-OHdG, 4-HNE) in aged LG acinar cells compared to young controls [8, 52, 74]. This imbalance arises from both heightened ROS production and a decline in endogenous antioxidant defense mechanisms.

Table 4. Comparative summary of animal models in lacrimal gland aging research: translational relevance and limitations.

Animal Model	Advantages	Limitations	Recommended Application	References
C57BL/6 mouse	Low cost; easy genetic manipulation; abundant aging models available	Stable hormone levels (no human-like menopause); simplified lacrimal gland structure; significant species differences compared to humans	Basic mechanisms, gene function studies, preliminary therapeutic interventions	[223]
Rabbit model	Large ocular structures; suitable for pharmacokinetics; multiple induced models (chemical/surgical)	Immune environment differs from humans; lacks natural aging models	Drug delivery, formulation testing, surgical model validation	[224, 225]
Non-human primate (NHP)	High anatomical and physiological similarity to humans; clinically relevant phenotype	High cost; ethical constraints; limited sample sizes; predominantly short-term induced models	Advanced efficacy validation; safety assessments for clinical therapies	[82]

Crucially, deficiency in key antioxidant enzymes exacerbates LG aging phenotypes. Sod1 knockout mice, a model of accelerated aging due to impaired ROS scavenging, exhibit pronounced LG pathology mirroring natural aging. This pathology includes acinar atrophy, stromal fibrosis, CD4+ T cell and monocyte infiltration, increased apoptotic cells, accumulation of secretory vesicles, mitochondrial swelling and degeneration, and significantly reduced basal and stimulated tear secretion. Elevated levels of oxidative damage markers (8-OHdG, 4-HNE) were directly observed within the LG tissue of these mice [74]. These findings provide direct experimental evidence that deficient ROS clearance is sufficient to drive LG dysfunction reminiscent of aging.

Furthermore, comparative analyses in aging C57BL/6 mice show a time-dependent increase in 8-OHdG-positive cells within both lacrimal and meibomian glands, correlating with the progression of dry eye signs [52]. Interventions modulating mitochondrial quality control, such as activating PINK1/Parkin-mediated mitophagy with rapamycin in middle-aged mice, can reduce LG ROS levels, decrease apoptosis, and improve tear secretion, underscoring the functional relevance of mitochondrial ROS and clearance pathways in LG aging [8].

Chronic disruption of circadian rhythms (chronic jet lag) in mice also induces hyperproliferation and dysfunction in LG, associated with significant ROS accumulation. This dysfunction was ameliorated by the antioxidant alpha-lipoic acid (ALA), further supporting a causal link between ROS dysregulation and LG pathology [83].

Collectively, these studies demonstrate that the accumulation of oxidative damage due to increased ROS generation and/or diminished antioxidant capacity is a key mechanistic feature of LG aging, contributing to cellular damage, inflammation, mitochondrial dysfunction, and impaired secretory function.

4.1.2 Interplay Between Oxidative Stress and Cellular Senescence, and Impact on Tear Secretion Function

Vicious Cycle Mechanism: Oxidative stress and cellular senescence are concurrently observed in aging lacrimal glands. Evidence suggests a potential interplay: elevated ROS can induce DNA damage and activate pathways like p53/p21 and p16^INK4a, leading to cell cycle arrest and senescence. Conversely, senescent cells exhibit mitochondrial dysfunction and altered metabolism, which can contribute to increased ROS production [8, 84-86]. Furthermore, the senescence-associated secretory phenotype (SASP), characterized by the secretion of factors like TNF-α and IL-1β, may contribute to a pro-oxidant microenvironment and inflammation within the gland tissue [39, 87].

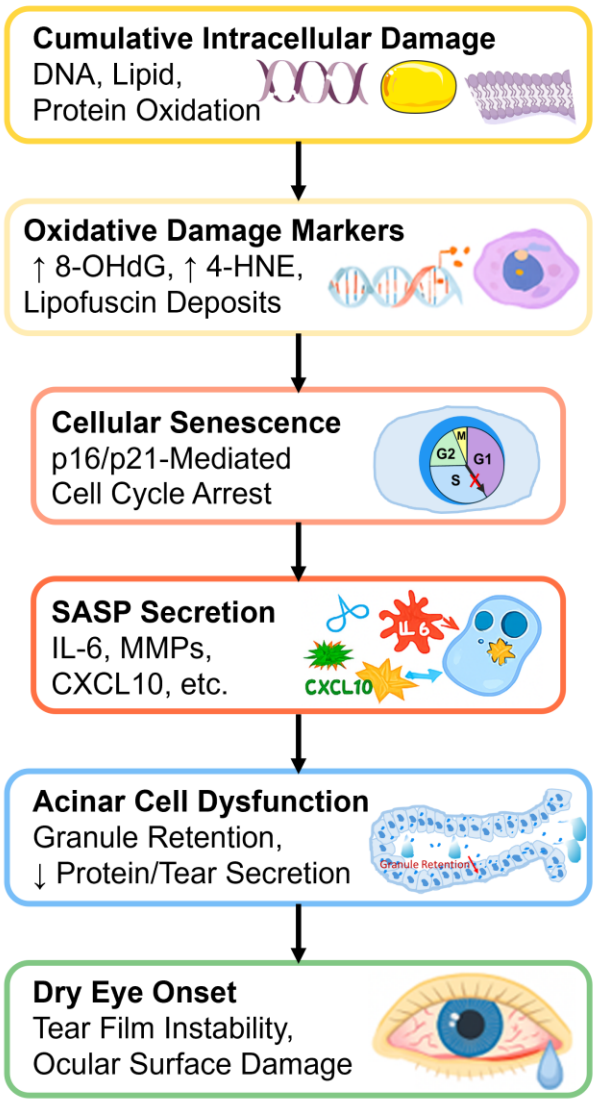

Figure 4. Cascade from oxidative cellular damage to tear secretion dysfunction in aging lacrimal glands. This schematic flowchart illustrates the proposed mechanistic pathway by which intracellular oxidative damage leads to lacrimal gland secretory dysfunction during aging. Accumulation of oxidative stress markers—including 8-hydroxy-2'-deoxyguanosine (8-OHdG), 4-hydroxynonenal (4-HNE), and lipofuscin granules—induces cellular senescence in acinar cells, characterized by elevated expression of $p16^{INK4a}$ and $p21^{Cip1}$. Senescent cells subsequently adopt a senescence-associated secretory phenotype (SASP), releasing pro-inflammatory and matrix-degrading factors such as interleukin-6 (IL-6), CXCL10, and matrix metalloproteinases (MMPs). These changes disrupt acinar cell function, leading to retention of secretory granules and a marked decline in both tear fluid and protein secretion. Ultimately, these impairments result in tear film instability and the clinical onset of aqueous-deficient dry eye disease (ADDE) in the elderly.

Functional Consequences: The combined burden of ROS-driven damage and senescence culminates in secretory dysfunction. Aged and $Sod1^{-/-}$ mice exhibit pronounced reductions in basal and stimulated tearing, accumulation of unemptied secretory granules, and diminished protein output [36, 62]. For instance, Yoon et al. reported tear production (normalized to body weight) was approximately 30-40% lower in 1-year-old and 2-year-old C57BL/6 mice compared to 8-week-old controls. Kojima et al. observed reduced reflex and basal tear volume in 50-week-old $Sod1^{-/-}$ mice relative to age-matched wild-type animals. Consistent with rodent findings, clinical studies in humans report age-related declines in Schirmer test scores and changes in tear composition. Reduced tear secretion correlates with histological observations in aged and $Sod1^{-/-}$ mice, including acinar atrophy, accumulation of secretory vesicles, and mitochondrial abnormalities [36, 52, 74]. Together, these data support a mechanistic link between oxidative stress, senescence-driven acinar dysfunction, and age-related tear deficiency (Fig. 4).

Limitations: Despite strong associative evidence, several caveats remain. Most mechanistic insights derive from rodents, which differ from humans in lacrimal architecture, immune milieu, and endocrine regulation [88]. The $Sod1^{-/-}$ model represents an extreme antioxidant deficit that may exaggerate ROS effects relative to physiological aging. Common readouts of oxidative damage (e.g., 8-OHdG, lipofuscin) are largely correlative and do not establish temporal precedence between ROS accumulation and senescence. Many datasets are cross-sectional rather than longitudinal, limiting causal inference. Finally, although antioxidant or mitochondria-targeted interventions ameliorate lacrimal pathology in animals, translation to humans remains challenging due to species-specific differences and the multifactorial nature of human lacrimal aging (hormonal, metabolic, and immunologic contributors) [74, 89]. Cautious extrapolation is therefore warranted, and additional human tissue studies and longitudinal analyses are needed to validate oxidative-stress–targeted therapies for age-related lacrimal gland dysfunction.

4.2 Immunosenescence and Chronic Inflammation

4.2.1 Impact of Systemic Immunosenescence on Lacrimal Gland Immune Homeostasis

Immunosenescence denotes age-related deterioration of innate and adaptive immunity and the emergence of a chronic, low-grade inflammatory state (“inflammaging”) [18, 49, 90]. A principal driver is thymic involution, which reduces the output of naïve lymphocytes—especially T cells—impairs central tolerance, and favors peripheral accumulation of antigen-experienced memory T cells [26, 39, 49]. In both humans and mice, aging is

associated with declines in B-cell numbers and naïve $CD4^+/CD8^+$ T cells, together with expansion of memory pools, reflecting reduced thymic export and prolonged survival of peripheral T cells [49].

Thymic atrophy may contribute to impaired negative selection and reduced thymic Treg output systemically, though local Treg dysfunction in aged lacrimal glands is primarily driven by inflammatory cues [39, 91]. Concomitantly, chronic stimulation elevates checkpoint receptors (e.g., PD-1) on T cells, promoting a senescent-like state with reduced responsiveness to novel antigens, alongside systemic elevations of IL-6/TNF-α [39, 49, 92]. Within the lacrimal gland, these systemic shifts correlate with immune dysregulation. Aged glands exhibit increased lymphocytic infiltration relative to young controls in mice, while human studies report frequent infiltrates without quantitative age-comparative data [14, 92-94]. Collectively, autoreactive lymphocyte accrual and Treg dysfunction, may predispose to chronic inflammation [39]. Table 5 summarizes key inflammatory and senescence-associated markers and their functional implications as mediators linking immunosenescence to local pathology.

Table 5. Comparative changes in key molecular markers in young vs. aged lacrimal glands and their functional impact.

Marker	Change with Aging	Functional Impact in Lacrimal Gland Aging	References
IL-6	↑ Elevated expression	Promotes chronic inflammation, acinar cell apoptosis, SASP amplification	[226]
TNF-α	↑ Elevated protein & mRNA	Drives lymphoid structure formation, goblet cell loss, immune activation; inhibition improves tear secretion and gland pathology	[59, 226]
MMP-9	↑ Increased activity	Degrades extracellular matrix and tight junctions, disrupts epithelial barrier and tear film stability	[227, 228]
IL-1β	↑In tears/ocular surface; lacrimal tissue evidence limited/inconsistent	Impairs myoepithelial contractility via downregulation of SMA/calponin; reduces tear output	[226]
SASP profile	↑ Amplified secretion	Creates pro-inflammatory milieu; impairs regeneration and promotes fibrosis	[55, 59]
Cathepsin S (CTSS)	↑ Elevated activity	Promotes ocular surface degeneration; blockade prevents goblet cell loss and corneal damage	[229]

Limitations: While immunosenescence is mechanistically linked to lacrimal gland aging, current evidence relies predominantly on associative data from murine models and cross-sectional human cohorts. This correlative nature precludes definitive causal attribution and complicates extrapolation of aging mechanisms to human physiology.

4.2.2 Permeability Changes in the Blood-Lacrimal Barrier and Immune Cell Infiltration

Under physiological conditions, a selective blood–lacrimal barrier restricts entry of serum proteins and immune cells into glandular tissue and tear fluid; large serum proteins (e.g., albumin) remain low in tears unless barrier integrity is compromised [39, 95]. With aging, barrier permeability increases at vascular and stromal levels, facilitating immune-cell ingress.

In aged mice, specialized high endothelial venules (HEVs) arise within the lacrimal gland. These peripheral node addressin (PNAd)–positive vessels, induced by locally elevated chemokines (CCL19, CCL21, CXCL13, CXCL12), support efficient transmigration of naïve and memory T cells into the parenchyma [14, 39]. Coordinated upregulation of chemokine and lymphotoxin pathways promotes lymphocyte clustering and ectopic follicle-like aggregates, indicating enhanced vascular selectivity for immune recruitment in aged glands [14].

Chronic inflammation further disrupts tissue barriers. Experimental IL-1β injection rapidly provokes massive immune-cell influx and acinar destruction, modeling acute barrier failure [39]. Systemic cholinergic blockade, by suppressing tearing, elevates inflammatory mediators and increases immune infiltration; withdrawal reverses these effects, underscoring barrier sensitivity to tear deficiency and epithelial stress [96].

As a result, aged glands with weakened barriers more readily admit and retain circulating immune cells. Even in older adults without overt dry eye, tear concentrations of IL-8, IL-6, TNF-α, and CCL5 (RANTES) are significantly higher than in younger individuals, consistent with increased permeability and immune infiltration [1, 71]. These changes sustain a state of persistent, low-grade inflammation within the aging lacrimal microenvironment.

Limitations: Although aging demonstrably disrupts blood-lacrimal barrier integrity and amplifies immune infiltration, the evidentiary foundation rests heavily on

rodent models (notably ectopic lymphoid structure formation in aged mice) and fragmented human data (e.g., cross-sectional tear cytokine elevations), which makes robust causal inference and quantification of human glandular barrier dysfunction challenging.

4.2.3 Changes in Immune Cell Composition in the Aged Lacrimal Gland

Aging remodels the lacrimal immune landscape, characterized by increased leukocyte burden, altered lineage composition, and dysregulated activation states [18, 38, 42, 97]. Focal lymphocytic aggregates—often adjacent to interlobular septa—correlate with parenchymal atrophy and fibrosis, consistent with tertiary-lymphoid-like organization in chronically inflamed tissue.

(1) $CD4^+$ T cells: Aged glands show enrichment of $CD4^+$ helper T cells with a shift from naïve to effector-memory and Th1-skewed phenotypes that produce IFN-γ and TNF. These cells upregulate inhibitory receptors (e.g., PD-1), reflecting chronic stimulation and partial exhaustion, yet they persist and maintain low-grade inflammation. A small naïve-like pool may remain, indicating incomplete conversion and ongoing recruitment in the aged niche [49, 94].

(2) $CD8^+$ T cells: Cytotoxic $CD8^+$ T cells, frequently memory-biased, increase with age and may contribute to epithelial injury, though direct evidence of perforin/granzyme-mediated damage in aged lacrimal glands remains limited. Although less extensively characterized than $CD4^+$ T cells in lacrimal aging, their cytolytic potential and crosstalk with Th1 inflammation suggest a substantive contribution to acinar injury [39, 49].

(3) Regulatory T cells (Tregs): $Foxp3^+$ Tregs persist but exhibit reduced suppressive capacity in aged glands. "Inflammatory Tregs" that produce IL-17 have been described, with impaired regulation and paradoxical promotion of local immunopathology. Adoptive-transfer observations indicate that age-associated Treg dysfunction facilitates inflammatory infiltration and tissue damage, consistent with diminished tolerance maintenance [39, 42, 94].

(4) B cells: B-cell infiltration increases markedly with age [14, 38, 39]. Both naïve and memory subsets expand, with evidence of local clonal proliferation and $GL7^+$ germinal-center-like microanatomy. These ectopic structures often lack full checkpoint control and can support local autoantibody production against glandular antigens, potentially impairing acinar function and amplifying tissue injury [98].

(5) Natural killer (NK) cells: Although relatively sparse, NK cells may undergo age-related phenotypic shifts, produce IFN-γ, and shape the inflammatory milieu in concert with T-cell–driven pathways; their precise role in lacrimal aging requires further definition [30, 41].

(6) Mast cells: Mast-cell numbers and activation increase from midlife onward, with higher frequencies of FcεRI^+ c-Kit^+ cells and enhanced degranulation in older glands [35, 39, 40]. Elevated mediators—including tryptase, histamine, IL-6, TNF-α, and IL-33—promote vascular permeability, recruit additional leukocytes, and perpetuate chronic inflammation [40, 99].

Taken together, the aged lacrimal gland displays a dysregulated immune microenvironment in which heightened innate (mast cells, NK cells) and adaptive (T/B cells) activation coexists with impaired regulatory control. This immune disequilibrium drives progressive structural degeneration and functional decline and is a defining feature of age-related lacrimal pathology [98].

Limitations: Human data on immune remodeling of aged lacrimal glands remain limited; rodent studies may overrepresent lymphoid aggregates, and cross-sectional designs constrain causal inference. Definitions of "aged" and analytic panels vary across studies, tissue dissociation can bias cell-type recovery, and sex and endocrine status are inconsistently reported. These factors complicate translation and highlight the need for standardized, multi-omic, and longitudinal human studies.

4.2.4 Expression and Role of Inflammatory Mediators in Lacrimal Gland Aging

The inflammatory milieu of the aged lacrimal gland comprises cytokines, chemokines, and matrix-remodeling enzymes produced by infiltrating immune cells (lymphocytes, macrophages, mast cells) and by stressed or damaged epithelial and stromal cells. These mediators sustain chronic inflammation, parenchymal injury, and fibrotic remodeling [39].

IL-1β. While IL-1β is a key pro inflammatory mediator in experimental lacrimal injury and ocular surface disease, consistent elevation of IL-1β within physiologically aged lacrimal gland tissue has not been demonstrated across studies. Prior intraglandular IL-1β injection experiments—performed in adult, not aged, mice—rapidly induce neutrophilic and lymphocytic influx with acinar damage, illustrating pathogenic potential rather than baseline elevation in aging. Recent ex vivo work indicates that IL-1β can impair lacrimal myoepithelial contractility via MMP-2 activation, offering a plausible mechanism by which episodic IL-1β exposure could contribute to hyposecretion [49, 100]. Accordingly, we treat IL-1β as a context dependent effector of inflammation, rather than a cytokine uniformly upregulated in physiological aging of the lacrimal gland. Evidence for increased IL-1β in physiologically aged

lacrimal glands is limited and inconsistent; much support derives from injury/cGVHD models or tear cytokine studies that also reflect ocular surface sources.

TNF-α. TNF-α, produced by Th1 cells and macrophages, is upregulated with age and exerts dual effects by propagating inflammation and enabling ectopic lymphoid organization [101]. TNF-α correlates with acinar apoptosis in inflammatory contexts, though causal evidence in physiological aging requires validation [39].

IFN-γ. IFN-γ, primarily derived from Th1 and NK cells, is a central mediator of age-associated glandular inflammation [41]. It induces epithelial apoptosis and impairs conjunctival goblet cell function; IFN-γ deficiency confers resistance to age-related loss of glandular and goblet cells, underscoring its pathogenic role [49]. Sustained IFN-γ production maintains a self-perpetuating cycle of injury and immune activation.

CXCL13 and related chemokines. CXCL13, a potent B-cell chemoattractant, is markedly upregulated with age and closely linked to the formation of ectopic lymphoid structures in the lacrimal gland [14, 39]. Together with CCL19 and CCL21, CXCL13 promotes lymphoid neogenesis and supports local autoimmune responses reminiscent of Sjögren's syndrome and dry eye.

Matrix metalloproteinases. MMP-2 and MMP-9 are elevated in aged and chronically inflamed glands, degrading extracellular matrix and basement membrane to facilitate immune-cell trafficking while contributing to acinar destruction and fibrosis. Pharmacologic MMP inhibition attenuates inflammation and improves tear secretion in preclinical models [100].

Additional cytokines and chemokines. IL-6, IL-8, CCL5, and IL-17 are also increased in aged lacrimal glands and tears, sustaining leukocyte recruitment and activation [41, 49, 94]. IL-17, produced predominantly by Th17 cells, synergizes with TNF-α to intensify tissue injury.

Collectively, these convergent pathways illustrate how systemic immunosenescence, increased blood–lacrimal barrier permeability, and ectopic lymphoid neogenesis permit the accumulation of immune cells and mediators within the aging gland. The resulting self-reinforcing inflammation drives progressive acinar injury, stromal fibrosis, and secretory decline. In summary, a trajectory of immunosenescence → local immune disequilibrium → persistent mediator release → cumulative tissue damage underlies the chronic inflammatory process characteristic of lacrimal gland aging [92].

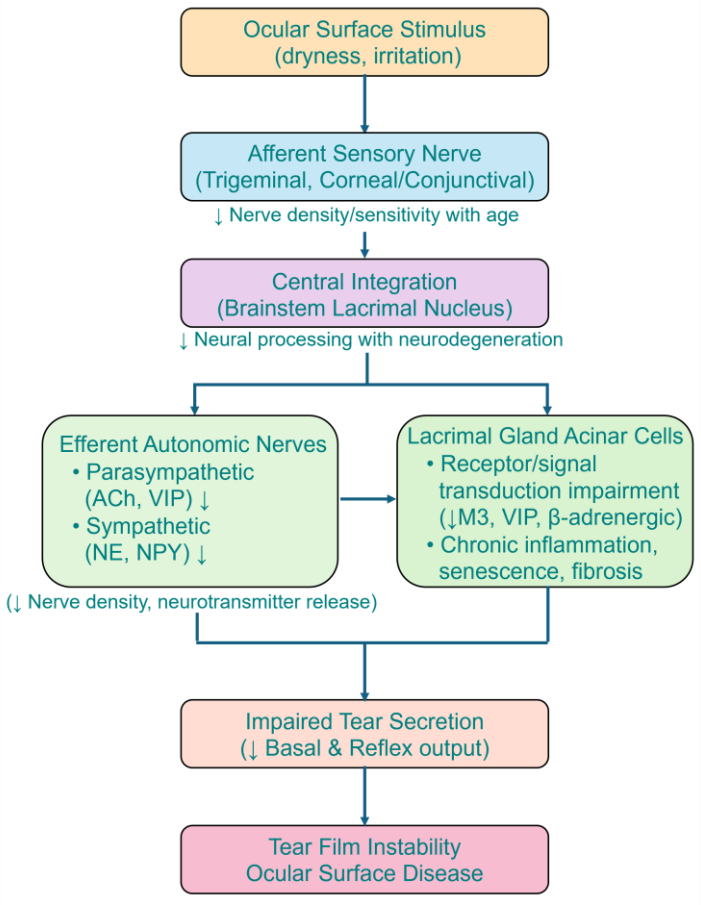

Figure 5. Schematic cascade of neuroregulatory impairment in age-related lacrimal gland dysfunction. Aging disrupts each step of the lacrimal functional unit. Reduced density and sensitivity of ocular surface sensory nerves weaken afferent input to the brainstem. Central integration of the tear reflex is impaired by neurodegeneration. Autonomic efferent output (both parasympathetic and sympathetic) declines due to loss of nerve fibers and reduced neurotransmitter release. At the lacrimal gland, age-related changes in receptor expression, signaling pathways, and cellular health further blunt secretory responses. Chronic inflammation and fibrosis exacerbate functional loss. The end result is diminished tear secretion, tear film instability, and a vicious cycle of ocular surface damage that perpetuates neuroregulatory decline.

4.3 Cellular and Molecular Mechanisms of Neural Regulatory Decline in Lacrimal Gland Aging

Age-related lacrimal gland dysfunction is driven by progressive impairment across the neural–lacrimal–ocular surface axis. As shown in Figure 5, aging perturbs the entire reflex arc—from ocular-surface sensory input, through central integration, to autonomic output and glandular responsiveness—resulting in a cascade of neuroregulatory deficits. The subsections below delineate each step of this pathway as a framework for understanding age-dependent declines in tear secretion and ocular-surface stability.

4.3.1 Structural and Functional Characteristics of Lacrimal Gland Innervation

The lacrimal gland is densely innervated by autonomic and sensory fibers. Parasympathetic input is anatomically and functionally predominant, arising from the facial nerve (VII) via the pterygopalatine ganglion; terminal branches envelop most acini [27, 35]. Parasympathetic fibers release ACh and VIP, which provide the principal secretomotor drive and robustly stimulate fluid and protein secretion [27, 102].

Sympathetic fibers originate from the superior cervical ganglion and reach the gland along perivascular routes. Although less numerous and variably distributed across species, they innervate subsets of acini and vascular elements and release NE [103]. Functionally, parasympathetic denervation produces a marked reduction in lacrimal output, whereas sympathectomy exerts comparatively modest effects on tear production [27, 102].

Sensory innervation derives from the ophthalmic division of the trigeminal nerve. Limited sensory endings penetrate the gland and contain neuropeptides such as substance P and calcitonin gene-related peptide (CGRP). These afferents constitute the sensory limb of the lacrimal functional unit (LFU), relaying ocular-surface stimuli to central nuclei to evoke reflex tearing [27, 102].

In summary, parasympathetic efferents are the dominant drivers of secretion, sympathetic fibers modulate baseline output and vascular tone, and trigeminal afferents furnish feedback control within the tear reflex arc.

4.3.2 Age-Related Changes in Neurotransmitter Synthesis, Release, and Degradation

Parasympathetic and sympathetic nerves regulate lacrimal gland function primarily via ACh, VIP, and NE, along with co-transmitters such as neuropeptide Y (NPY) [27, 104]. The synthesis, vesicular packaging, evoked release, and enzymatic clearance of these transmitters undergo substantive age-related alterations that compromise secretomotor control:

(1) ACh**:** ACh is synthesized in parasympathetic terminals by choline acetyltransferase, packaged into synaptic vesicles, released upon depolarization, and rapidly hydrolyzed by acetylcholinesterase (AChE). Aging is associated with a marked decline in cholinergic innervation and release capacity: cholinesterase-positive fibers are reduced in 24-month-old rat glands [105], and depolarization-evoked ACh release is severely attenuated in aged mouse glands [35, 39]. Chronic inflammation further depresses cholinergic signaling [36].

(2) VIP: VIP is coreleased with ACh from parasympathetic neurons, signals through VPAC receptors, and is degraded by peptidases in vivo. VIP robustly stimulates lacrimal secretion and contributes to regulation of tear-film osmolarity [27]. With aging, VIP-immunoreactive networks encircling acini are substantially diminished [35], and exogenous VIP elicits blunted secretory responses in aged rats compared with young controls [36, 39], consistent with impaired VIPergic signaling, likely due to reduced peptide availability and/or post-receptor defects.

(3) NE: NE is synthesized in sympathetic terminals by tyrosine hydroxylase and dopamine β-hydroxylase, stored in vesicles, and acts on adrenergic receptors—particularly α_1 receptors—to modulate protein secretion and vasomotor tone [36]. Clearance occurs largely via Na^+-dependent reuptake and degradation by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Aging decreases tyrosine hydroxylase-positive sympathetic fibers, implying reduced NE availability; cotransmitters such as NPY may likewise decline [35, 39] . Although sympathetic input minimally affects basal aqueous output, its attrition can alter tear-protein composition and vascular regulation.

Collectively, age-related deficits in parasympathetic (ACh, VIP) and sympathetic (NE) transmitter dynamics blunt neural control of the gland, yielding reduced basal and stimulus-evoked tearing in response to cholinergic or adrenergic drive [35, 49, 105].

4.3.3 Impairments in Neurotransmitter Signal Transduction Pathways with Age

Effective lacrimal secretion depends on neurotransmitter receptors on acinar cells—muscarinic M_3, adrenergic α_1/β, and VIP/VPAC subtypes—and on intact downstream signaling cascades. In young glands, receptor expression is high and transduction is efficient: ACh–M_3 engagement couples to Gq/11–phospholipase C (PLCβ), generates IP_3-dependent Ca^{2+} mobilization, and activates PKC; VIP–$VPAC_{1/2}$ activates adenylyl cyclase (AC), elevates cAMP, and stimulates PKA; NE acting on α_1D or

β-adrenergic receptors triggers PLC/Ca^{2+} or AC–cAMP–PKA pathways, with additional contribution from NO/cGMP signaling [36, 106]. These inputs converge on the exocytic machinery and ion/water transporters to drive protein and fluid secretion.

With aging, multiple defects emerge. Receptor availability and function decline: functional assays demonstrate that by midlife (8–12 months in mice) secretory responses to muscarinic and α_1-adrenergic agonists are significantly blunted—preceding overt nerve-fiber loss—implicating reduced M_3 and α_1 signaling due to lower receptor density, altered G-protein coupling, or desensitization [35, 39]. Aged rats likewise exhibit attenuated tearing after exogenous VIP, consistent with diminished VPAC-mediated signaling [36]. Downstream amplification is blunted: aged acinar cells display weaker second-messenger responses; for example, a β-adrenergic agonist combined with phosphodiesterase inhibition (to raise cAMP) elicits substantially less protein secretion in aged glands than in young, indicating reduced responsiveness of PKA-dependent effectors and the secretory apparatus [107]. Similarly, ACh-evoked Ca^{2+} mobilization is diminished, likely constraining PKC activation and granule exocytosis.

In aggregate, aging weakens receptor–effector coupling in the lacrimal gland: the expression and function of M_3, α/β-adrenergic, and VIP/VPAC receptors are reduced, and canonical amplifiers (Ca^{2+}, PKC, cAMP/PKA) are dampened, producing a muted secretory response to neural stimulation [39, 106, 107]. Consistent with these signaling deficits, aged glands exhibit reduced basal and agonist-evoked tearing to cholinergic and adrenergic stimulation, and loss of cholinesterase-positive fibers corroborates diminished cholinergic drive [35, 49, 105].

4.3.4 Functional Decline Precedes Structural Deterioration: "Intact" Structure but Weakened Response

A consistent observation in lacrimal gland aging is that secretory dysfunction emerges before overt structural degeneration—i.e., "functional decline precedes structural deterioration." In murine models, tear secretory responses to neural stimulation (electrical-field stimulation, muscarinic agonists, adrenergic agonists) are already diminished by 8–12 months of age (midlife), while overall innervation density and routine histology remain comparable to young adults. Marked axonal loss and pronounced histopathology—acinar atrophy and lymphocytic aggregation—typically arise later, at ~24–32 months [35, 39].

These findings indicate that early hyposecretion primarily reflects neurotransmitter signaling defects and cellular stress, though incipient structural changes (e.g., partial nerve loss) may coexist. Consistent with this interpretation, lipofuscin accumulation and mast-cell infiltration—features associated with cellular senescence—are detectable by midlife, preceding obvious structural abnormalities [8, 39].

Accordingly, lacrimal gland aging includes an early dysfunction-predominant phase, in which neuroglandular signaling and tear output decline ahead of visible tissue deterioration. A similar pattern is evident in early dry eye disease, underscoring the primacy of early molecular dysfunction in the pathogenesis of age-related tear deficiency and highlighting opportunities for intervention prior to irreversible structural damage [39].

4.3.5 Impaired Coordination in the Neural–Lacrimal–Ocular Surface Functional Unit

Lacrimal secretion is governed by a tripartite lacrimal functional unit (LFU) integrating the ocular surface, sensory and autonomic nerves, and the lacrimal gland [27, 100]. Afferent fibers from the cornea and conjunctiva convey ocular-surface status to central nuclei, which in turn issue autonomic efferent commands to the gland to elicit tearing and preserve ocular-surface homeostasis [27, 102].

Aging disrupts coordination across multiple nodes of this reflex arc: (1) Weakened afferent arm: Corneal and conjunctival sensory nerve density and sensitivity decline with age, diminishing detection and transmission of desiccating stimuli to the brainstem lacrimal centers [35, 41, 102]. (2) Altered central integration: Age-related changes in central pathways, including brainstem neurons mediating the lacrimal reflex, reduce processing efficacy and attenuate outgoing secretomotor drive, even when sensory input is present [39, 41]. (3) Impaired efferent arm: The density and function of autonomic efferents—especially parasympathetic fibers—decrease with age, as does neurotransmitter release, thereby weakening glandular responsiveness to neural stimulation [35, 39]. (4) Intrinsic Gland Dysfunction: Acinar cell sensitivity to neural cues declines owing to receptor and post-receptor signaling defects, compounded by chronic inflammation and tissue remodeling (e.g., fibrosis), leading to reduced secretory output despite intact firing of upstream neurons [39].

Together, these alterations dysregulate the tear reflex. When the ocular surface becomes dry, the feedback loop fails to generate an adequate secretory response, destabilizing the tear film and initiating a self-reinforcing cycle: tear deficiency exacerbates epithelial injury, which further degrades sensory input and depresses secretion [39, 102]. Chronic inflammation amplifies this loop, as immune-cell infiltration and mediators (e.g., IL-1,

TNF-α) impair neurotransmission and acinar function within the gland [36]; ocular-surface inflammation can likewise damage corneal nerve terminals. Thus, aging produces not only local glandular pathology but a systems-level failure of the neural–lacrimal–ocular surface network central to age-related dry eye. A comparative schematic of neural signaling in young versus aged glands is shown in Figure 6.

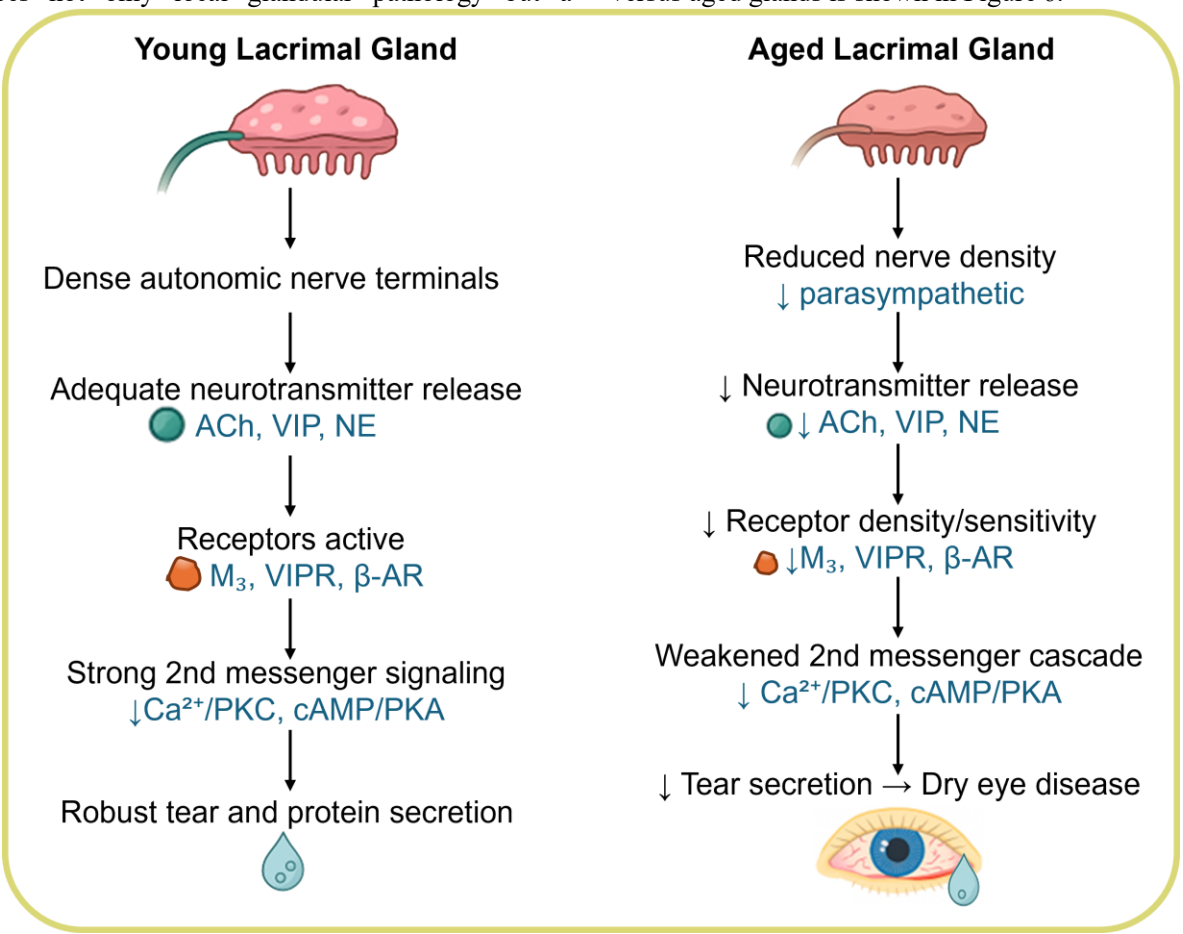

Figure 6. Comparative schematic of neural signaling pathways in young versus aged lacrimal glands. This flowchart illustrates the progressive decline of neuroregulatory mechanisms in aging lacrimal glands, emphasizing the stepwise functional deterioration across the neural signal cascade. In the young gland (left panel), autonomic nerve terminals—both parasympathetic and sympathetic—are densely distributed and actively release neurotransmitters such as acetylcholine (ACh), vasoactive intestinal peptide (VIP), and norepinephrine (NE). These neurotransmitters bind to well-expressed acinar cell receptors (M_3 muscarinic receptors, VIP receptors, β-adrenergic receptors), triggering robust second messenger signaling via Ca^{2+}/PKC and cAMP/PKA pathways, ultimately resulting in efficient tear and protein secretion. In contrast, the aged gland (right panel) exhibits reduced nerve fiber density—especially in parasympathetic branches—along with impaired neurotransmitter synthesis and release. Acinar cell receptors show downregulated expression or diminished sensitivity, and intracellular signaling cascades are blunted, leading to decreased Ca^{2+} mobilization and reduced cAMP/PKA activation. These cumulative deficits result in insufficient secretory response despite preserved gross anatomy, explaining the age-associated decline in tear secretion and the onset of dry eye disease.

Limitations: Current understanding of age-related neuroregulatory decline derives primarily from animal models. Functional mapping of lacrimal neural circuits in aging—particularly in large animals and humans—remains limited, and most evidence is cross-sectional rather than longitudinal [35]. Establishing causality and enabling clinical translation will require interventional studies (e.g., targeted neural stimulation or restoration in aged glands) and validation in higher animal models or human cohorts [36].

4.4 Age-Related Changes in Molecular Markers

4.4.1 Apoptosis and Senescence Markers

Evidence indicates that aging of the lacrimal gland is accompanied by increased apoptosis and activation of senescence programs. Aged acinar cells exhibit apoptotic markers—TUNEL positivity and cleaved caspase-3—consistent with chronic low-grade inflammatory and oxidative stress [36]. Proinflammatory cytokines such as

TNF-α and Fas ligand may contribute to apoptotic signaling in inflammatory contexts, potentially accelerating acinar loss [74]. Senescence-associated damage markers, including 8-OHdG (DNA oxidation) and 4-HNE (lipid peroxidation), are elevated with age [49], indicating persistent oxidative injury and engagement of senescence pathways. The contributions of p16^INK4a upregulation and telomere attrition to lacrimal gland senescence are increasingly recognized, although mechanistic details remain under investigation.

4.4.2 ECM Remodeling and Fibrosis Factors

Fibrosis is a defining histopathologic feature of the aging lacrimal gland. Transforming growth factor-β (TGF-β) and matrix metalloproteinases (MMPs) are principal regulators of extracellular matrix (ECM) remodeling and fibrotic progression. With age, glands exhibit increased collagen deposition and matrix stiffening [49]. TGF-β, a profibrotic cytokine, is overexpressed and drives fibroblast–to–myofibroblast differentiation (marked by α-SMA expression) and excessive ECM synthesis. In aged human tissue, both TGF-β and vimentin are elevated, whereas interventions such as melatonin attenuate their expression and reduce fibrosis [39, 108]. MMP-2 and MMP-9 also display age-related dysregulation: while they contribute to homeostatic turnover in youth, excessive activity degrades basement membranes and interstitial matrix, facilitating immune-cell ingress and perpetuating aberrant repair that culminates in fibrosis. Additional contributors include tissue inhibitors of metalloproteinases (TIMPs), fibronectin, and laminin, although their age-dependent dynamics in the lacrimal gland remain to be delineated [109].

4.4.3 Lipid Metabolism Changes

Adipose infiltration is a recurrent feature of the aged lacrimal gland, indicating fibro-adipogenic remodeling and altered lipid handling within the stromal compartment [18]. Mechanistically, activation of peroxisome proliferator-activated receptor-γ (PPAR-γ)–dependent adipogenic programs in mesenchymal/stromal cells has been proposed, biasing differentiation toward adipocytes at the expense of reparative fibroblasts [45]. The resultant adipocytes secrete adipokines (e.g., leptin, adiponectin) and proinflammatory mediators that reshape the immunometabolic milieu, promote macrophage recruitment and polarization, and potentiate TGF-β–driven fibrogenesis [39, 110]. Despite these observations, direct evidence for specific dysregulation of lipid-metabolic pathways in lacrimal aging—such as alterations in fatty-acid uptake/β-oxidation, de novo lipogenesis, or lipophagy gene networks—remains limited; systematic transcriptomic and lipidomic studies are needed to establish causal mechanisms.

4.4.4 Immune Infiltration and Inflammatory Mediators

Chronic immune cell infiltration is a hallmark of the aged lacrimal gland [39]. Histologic surveys indicate that approximately 63% of specimens from middle-aged and elderly individuals contain focal lymphoid aggregates, a pattern mirrored by significant lymphocyte increases in aged mice [14, 49]. The infiltrates consist predominantly of T cells (especially $CD4^+$), B lymphocytes/plasma cells, and innate effectors (monocytes/macrophages, neutrophils) [18, 74]. These cells upregulate antigen-presentation pathways and release a broad array of mediators—including MHC class II, IFN-γ, IL-1β, and TNF-α—that sustain inflammation [101]. The resulting milieu not only injures glandular parenchyma but also perturbs innervation and secretory control [36, 39]. Mast cells are increased in aged glands and act as key amplifiers of inflammation. Through release of histamine and other mediators, they recruit additional lymphocytes and macrophages, enhance vascular permeability, and accelerate tissue damage [39, 40]. Notably, mast cell–deficient aged mice exhibit reduced inflammatory burden and preservation of acinar architecture, implicating mast cells as proximal drivers of immune pathology in aging [40].

In summary, the aged lacrimal gland exists in a state of persistent, low-grade inflammation ("inflammaging") [18], characterized by sustained immune infiltration and elevated inflammatory mediators. Although typically less severe than overt autoimmunity, this chronic process cumulatively promotes parenchymal damage and progressive secretory decline.

5. Endocrine, Metabolic, and Lifestyle Factors in Lacrimal Gland Aging

5.1 Regulation of Lacrimal Gland Function by Sex Hormones and Their Age-Related Changes

Androgens (e.g., testosterone, dihydrotestosterone) are essential for lacrimal gland homeostasis. Androgen receptors (AR) are widely expressed in acinar and ductal epithelia [111, 112]. Androgen signaling supports protein synthesis and secretion, preserves glandular architecture, and modulates local immunity with demonstrable anti-inflammatory effects within the gland [3, 111, 113]. Androgens also sustain meibomian-gland lipid output, contributing to tear-film stability.

With aging, circulating androgen levels decline—gradually in men and abruptly in women after menopause

[114]. DHEA and its sulfate (DHEA-S), major adrenal androgens, are significantly reduced in older adults of both sexes and correlate with worse dry eye metrics and lower tear secretion [115]. Given women's lower baseline androgen exposure, the postmenopausal fall can reduce lacrimal performance below the threshold required for ocular-surface homeostasis [113, 114]. Androgen deficiency therefore constitutes a substantial contributor to age-related hyposecretion and increased dry eye risk, particularly in postmenopausal women. Early clinical studies further indicate that testosterone supplementation can improve tear-film stability and alleviate symptoms in androgen-deficient patients [116].

Estrogens (e.g., 17β-estradiol) exert more complex, context-dependent effects. Estrogen receptors (ERα, ERβ) are present in male and female lacrimal glands without major sex differences in expression [117]. At physiological levels, estrogens can promote epithelial survival and dampen inflammation—for example, estrogen supplementation reduces glandular inflammation in Sjögren's syndrome models. However, outcomes vary with dose, timing, and hormonal milieu: some studies report pro-inflammatory actions, whereas others show protective or negligible effects. High-dose or unopposed estrogen in certain hormone-replacement regimens may increase dry eye risk, while combined estrogen–progesterone therapy can enhance tear secretion [117].

In summary, sex hormones are pivotal regulators of lacrimal function. Age-related androgen deficiency—especially after menopause—drives secretory decline and dry eye susceptibility, whereas estrogen effects are bidirectional and context sensitive: physiological signaling may be protective, but imbalance or excessive supplementation can be detrimental.

5.2 Impact of Thyroid Hormone and Glucocorticoid Level Changes on Lacrimal Gland Homeostasis

5.2.1 Thyroid Hormones

Thyroid hormones (T3, T4) exert broad control over cellular metabolism and neural function, and the lacrimal gland is a recognized target tissue. Thyroid hormone receptors—particularly TRβ1—are expressed in human and animal lacrimal tissue [39, 118]. Under physiological conditions, thyroid hormones sustain the metabolic activity and secretory capacity of lacrimal epithelia and help maintain balanced neural innervation.

Hypothyroidism is associated with reduced tear production, potentially through metabolic and immune alterations. It frequently coexists with autoimmune thyroiditis (e.g., Hashimoto's), increasing susceptibility to immune-mediated lacrimal injury and accelerating functional decline [39, 118].

Hyperthyroidism and Thyroid Eye Disease (TED): Dry eye is common in hyperthyroid states, particularly in TED [119]. Mechanisms include increased evaporative loss due to eyelid retraction and proptosis, as well as direct autoimmune effects on the gland. Human lacrimal acinar cells express the thyrotropin receptor (TSHR), rendering them susceptible to TSHR-targeting autoantibodies in Graves' disease. Clinically, lacrimal gland volume is increased in active TED—consistent with inflammation—and may shrink with fibrosis in inactive but symptomatic disease [120]. Thus, both thyroid hormone imbalance and immune dysregulation perturb lacrimal metabolic and neural homeostasis, contributing to age-related functional decline.

5.2.2 Glucocorticoids

Glucocorticoids (cortisol) are critical for stress response and immune regulation, with glucocorticoid receptors are widely distributed in lacrimal cells [39]. Physiologically, cortisol exerts anti-inflammatory and immunosuppressive effects; acute elevations help restrain excessive inflammation. In contrast, chronic elevation—due to prolonged psychological stress or Cushing's syndrome—impairs lacrimal function. Sustained stress modifies autonomic balance via limbic–HPA pathways and suppresses basal tearing [121]. Disruption of circadian cortisol rhythms and persistent hypercortisolemia further inhibit parasympathetic stimulation of the gland, reducing tear secretion [121, 122].

In older adults, hypothalamic–pituitary–adrenal (HPA) axis changes yield higher baseline cortisol and attenuated diurnal variation [123, 124]. Although tissue sensitivity to cortisol may decline with age, persistent elevation is linked to cognitive and immune dysfunction [124]. When increased cortisol is not counterbalanced by androgens/DHEA (i.e., a high cortisol-to-androgen ratio), it fosters low-grade systemic inflammation ("inflammaging") [125]. In the lacrimal gland, chronic mild hypercortisolemia weakens immune surveillance and perturbs epithelial metabolism and function, thereby accelerating degenerative change and predisposing to chronic inflammation [39].

5.3 Role of Insulin Signaling Pathway in the Lacrimal Gland and Mechanisms in Elderly Diabetic Patients

5.3.1 Insulin Signaling and Lacrimal Gland Function

Beyond its role in glucose homeostasis, insulin functions as a trophic signal for the lacrimal gland. Lacrimal epithelial cells express insulin receptors (IR) and insulin-like growth factor-1 receptors (IGF-1R); receptor engagement activates the canonical PI3K–AKT–mTOR

pathway, supporting acinar cell survival, proliferation, and protein biosynthesis, thereby promoting tear secretion [112, 126, 127]. In animal models of lacrimal hypofunction, insulin supplementation partially restores expression of key secretory proteins and vesicle biogenesis, improving tear output [128].

5.3.2 Impact of Insulin Deficiency and Diabetes

Deficient or resistant insulin signaling compromises lacrimal structure and function [127]. In streptozotocin-induced diabetic rats, stimulus-evoked phosphorylation of the insulin receptor is impaired, indicating impaired downstream signaling in hyperglycemic or insulin-deficient states [112]. Clinically, tear-film dysfunction is a prevalent diabetic complication in older adults: epidemiologic studies report a 15–33% prevalence of dry eye in individuals over 65 with diabetes, exceeding age-matched nondiabetic rates and correlating with higher HbA1c levels [126].

5.3.3 Mechanisms of Diabetes-Associated Lacrimal Gland Dysfunction

Diabetes-associated dry eye arises from convergent neuropathic, metabolic, inflammatory, and biochemical insults [127]. Autonomic neuropathy is associated with impaired reflex tearing in diabetic patients, as evidenced by blunted tear production during hemodialysis [129]. Direct cytotoxic effects of hyperglycemia further destabilize acinar function: activation of the polyol pathway drives intracellular sorbitol accumulation, osmotic stress, and mitochondrial dysfunction, precipitating early acinar atrophy and reduced secretory capacity [126, 130].

Chronic inflammation compounds these effects. Elevated glucose stimulates release of IL-1α, IL-1β, TNF-α, and MMP-9 from lacrimal and ocular-surface epithelia, while tear hyperosmolarity amplifies epithelial injury and destabilizes the tear film [126, 131]. Advanced glycation end products (AGEs) detected in diabetic tears mirror systemic metabolic dysregulation and contribute to local inflammatory and structural damage within the gland [126].

Systemic metabolic disturbances further impair lacrimal function via insulin-signaling disruption. Pei et al. showed that db/db mice exhibit dampened circadian rhythms, increased glandular inflammation, and reduced tearing despite systemic insulin therapy, implicating local insulin resistance as a key driver [127]. High-fat diets exacerbate dysfunction by suppressing insulin–PI3K/AKT signaling, promoting lipid accumulation, oxidative stress, and fibrosis [18, 132]. In addition, Plin2 has been identified as a critical mediator linking disordered lipid metabolism to inflammatory aging in the lacrimal gland, highlighting a potential therapeutic target for metabolic-induced dysfunction [18]. Collectively, these processes accelerate structural degeneration and functional decline in diabetic patients [127].

5.3.4 Circadian Rhythm Disruption

Under physiological conditions, the lacrimal gland functions as a robust peripheral clock: in young mice its mass, tear output and hundreds of transcripts—including the core oscillators *Bmal1* and *Per2* and the secretory effector *Irs1*—exhibit high-amplitude, light-entrained 24-h rhythms [133, 134]. Aging blunts this program: temporal transcriptomics show that >60% of rhythmic genes dampen or phase-shift while oxidative-stress and inflammatory pathways rise, and systemic clock-to-clock communication weakens [41, 135]. The compromised oscillator heightens susceptibility to chronodisruption: light-cycle phase advance, high-fat feeding, or sleep loss rapidly rewire residual rhythms, increase ROS, drive immune-cell infiltration, and depress tearing [134, 136, 137]. These perturbations create a vicious cycle in which circadian disruption accelerates tissue injury and inflammation, further eroding rhythmicity. Importantly, this loop is plastic: fecal microbiota transplantation from young donors partially restores PER2 oscillations, reduces oxidative stress, and improves secretion in aged mice [4], underscoring circadian synchronization—via light, behavioral, or microbiome-targeted interventions—as a promising lever to slow lacrimal gland aging and its dry eye sequelae [138].

5.4 Impact of Age-Related Changes in Adrenally Derived Hormones on Lacrimal Gland Inflammatory Susceptibility and Metabolism

5.4.1 DHEA and Immune Modulation

Dehydroepiandrosterone (DHEA), an adrenal androgen precursor, can be converted to active sex steroids (testosterone, estradiol) and also exerts direct immunomodulatory effects. Circulating DHEA-S peaks in adolescence and declines progressively with age [139]. DHEA influences immune balance by enhancing T-cell IL-2 production and counteracting certain glucocorticoid-mediated suppressive pathways, thereby modulating cytokine profiles and innate–adaptive cross-talk [140]. Age-related loss of DHEA is therefore associated with diminished immune resilience and heightened inflammatory susceptibility.

Low DHEA-S levels correlate with dry eye burden: both elderly women with Sjögren's syndrome and older adults in the general population exhibit significantly

lower serum DHEA-S than younger controls [115, 141]. In older men, lower DHEA-S shows a modest association with worse symptoms and reduced Schirmer values [142]. These observations suggest that DHEA deficiency increases lacrimal vulnerability through loss of androgenic support and reduced immunoregulation. Trials of exogenous DHEA have yielded mixed results, underscoring the need to clarify local intracrine metabolism and receptor signaling within the lacrimal gland.

5.4.2 Cortisol and the Cortisol/DHEA Ratio

Adrenocorticosteroids, particularly cortisol, undergo age-related changes with implications for lacrimal homeostasis. Older adults often exhibit elevated basal cortisol with blunted diurnal variation [123]. In parallel, the age-dependent fall in DHEA coupled with stable or slightly increased cortisol produces a rising cortisol-to-DHEA ratio—a composite marker of pro- versus anti-inflammatory balance in aging [123, 125]. A high ratio is associated with systemic chronic low-grade inflammation ("inflammaging") and may heighten vulnerability to inflammatory processes [143]. This endocrine tilt—combining androgen deficiency with glucocorticoid-driven immune dysregulation—may contribute to the higher prevalence of autoimmune dry eye phenotypes (e.g., Sjögren's syndrome) in the elderly. In summary, the aging adrenal profile—characterized by DHEA decline and relatively increased cortisol—is associated with systemic inflammation and may represent a risk factor for lacrimal dysfunction, though gland-specific mechanistic evidence remains limited [141, 143]. Table 6 outlines the mechanisms by which systemic endocrine and metabolic factors influence lacrimal function and their trajectories with aging.

Table 6. Systemic Endocrine and Metabolic Factors Influencing Lacrimal Gland Aging.

Factor	Receptor expression & distribution	Mechanism of action	Age-related alterations & impact	Representative references*
Androgens (testosterone, DHT)	Androgen receptor (AR) abundant in acinar & ductal epithelia	Stimulate tear-protein synthesis, maintain structure, dampen inflammation, enhance meibomian lipid output	Age-/menopause-related androgen decline → ↓ tear secretion, unstable film, ↑ dry-eye risk (especially post-menopausal women)	[21, 117, 230, 231]
Estrogens (17β-estradiol, etc.)	ERα and ERβ present in gland (no strong sex bias)	Biphasic: low doses cytoprotective, high doses pro-inflammatory; modulate epithelial gene expression	Post-menopausal estrogen loss; estrogen-only HRT may worsen DED whereas combined HRT can help	[21, 232, 233]
Thyroid hormones (T3/T4)	THRβ1 in epithelia; TSH receptor on acinar cells (Graves' target)	Drive metabolic & secretory activity; auto-Abs to TSHR induce inflammatory dacryoadenopathy	Hypothyroidism and Graves' become common with age → reduced secretion or immune-mediated damage	[118, 234]
Glucocorticoids (cortisol)	Glucocorticoid receptor widely expressed in epithelial & immune cells	Acute GC dampens inflammation; chronic excess suppresses neural reflexes & remodels tissue	Blunted circadian rhythm and higher baseline cortisol in elderly → chronic low-grade inflammation, reduced basal tearing	[235]
Insulin / IGF-1	Insulin & IGF-1 receptors on acinar membranes	PI3K–Akt–mTOR signaling supports growth, survival & protein secretion	Insulin resistance / diabetes mellitus (common in elderly) → oxidative stress, neuropathy & ≈50 % higher DED prevalence	[126, 236]
Adrenal androgen (DHEA / DHEA-S)	Acts via local conversion to testosterone (AR) or directly on immune cells	Supplements local androgen pool, immunomodulation (IL-2↑, cortisol antagonism), anti-apoptotic	DHEA-S peaks at ~30 y then falls to ≤20 % by 60 y → increased susceptibility to lacrimal inflammation/atrophy; supplementation under investigation	[19, 20, 237]

* These studies or reviews documented the receptor distribution, mechanistic effects, and/or age-related consequences for each systemic factor.

5.5 Influence of Nutritional Metabolism and Lifestyle Factors on the Lacrimal Gland's Metabolic-Inflammatory Axis

Accumulating evidence indicates that modifiable lifestyle and metabolic factors—particularly cigarette smoking, metabolic syndrome, and diabetes—accelerate lacrimal gland aging and amplify inflammation [44, 127, 136, 144]. Epidemiologic and experimental studies link tobacco exposure and suboptimal diet to heightened oxidative stress and chronic ocular-surface inflammation [145]. Metabolic syndrome and diabetes are strongly associated with tear-film hyperosmolarity, localized inflammatory activation, and significantly diminished lacrimal function compared with healthy controls [146].
Data from older cohorts and aged animal models indicate that targeted lifestyle modification can attenuate age-related declines in lacrimal function [147]. Smoking is a modifiable risk factor for dry eye in older adults: smokers exhibit reduced tear-film stability and more severe symptoms [148], whereas cessation correlates with higher tear output and symptom improvement [149]. Metabolic health exerts comparable effects; older adults with metabolic syndrome show markedly reduced secretion and a higher prevalence of lacrimal dysfunction than metabolically healthy peers [150]. Among elderly patients with diabetes, poor glycemic control associates with exacerbated symptoms and signs, whereas stringent glucose regulation is protective for lacrimal performance [151].

Comprehensive programs combining structured physical activity with dietary optimization also confer benefit [147]. In older patients with dry eye, obesity, and hypertension, a six-month regimen of high-intensity interval training plus a Mediterranean diet significantly increased tear production and ameliorated clinical signs [152]. Consistent with human data, aging animal studies show that long-term caloric restriction augments tear secretion, attenuates fibrosis and oxidative injury, and preserves glandular function [44, 145].

6. Animal Models and Clinical Relevance to Lacrimal Gland Aging

6.1 Differences in Lacrimal Gland Anatomy Across Species

Substantial interspecies differences in lacrimal gland anatomy, histology, and immune architecture critically influence the selection and interpretation of animal models for aging research. In humans, the lacrimal gland resides superolaterally within the orbital lacrimal fossa and comprises a large main (orbital) lobe, a smaller palpebral lobe, and numerous accessory glands (Krause, Wolfring) in the conjunctiva [45, 153]. Histologically, it is a compound tubuloacinar gland with serous-predominant acini arranged in lobules with prominent central lumina. Epithelial cells express keratins 8/18 and aquaporins AQP1, AQP3, and AQP5. Immunologically, the human gland is enriched for sIgA-producing plasma cells, whereas mast cells are uncommon [39, 45, 154].

Rodents (mice, rats) possess an exorbital and an intraorbital lacrimal system and a Harderian gland absent in humans. Rodent acini are smaller, densely packed, and less clearly luminal, with a comparatively sparse ductal network. Keratin-8 expression is lacking, and the aquaporin profile is dominated by AQP1, AQP4, and AQP5. The immune landscape diverges as well, with fewer plasma cells, more mast cells, and pronounced sex-dependent differences in gland size and cellularity [39, 154].

In rabbits, a relatively small main lacrimal gland is accompanied by a large Harderian gland that supplies a major fraction of lipid-rich secretions—an important mechanistic distinction from humans despite histologic overlap in acinar organization [155].

Canine lacrimal anatomy and function more closely parallel the human system. Dogs have an intraorbital main gland and a nictitating membrane gland that together contribute substantial tear volume. Serous acinar structure, tear protein composition (lysozyme, sIgA), and immune complexity are highly similar to human tissue, with abundant plasma cells and robust lymphoid architecture. Aged dogs frequently develop T cell–mediated lacrimal inflammation reminiscent of human Sjögren's syndrome, supporting their use as translational models of immune aging despite shorter lifespan constraints [26, 39].

Non-human primates (e.g., macaques) most closely mirror human lacrimal gland location, development, innervation, hormonal responsiveness, and tear composition [82, 156]. Their immune repertoire—including plentiful sIgA-secreting plasma cells and regulatory T cells—further enhances translational relevance. Nevertheless, high cost, ethical considerations, and limited availability of aged subjects restrict routine deployment.

In summary, while the basic cellular organization of the lacrimal gland is conserved across mammals, salient anatomical, molecular, and immunological differences remain [154, 157]. These similarities and distinctions delineate the strengths and extrapolation limits of each model for studying human lacrimal gland aging, dry eye pathogenesis, and therapeutic interventions. Model selection should align with the mechanistic question and intended translational endpoint.

6.2 Major Animal Models for Lacrimal Gland Aging Research

6.2.1 Naturally Aged Models

Naturally aged mice (e.g., C57BL/6 or BALB/c, 18–24 months) serve as the primary reference model for physiological lacrimal gland aging. These models recapitulate key features of human aging, including acinar atrophy, stromal fibrosis, ductal ectasia with cystic change, and persistent low-grade lymphocytic infiltration—predominantly $CD4^+$ and $CD8^+$ T cells and B cells concentrated around ducts. Additional age-associated findings include reduced autonomic innervation and microvascular aging [26, 35, 41, 49]. At the molecular level, aged glands show upregulation of proinflammatory mediators (IL-1β, TNF-α, CXCL13) and senescence pathways (p16^INK4a, p21^Cip1), with concomitant downregulation of regenerative/lineage programs (Pax6, Sox9); these changes correlate with reduced tear secretion and a dry-eye phenotype [26, 39]. Strengths include high fidelity to organismal aging and compatibility with genetic or pharmacologic manipulation, whereas limitations encompass the time and cost required to age cohorts and incomplete modeling of human-specific variables (e.g., menopause).

6.2.2 Chronic Graft-versus-Host Disease (cGVHD) Models

Allogeneic cGVHD models (e.g., BALB/c recipients following bone marrow transplantation) induce immune-mediated lacrimal injury within weeks and reproduce aging-associated features, including periductal mononuclear aggregates, parenchymal destruction, oxidative stress with accumulation of 8-OHdG and 4-HNE, and activation of senescence/stress pathways (p16^INK4a, p38 MAPK) [158]. These models provide evidence consistent with the "inflammaging" hypothesis, indicating that immune dysregulation alone can accelerate lacrimal aging and impair tear secretion [159]. Strengths include rapid, reproducible pathology suitable for interventional testing; limitations include surgical complexity, a tendency toward acute, systemic involvement, and partial divergence from the tempo of physiological aging.

6.2.3 Heterochronic Parabiosis Models

Heterochronic parabiosis (HPB), which surgically joins the circulatory systems of young and aged mice, is widely used to interrogate systemic regulation of tissue aging. Its application to lacrimal gland biology was limited until recently. Scholand et al. employed HPB to test whether youthful systemic factors could ameliorate age-related dacryoadenitis [160], Contrary to a rejuvenation paradigm, young lacrimal glands developed pronounced lymphocytic infiltration and upregulation of inflammatory transcripts (*Il1b*, *Ifng*, *Ciita*, *Cxcl9*), recapitulating aged phenotypes. Flow-cytometric analyses revealed an expansion of marginal zone–like B (MZB) cells originating from aged partners, indicating chemotactic dominance of pro-aging systemic cues. Notably, aged lacrimal glands were largely refractory to rejuvenation, suggesting that local microenvironmental imprinting and structural deterioration may outweigh circulatory influences in governing inflammatory aging of the gland.

While HPB remains valuable for dissecting the actions of circulating factors, outcomes are tissue-specific and can be detrimental. For example, young blood can enhance cognition and stem-cell activity in the central nervous system [161], whereas aged blood rapidly imposes pro-aging phenotypes across multiple tissues [162, 163]. These observations indicate that pro-youthful and pro-aging signals coexist within the circulation and that organ susceptibility varies. In the lacrimal gland, HPB highlights the predominance of local inflammatory memory and architectural decline, arguing for therapeutic strategies that prioritize tissue-specific micro-environmental modulation rather than reliance on systemic rejuvenation alone.

6.2.4 Autoimmune Models

The Non-Obese Diabetic (NOD/LtJ) mouse is an established autoimmune model of Sjögren's syndrome–like dacryoadenitis affecting the lacrimal gland. NOD/LtJ mice develop early, progressive lymphocytic infiltration with ectopic follicle formation, acinar destruction, stromal fibrosis, and marked reductions in tear output, accompanied by a pronounced type-I interferon signature and Th1/Th17-skewed responses (IFN-γ, IL-17, CXCL13) [26, 164]. This model is valuable for interrogating immune-mediated gland degeneration and for screening immunomodulatory interventions, but it is constrained by the early onset of pathology, the absence of classic human autoantibodies, and the confounding influence of diabetes [164, 165].

In summary, model selection should align with the scientific objective: naturally aged mice best recapitulate physiological aging; cGVHD models emphasize immune-driven degeneration; parabiosis isolates systemic influences; and autoimmune models simulate accelerated, inflammatory damage characteristic of Sjögren's syndrome. These approaches are complementary, and interpretation must consider species differences, induction modalities, and phenotypic expression.

6.3 Extrapolation Capacity and Phenotypic Comparison of Animal Models to Human Lacrimal Gland Aging Mechanisms

6.3.1 Anatomical and Histological Comparisons

Among commonly used laboratory species, the rabbit lacrimal gland closely approximates human anatomy and histology. Rabbits possess large compound tubuloacinar glands with clear lobular septation and acini displaying well-defined luminal architecture. Tissue composition (serous/mucous balance), ductal organization, basal and reflex tearing, and parasympathetic innervation patterns are broadly concordant with humans. Consequently, rabbit studies frequently align with human observations in investigations of lacrimal degeneration, regeneration, and therapeutic response [88, 155]. Nonetheless, species-specific immune regulatory features and the non-primate status of rabbits constrain their utility for dissecting complex human immunopathology.

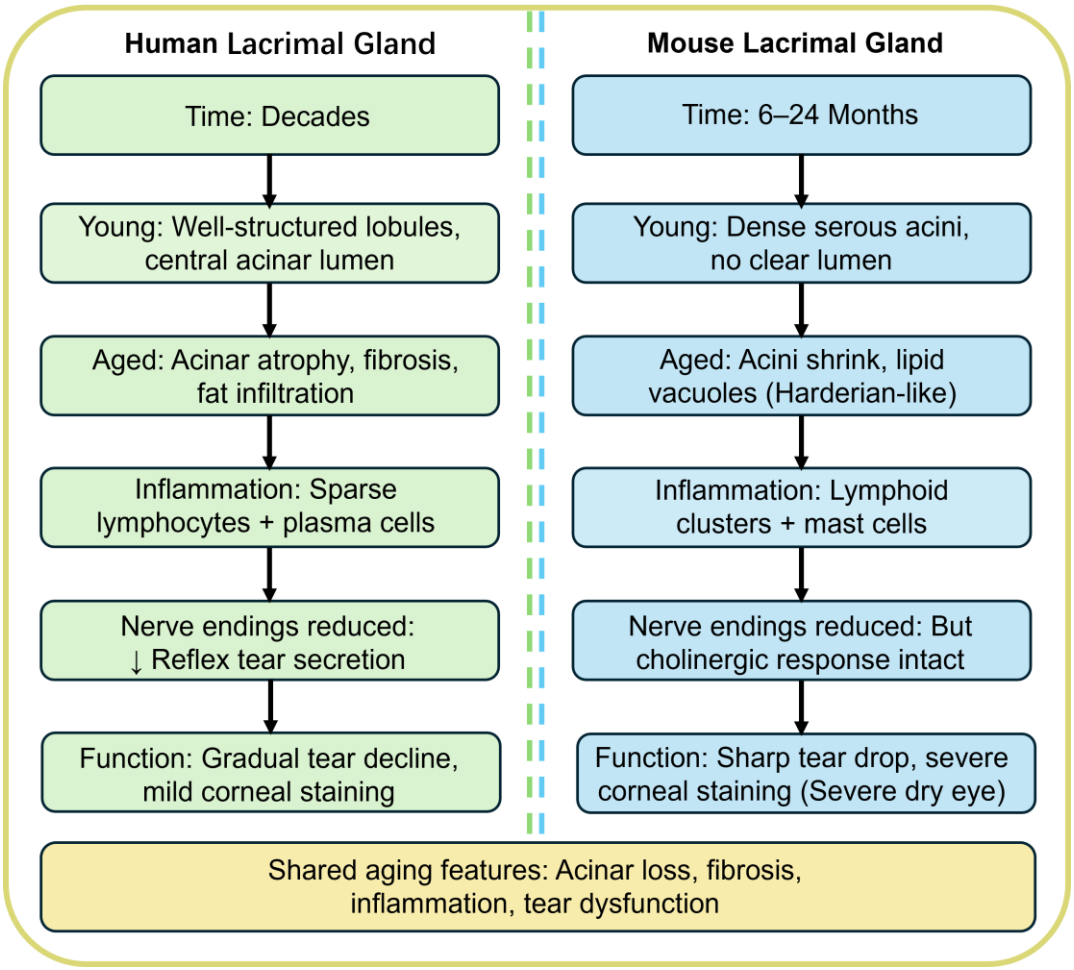

Figure 7. Comparative aging features of the human and mouse lacrimal glands. This diagram summarizes the histopathological and functional differences in lacrimal gland aging between humans and mice. The left column outlines human aging over decades, starting from well-organized lobular structures in youth to acinar atrophy, fibrosis, and fat infiltration in old age. Inflammatory changes include sparse lymphocyte infiltration and prominent plasma cells. Aging is also accompanied by reduced autonomic innervation and diminished reflex tear secretion, ultimately leading to a gradual decline in tear production and mild ocular surface staining. The right column illustrates the compressed aging timeline in mice (6 to 24 months), characterized by dense acini in youth and progressive shrinkage with lipid vacuole accumulation in aged glands—resembling Harderian gland–like changes. Inflammation in aged mice is more pronounced, with distinct lymphoid clusters and increased mast cell infiltration. Although neural decline occurs, residual cholinergic responsiveness remains detectable. Functionally, aged mice exhibit an abrupt drop in tear secretion and more severe dry eye phenotypes, including increased corneal staining. This cross-species comparison highlights both shared aging hallmarks (e.g., acinar loss, fibrosis, tear dysfunction) and model-specific features, offering insight into the translational value and limitations of mouse models for studying human lacrimal gland aging.

By contrast, rodent models (mice, rats) present greater extrapolation limits due to structural and immunologic divergence: acini are smaller and densely packed with less conspicuous lumina, the ductal network is comparatively sparse, and immune cell composition differs (fewer plasma cells, more mast cells). Rodents also harbor glands absent in humans—such as the Harderian gland in mice and rats—and rabbits possess prominent palpebral-associated glands, further complicating translation **(**Fig. 7**)** [39, 154]. Phenotypically, aged mouse glands often exhibit extensive lymphoid aggregates and sclerosing lipid deposition, whereas human glands more typically show focal infiltration with fibrofatty replacement and comparatively modest lipid accumulation. In addition, rodent glands display pronounced sex-hormone sensitivity with large sex differences in size and cellularity, challenging direct extrapolation from single-sex studies to mixed-sex human populations.

6.3.2 Cellular and Molecular Mechanisms

Despite anatomical divergence, rodents and humans share conserved molecular hallmarks of lacrimal aging: accumulation of oxidative stress; chronic inflammatory activation with increased IL-6, IL-8, and TNF-α; declining cholinergic signaling evidenced by reduced choline acetyltransferase (ChAT)–positive fibers; elevated senescence markers (p16^INK4a, SA-β-Gal); and reduced epithelial progenitor/regeneration markers (KRT5, p63, SOX9) [36, 39, 40]. These shared programs underpin the translational value of rodent studies for elucidating core mechanisms. Clinically relevant outcomes—declines in tear output and tear-film stability—are likewise reproduced in rodent aging models.

6.3.3 Histopathological and Immune Patterns

In aged mouse glands, chronic low-grade inflammation characterized by scattered lymphocytic infiltration and mild acinar atrophy parallels changes reported in human autopsy specimens, though severity is typically greater in mice owing to shorter lifespan [26]. Mouse models also recapitulate ductal cyst formation observed in older human glands, whereas extensive fibrosis and adipose replacement are less pronounced except in very old rodents—features better modeled in larger species such as dogs [39]. Autoimmune strains (e.g., NOD mice) mirror severe Sjögren's-like pathology with dense lymphoid follicles and acinar destruction, aligning more closely with pathologic than physiologic human aging [26, 35, 49].

Immune-infiltration patterns reveal both overlap and divergence. Age-related lymphocytic infiltration is predominantly T-cell–mediated in humans and rodents; however, humans retain higher numbers of sIgA-secreting plasma cells, whereas rodents exhibit lower baseline and reactive plasma-cell counts. In Sjögren's syndrome, human glands feature $CD4^+$ T cells, B cells, germinal centers, and IgG^+ plasma cells, while NOD mice display comparatively greater $CD8^+$ T-cell and cytotoxic activity [11].

6.3.4 Functional and Clinical Phenotypes

Animal models—naturally aged, cGVHD, and NOD—consistently reproduce reduced tear output and tear-film instability, recapitulating the clinical profile of human age-related dry eye [39]. In NOD and cGVHD mice, severe dryness and corneal pathology—punctate epitheliopathy, epithelial-barrier compromise, and stromal involvement—parallel findings in Sjögren's syndrome. Concordant shifts in tear composition—lower total protein, lactoferrin, and lysozyme, altered IgA, and changes in antimicrobial peptides—provide a plausible mechanistic explanation for the increased susceptibility to ocular-surface infection observed in older populations.

6.3.5 Summary and Translational Strategy

Overall, translational fidelity follows an approximate gradient of rabbit > rodents: rabbits offer closer anatomical and physiological concordance with humans, whereas rodents are indispensable for defining conserved cellular and molecular pathways [26]. A tiered validation strategy is recommended: (1) employ rodents for mechanistic interrogation and initial efficacy testing; (2) confirm anatomic and functional endpoints in larger species (rabbits, dogs); and (3) integrate evidence across models with targeted human tissue studies to optimize clinical translation. Interpretation should explicitly consider interspecies differences in anatomy, immune composition (notably plasma-cell abundance), pathology, and the extent to which each model amplifies or simplifies disease features [39].

6.4 Application Value of Animal Models in Drug Intervention, Genetic Manipulation, and Tissue Regeneration Research

6.4.1 Mouse Models: Genetic Manipulation and Mechanistic Screening

Mouse models constitute the predominant experimental platform for lacrimal gland aging owing to their genetic tractability and well-characterized inbred backgrounds.

Transgenic, knockout, and CRISPR-based approaches enable causal testing of candidate pathways and the creation of accelerated-aging or autoimmune dry eye phenotypes (e.g., MRL/lpr mice that spontaneously develop severe Sjögren's-like dacryoadenitis) [166, 167]. These systems support efficient screening of immunosuppressants, anti-inflammatory agents, antioxidants, neuroprotective compounds, and hormone-based strategies for their effects on glandular degeneration and dry eye endpoints. Murine models are also well suited for studies of tissue regeneration and gene therapy. Local delivery of recombinant growth factors or gene vectors to aged glands can partially restore architecture and function, providing proof-of-concept for translational approaches [168].

Key limitations include the small size of the murine lacrimal gland—which complicates microsurgical manipulation and tear collection—and inherent interspecies differences in anatomy, endocrine regulation, and immune composition. Consequently, promising findings in mice should be validated in larger species to refine dosing, delivery, and pharmacodynamic readouts prior to clinical translation.

6.4.2 Rat Models: Functional Assessment and Local Drug Delivery

Rat lacrimal glands are severalfold larger than those of mice, enabling precise microsurgical access, orthotopic injury modeling (e.g., duct ligation, partial excision), and targeted intraglandular drug delivery. Established injury–regeneration paradigms in rats support quantitative evaluation of structural recovery and therapeutic efficacy. The larger gland size also permits repeated tear sampling and pharmacokinetic/pharmacodynamic comparisons (e.g., topical versus systemic exposure). Owing to their larger size and more distinct autonomic neuroanatomy, rat models are well suited for probing neural regulation through targeted denervation, ganglionectomy, or electrical stimulation, and are frequently used to assess anti-inflammatory and antioxidant interventions [169]. Key limitations include a more restricted genetic toolkit and fewer immunologic reagents relative to mice, although CRISPR-based editing and viral-vector methods are expanding experimental capability.

6.4.3 Rabbit Models: Surgical Innovation and Clinical Analogue

Rabbit models are particularly valuable in ophthalmic research owing to anatomical and physiological concordance with humans (globe size, gland size, and tear production/drainage) [168]. Their relatively large lacrimal glands permit extensive microsurgical manipulation—such as partial or bilateral excision and duct ligation—facilitating robust deficiency models for evaluating tissue substitutes, grafts, and regenerative therapies [170]. Recent studies show that transient duct obstruction followed by reopening induces epithelial proliferation and partial functional recovery in rabbits, a response that is of significant interest as a model for probing reparative processes with potential relevance to human regeneration. Rabbits are also well suited for assessing surgical innovations and advanced imaging modalities. Principal limitations include higher husbandry costs, longer breeding cycles, and interspecies differences in immune systems and antibody repertoires that constrain detailed immunologic interrogation.

In summary, each animal model offers distinct strengths: mice are optimal for initial mechanistic and genetic screening; rats enable rigorous functional assessment and localized drug delivery; and rabbits provide a pragmatic platform for preclinical validation, surgical development, and regenerative studies. Strategic, complementary deployment of these models supports comprehensive mechanistic inquiry and enhances the translational trajectory of interventions for lacrimal gland aging and dysfunction.

6.5 Limitations and Future Perspectives in Modeling Human Lacrimal Gland Aging Mechanisms

6.5.1 Lifespan and Time Scale Discrepancies

The abbreviated lifespan of laboratory species—particularly rodents—limits their capacity to model the decades-long trajectory of human lacrimal gland aging. In humans, age-linked declines across multiple systems (e.g., immunosenescence, neurodegeneration) accumulate gradually and interact over years, whereas in animals these processes are condensed into months. As a result, late-stage histopathologies seen in older adults (e.g., epithelial dysplasia, hyperplastic nodules) are infrequently observed in mice, largely because the animals do not survive long enough to develop them. Moreover, an 18-month-old mouse, commonly designated "aged," is physiologically closer to a middle-aged human—a period when many age-related disorders are only beginning to emerge [35, 39, 41]. This temporal mismatch complicates cross-species comparisons and underscores the need for careful physiological age calibration, life-course modeling, and complementary use of longer-lived species when late-onset pathology is central to the research objective.

6.5.2 Immune System Differences

Substantial cross-species differences in immune composition and regulation shape lacrimal aging phenotypes. Rodents harbor fewer sIgA-secreting plasma cells and exhibit attenuated IgA responses within the lacrimal gland, potentially blunting detection of mucosal immune perturbations that are prominent in humans [11]. Human immunosenescence is marked by pronounced T-cell remodeling—expansion of effector-memory subsets alongside diminished regulatory T-cell function. While aged mice also exhibit T cell shifts, the specific patterns and drivers (e.g., the role of lifelong antigenic exposure in humans) differ, and these changes are often less pronounced in mice maintained under specific pathogen-free conditions [49]. Moreover, autoimmune dry eye in humans (e.g., Sjögren's syndrome) is polygenic and HLA-associated, whereas rodent models such as NOD mice rely on distinct susceptibility loci and do not fully recapitulate the human immunoregulatory gene set or autoantibody repertoire [164]. These disparities warrant cautious extrapolation and emphasize the need for cross-model validation in translational studies.

6.5.3 Endocrine and Metabolic Factors

Human lacrimal function is strongly modulated by endocrine status, particularly sex-steroid signaling. Postmenopausal declines in estradiol among women and age-associated androgen loss in men are each associated with reduced tear secretion and higher dry eye prevalence [21, 171]. By contrast, rodents do not undergo a true menopause and exhibit only modest age-related androgen decreases, limiting the ability of murine models to reproduce the abrupt endocrine transitions characteristic of human aging. In addition, standard laboratory mice do not spontaneously develop metabolic syndrome or type 2 diabetes—major contributors to human lacrimal dysfunction—necessitating artificial induction (e.g., diet or toxin models) for mechanistic study [39, 127].

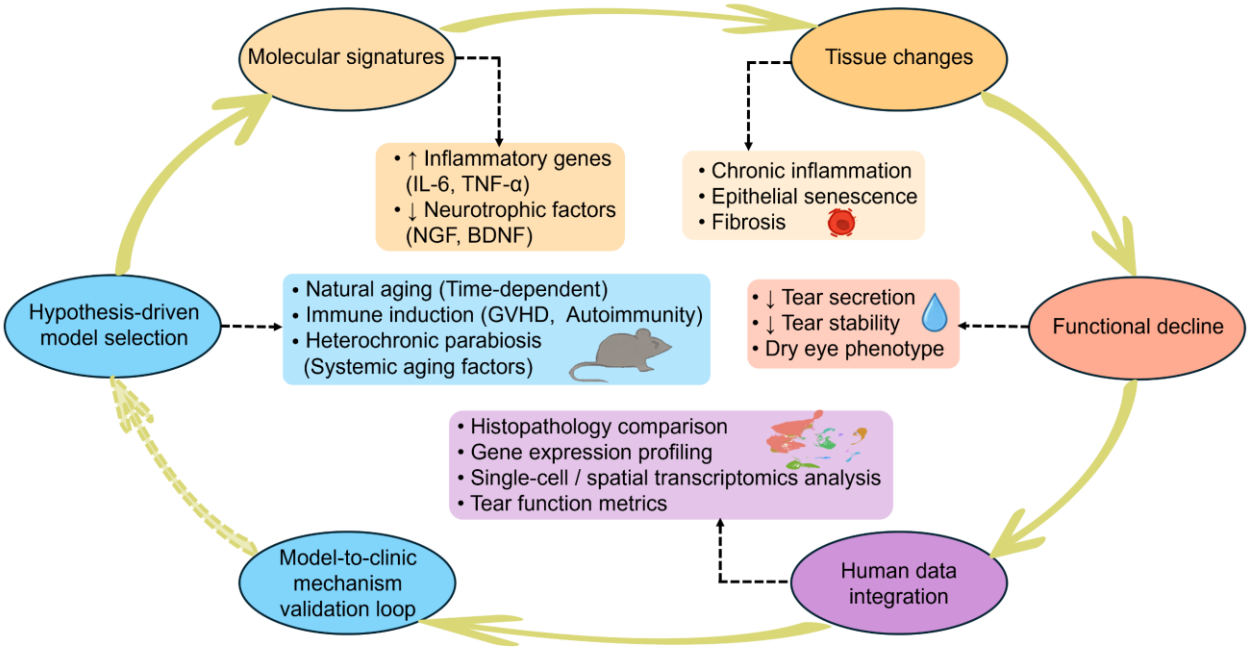

Figure 8. Experimental validation workflow for lacrimal gland aging mechanisms using animal models. This schematic flowchart outlines the methodological framework for utilizing animal models to investigate and validate mechanisms of lacrimal gland aging. Researchers select model systems based on specific hypotheses, including natural aging (time-dependent degeneration), immune-mediated induction (e.g., chronic graft-versus-host disease or autoimmune conditions), and heterochronic parabiosis to assess the influence of systemic aging factors. These models induce pathological changes in the lacrimal gland, including tissue-level alterations such as chronic inflammation, epithelial senescence, and fibrosis, accompanied by molecular signatures such as upregulated inflammatory genes and decreased neurotrophic factors. These changes result in functional decline, reflected in reduced tear production, tear film instability, and dry eye phenotypes (e.g., corneal staining, increased blink rate). Data from animal models are integrated with human clinical and histopathological data through comparative analyses of tissue structure, gene expression profiles, single-cell and spatial transcriptomics, and tear function metrics. This model-to-clinic feedback loop enables iterative refinement of aging hypotheses and validation of mechanistic targets with translational relevance, thereby bridging preclinical findings with human disease contexts.

6.5.4 Environmental and Life History Factors

Humans encounter diverse climates, airborne pollutants, pathogens, and lifestyle exposures (e.g., smoking, prolonged screen use) that shape lacrimal aging trajectories and dry-eye risk [172, 173]. By contrast, laboratory animals are maintained in controlled, specific-pathogen-free environments, yielding phenotypes that emphasize intrinsic, programmed aging while underrepresenting exogenous stressors. Genetic uniformity and standardized housing further constrain interindividual variability, limiting extrapolation to heterogeneous human populations.

6.5.5 Physical and Technical Constraints

Murine lacrimal glands are small and extraorbital/subcutaneous, complicating in vivo imaging, repeated sampling, and longitudinal functional assessment; consequently, most datasets derive from cross-sectional end-point analyses. Larger species (e.g., dogs) permit serial imaging or biopsies but are limited by cost and ethical constraints. "Humanized" platforms—engrafting human immune cells or tissues into immunodeficient mice—are emerging but remain technically demanding and prone to rejection and cross-species artifacts [39].

Table 7. Comparative Overview of Animal Models for Lacrimal Gland Aging: Features, Applications, Limitations, and Representative References.

Model type	Core features	Major applications	Key limitations	Representative ref.*
Naturally aged rodents	Gradual, spontaneous degeneration; hallmark changes include acinar atrophy, fibrosis and low-grade inflammatory infiltration	Elucidating physiological aging mechanisms; baseline comparator for anti-aging interventions	Long study duration; does not capture human menopause or metabolic co-morbidities (e.g., T2-DM)	Rocha et al., "The Aging Lacrimal Gland: Changes in Structure and Function"[36]
Auto-immune models (NOD, MRL/lpr mice)	Early-onset Sjögren-like lymphocytic infiltration and gland destruction driven by single-gene defects	Immunopathogenesis studies; immunomodulatory drug screening; modelling Sjögren's syndrome	Rapid onset; limited auto-antibody spectrum compared with humans; less suitable for idiopathic late-age disease	Rattner et al., "Normal and Sjögren's-syndrome models of the murine lacrimal gland studied at single-cell resolution" [11]
cGVHD models	Rapid immune-mediated damage with oxidative stress and periductal fibrosis	"Inflamm-aging" mechanisms; anti-fibrotic and anti-inflammatory therapy testing	Acute phenotype; requires bone-marrow transplantation; systemic confounders	Yamane et al., "Functional Role of Lacrimal-Gland Fibroblasts in a Mouse Model of Chronic GVHD" [238]
Parabiosis (hetero-/isochronic)	Shared circulation between young ↔ old mice; reveals circulating pro- and anti-aging factors	Discovery of systemic rejuvenation or pro-aging molecules; proof-of-concept for systemic interventions	Surgical stress; mixed pathology; limited direct clinical endpoints	Scholand et al., "Heterochronic Parabiosis Causes Dacryoadenitis in Young Lacrimal Glands" [160]
Rabbit models	Closer lacrimal anatomy and tear composition to humans; large gland facilitates surgery & imaging	Pre-clinical validation of drugs, tissue engineering, surgical techniques, drug-delivery devices	Higher cost & housing; longer lifespan; fewer immunological reagents	Baranauskas et al., "Rabbit Models of Dry-Eye Disease: Comparative Analysis" [239]
Dog / non-human-primate models	Spontaneous age-related or immune-mediated KCS in dogs; primates share closest ocular anatomy & aging pattern with humans	Late-stage translational testing, regenerative studies, surgical training	High cost, ethical constraints, limited availability	Yasui et al., "Secretory Epithelium in the Canine Lacrimal Gland" [240]; Gong et al., "A New Non-human-Primate Model of Desiccating-Stress-Induced Dry-Eye Disease" [82]

* Each reference substantiates the "Core features" and/or "Major applications" column for the corresponding model.

6.5.6 Future Directions and Integrative Summary

Given these limitations, strategic integration of models is essential. Naturally aged rodents remain indispensable for mechanistic discovery yet do not fully capture abrupt hormonal transitions or complex metabolic comorbidities characteristic of human aging. Autoimmune and cGVHD models elucidate immunopathogenesis and "inflammaging," though they overrepresent early-onset, high-grade inflammation. Parabiosis isolates circulatory

influences but remains largely experimental. Large-animal platforms—rabbits, dogs, nonhuman primates—offer superior anatomical and functional fidelity for surgical and regenerative studies, albeit with constrained throughput due to cost and ethics. Accordingly, translational progress is best supported by a multi-model, multi-scale pipeline: rodents for discovery → larger animals for validation → human organoids/explants for preclinical bridging. Comparative single-cell and spatial omics across these tiers will refine target selection and enhance the clinical relevance of preclinical findings (Fig. 8; Table 7).

7. Prospects for Intervention and Therapeutic Strategies

As mechanistic understanding of lacrimal gland aging advances, a broad portfolio of targeted and regenerative therapies is emerging. Defining pathological features include acinar degeneration (e.g., atrophy, ductal obstruction, fibrosis), chronic leukocyte infiltration (lymphocytes, mast cells), oxidative-stress–associated cellular injury (e.g., lipofuscin accumulation), loss of autonomic innervation, and stem/progenitor cell exhaustion [36, 49, 52]. Interventions aimed at these interlinked pathways demonstrate activity in preclinical models and, increasingly, in early-phase human studies. This section reviews contemporary approaches—anti-inflammatory, antioxidant, hormonal, neurotrophic, and regenerative—organized by their mechanistic targets within the aging lacrimal gland. To calibrate developmental stage, all modalities are classified by Evidence Stage throughout this chapter: preclinical (animal/in vitro only), feasibility (human proof-of-concept or advanced preclinical), and early-phase clinical (Phase I–II or preliminary clinical use). Figure 9 and Table 8 present these stages with color badges for rapid reference.

Table 8. Emerging Therapeutic Strategies for Lacrimal Gland Aging: Mechanisms, Targets, Key Evidence, and Clinical Stage.

Strategy	Mechanism/Target	Key evidence/recent advances*	Current clinical stage	Evidence Stage
Anti-inflammatory agents (cyclosporine A, lifitegrast, statins, mast-cell stabilizers)	Suppress T-cell, cytokine and mast-cell–driven chronic inflammation in the gland and ocular surface	• 0.1 % cyclosporine nano-emulsion improved signs in a multicentre RCT [241] • Lifitegrast OPUS-3 phase-III trial reduced symptom scores within 14 days [242] • Population studies link systemic statin use to lower DED risk and better meibomian function [243] • Aged-mouse work shows mast-cell deficiency lessens lacrimal inflammation, suggesting benefit of mast-cell stabilizers [40]	CsA & lifitegrast - approved; statins & mast-cell stabilizers - pre-clinical /repurposing	●
Mesenchymal stem-cell (MSC) therapy	Paracrine immunomodulation, anti-fibrotic signals, acinar regeneration	Double-blind RCT: allogeneic adipose-derived MSCs injected into the lacrimal gland of Sjögren patients increased Schirmer scores and reduced inflammatory infiltration [211]	Phase I–II	●
Gene therapies (HMGB1-Box A, anti-HSP47 siRNA, CRISPR concepts)	Boost anti-senescence pathways; silence pro-fibrotic or inflammatory genes	• HMGB1-Box A delivery to lacrimal-gland organoids attenuated cellular senescence and improved secretion in mice [198] • Topical vitamin-A–liposome siRNA targeting HSP47 reversed fibrosis and restored tearing in cGVHD mice [244]	Pre-clinical / proof-of-concept	●
Senolytics (navitoclax [ABT-263], dasatinib + quercetin)	Selective clearance of senescent cells and suppression of SASP	ABT-263 treatment in a cGVHD model preserved lacrimal structure and tear volume; D+Q highlighted as a general ocular senolytic strategy [245, 246]	Pre-clinical	●
Hormone replacement (androgens, estrogens, DHEA)	Restore androgen- or estrogen-dependent secretory and immune balance	• Pilot RCT of topical testosterone gel improved TBUT and symptoms in DED patients [247] • Reviews confirm androgen therapy increases tear secretion and reduces inflammation [171]	Phase II / clinical use (topical androgens); DHEA investigational	●/●

		• DHEA supplementation trials show mixed results but remain under study [171]		
Organoid or engineered-tissue transplant	Replace lost acinar epithelium and re-establish secretion	Human lacrimal-gland organoids engrafted into mouse glands survived ≥ 14 days and expressed AQP5, restoring tear output [192]	Pre-clinical / feasibility	●
Neural stimulation (TrueTear® intranasal device, NGF, electrical cues)	Enhance parasympathetic/sensory drive; promote nerve remodeling	• TrueTear RCTs (OCUN-009/-010) and meta-analysis show immediate Schirmer increases and symptom relief [248] • Recombinant human NGF promotes ocular-surface and neural repair in pre-clinical models [249]	TrueTear – approved; NGF & electrical implants – Phase II	●
Organoid-on-chip / microfluidics	In-vitro platforms recreating gland micro-environment for drug screening and mechanistic studies	Next-generation lacrimal-gland-on-chip systems replicate secretion dynamics and permit high-throughput testing of aging modifiers [250]	Pre-clinical / platform technology	●

* Key evidence cites one or more pivotal pre-clinical studies, clinical trials or reviews that substantiate the mechanism and status of each strategy. Evidence Stage definitions: ● early-phase clinical (Phase I–II or preliminary clinical use); ●feasibility (human proof-of-concept or advanced preclinical); ● preclinical (animal/in-vitro only).

7.1 Anti-Inflammatory and Immunomodulatory Strategies

7.1.1 Rationale for Anti-Inflammatory Therapy

Chronic low-grade inflammation is a defining feature of the aged lacrimal gland, characterized by increased lymphocyte infiltration and progressive secretory decline [41]. Cytokines released within this milieu can also injure parasympathetic terminals, further degrading neural regulation of tear secretion [36, 39]. Accordingly, modulation of inflammatory signaling represents a central strategy to mitigate lacrimal gland aging.

7.1.2 Key Therapeutic Approaches

Multiple pharmacologic modalities have been evaluated to attenuate persistent inflammation and immune dysregulation in aging lacrimal tissue. Topical cyclosporine A (CsA) remains the most widely used immunomodulator; by inhibiting calcineurin-dependent T-cell activation, CsA reduces inflammatory infiltration in the lacrimal gland and on the ocular surface. Numerous clinical studies have demonstrated that CsA significantly improves tear secretion and alleviates symptoms in patients with age-related dry eye, supporting its efficacy for both symptomatic relief and improvement of glandular function [108, 174].

Statins, although developed for dyslipidemia, exhibit pleiotropic anti-inflammatory and immunomodulatory actions, lowering circulating IL-6, IL-8, TNF-α, and C-reactive protein [175]. In ophthalmic contexts, statins reduce inflammation associated with meibomian gland dysfunction and partially improve dry eye symptoms [176]. Direct evidence in lacrimal gland aging is limited; nonetheless, their systemic anti-inflammatory profile suggests potential adjunctive value in selected patients.

Mast-cell–targeted therapy is biologically plausible given age-related increases in glandular mast cells. Mast cell stabilizers (e.g., sodium cromoglicate) prevent degranulation, thereby limiting histamine-driven recruitment of leukocytes and dampening inflammatory cascades. By curbing this amplification loop, such agents may indirectly preserve acinar architecture and neural function in aging glands; recent animal data support reduced immune infiltration and tissue injury with mast-cell inhibition [36, 39].

Emerging immunomodulators include integrin antagonists (e.g., lifitegrast), which inhibit leukocyte adhesion and trafficking and have demonstrated anti-inflammatory effects in clinical dry eye trials. These agents may be leveraged to address age-related lacrimal hypofunction by limiting immune-cell recruitment to the gland.

In summary, anti-inflammatory and immunomodulatory therapies target the persistent "inflammaging" state characteristic of the older lacrimal gland. By reducing immune-cell infiltration and preserving acinar and neural elements, these interventions offer the potential to slow—and in some cases ameliorate—age-associated declines in tear secretion.

7.2 Antioxidant and Senolytic Strategies

7.2.1 Rationale for Antioxidant and Senolytic Therapy

Oxidative stress is a central driver of lacrimal gland aging, with age-related accumulation of ROS promoting progressive cellular injury and dysfunction [8, 36]. Consistent with this, SOD1-deficient mice exhibit

accelerated acinar atrophy, inflammation, and secretory decline, underscoring the importance of endogenous antioxidant defenses [74, 177]. In parallel, senescent cells—defined by persistent cell-cycle arrest and a proinflammatory, tissue-remodeling SASP—accumulate with age, implicating targeted clearance (senolysis) as a geroscience-informed therapeutic avenue.

7.2.2 Representative Therapeutic Strategies

A range of antioxidant and senolytic agents has been evaluated for mitigating ROS burden and senescence-associated pathology in the aging lacrimal gland. N-acetylcysteine (NAC) replenishes intracellular glutathione and directly scavenges ROS. Clinically, topical NAC is used for filamentary keratitis and to modify tear rheology; preclinical studies indicate that sustained oral NAC lowers oxidative load in aged lacrimal tissue and improves tear-film stability. Although direct evidence specific to lacrimal aging remains limited, NAC is broadly considered supportive in dry eye management and oxidative-stress reduction [178, 179].

Melatonin exhibits potent antioxidant and anti-inflammatory activities. In aged mouse models, topical melatonin reduces IL-1β, IL-6, and TNF-α in the lacrimal gland, increases AQP5 expression, and enhances tearing [106]. Mechanistically, melatonin suppresses NLRP3 inflammasome activation via SIRT1, attenuating sterile inflammation; ex vivo studies further suggest anti-fibrotic effects in aged human lacrimal tissue [108, 180].

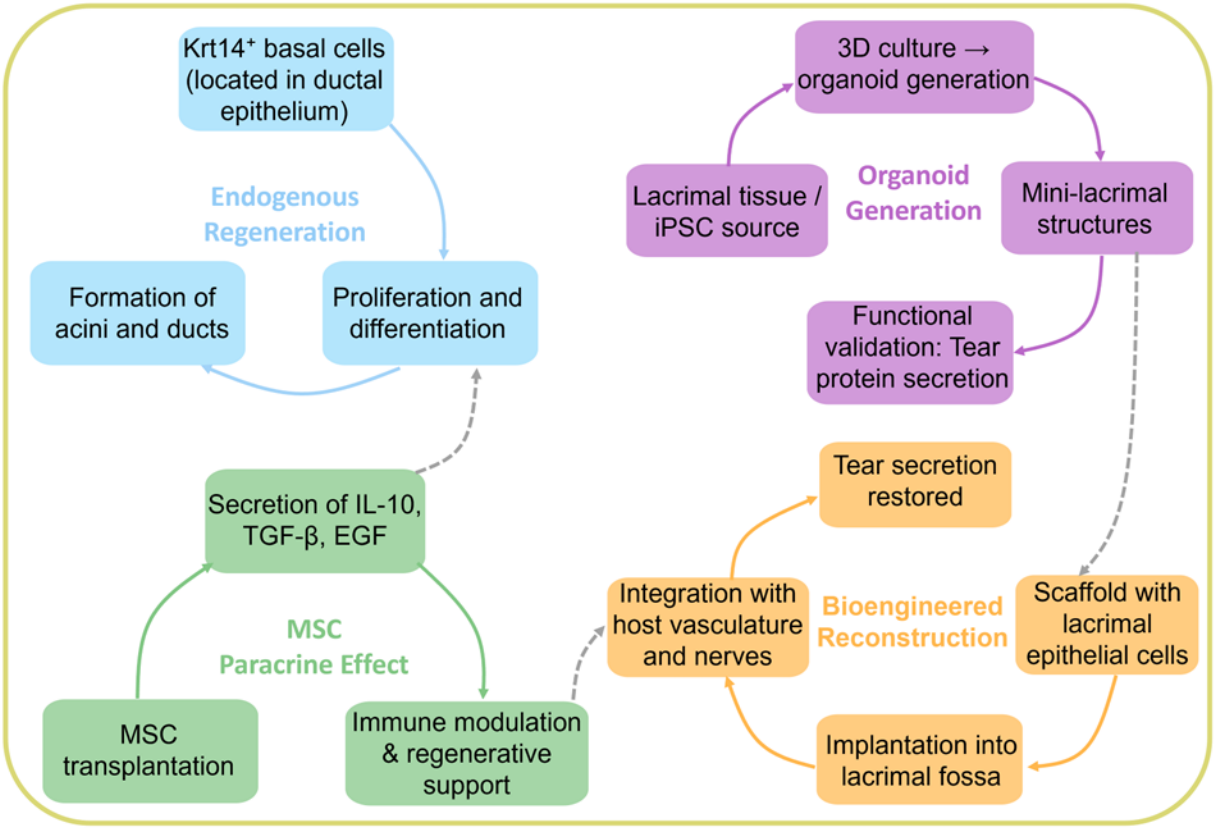

Figure 9. Integrated schematic of regenerative strategies for restoring lacrimal gland structure and function. This figure presents a comprehensive overview of current and emerging strategies for lacrimal gland regeneration. The top left module illustrates the endogenous regeneration pathway, wherein Krt14⁺ basal cells located in ductal epithelium proliferate and differentiate to regenerate acinar and ductal structures. The upper right module depicts the generation of lacrimal organoids derived from human lacrimal tissue or induced pluripotent stem cells (iPSCs), through three-dimensional (3D) culture, forming mini-glandular structures capable of tear protein secretion upon functional validation. The lower left module describes the paracrine role of transplanted mesenchymal stem cells (MSCs), which secrete immunomodulatory and regenerative factors such as IL-10, TGF-β, and EGF. These factors enhance endogenous stem cell activity and support integration of bioengineered grafts. The bottom right module highlights bioengineered lacrimal gland reconstruction: scaffolds seeded with lacrimal epithelial cells are implanted into the lacrimal fossa, where they integrate with host vasculature and innervation, eventually restoring tear production. Inter-module arrows indicate synergistic interactions, such as MSC-derived factors promoting both stem cell activation and graft integration. This unified framework underscores the multi-tiered approach toward achieving functional lacrimal gland regeneration.

Senolytic agents aim to remove senescent cell populations that propagate chronic inflammation and matrix remodeling. The BCL-2 family inhibitor navitoclax (ABT-263) decreases senescence markers and alleviates fibrosis and inflammation in cGVHD-related dry eye models, with associated structural and functional improvement at the gland level [181]. Classic combinations such as dasatinib plus quercetin (D+Q) have likewise shown activity in preclinical dry eye paradigms [182]. Conceptually, pairing senolytics with antioxidants may provide complementary benefits by reducing SASP-mediated inflammatory signaling while limiting ongoing ROS-driven damage.

In summary, antioxidant and senolytic interventions target two interlocking mechanisms of lacrimal gland aging—excess ROS and persistence of senescent cells. Agents such as NAC and melatonin protect acinar cells and the surrounding microenvironment, whereas senolytics (e.g., navitoclax, D+Q) reduce proinflammatory cell reservoirs, thereby mitigating the SASP-driven tissue damage that impedes regeneration [180]. Taken together, these strategies show promise in preclinical studies and warrant prospective evaluation in early-phase clinical trials for age-related dry eye disease.

7.3 Hormone Replacement Therapy and Neuromodulation Support

7.3.1 Hormone Replacement Strategies

Endocrine regulation is integral to lacrimal gland function, and age-related declines in sex steroids—particularly androgens—are implicated in the pathogenesis of dry eye, most notably in postmenopausal women [36]. Androgens sustain secretory activity in both the lacrimal and meibomian glands; their loss reduces aqueous and lipid output and destabilizes the tear film.

Topical androgens and local hormonal therapy. Androgen receptors are expressed on lacrimal epithelial cells, and topical or transdermal delivery has shown promise for symptom relief and tear-film stabilization. Small clinical studies report that 0.03% testosterone ointment and testosterone eye drops increase tear-film lipids and improve symptoms [171, 183]. A synthesis of six trials suggested potential benefit with topical or transdermal androgens [171]. In a double-blind randomized controlled trial, transdermal androgen gel applied to the eyelid margin significantly improved both symptoms and clinical signs in older patients without serious adverse events [184]. Although no androgen eye drop is currently approved, new formulations (e.g., testosterone nanoemulsions) are in development.

Systemic hormonal modulation. In patients with pronounced endocrine imbalance, systemic hormone replacement can be considered. A small study showed that combined estrogen plus methyltestosterone improved symptoms in postmenopausal women more than estrogen alone [185], underscoring the importance of estrogen–androgen balance. Clinical experience also supports testosterone supplementation in aging men with low levels and reduced tear secretion. Other endocrine axes (e.g., thyroid, prolactin) may influence lacrimal function. Systemic therapy should be individualized, as estrogen monotherapy may exacerbate inflammation; selective androgen receptor modulators could offer more targeted options going forward.

7.3.2 Neuromodulation and Neurotrophic Support

Parasympathetic input is essential for maintaining lacrimal secretion, yet aging reduces both neuronal populations and neurotransmitter release capacity [41]. Several neuromodulatory approaches aim to augment neural drive to the aging gland:

Pharmacologic stimulation. Muscarinic agonists such as pilocarpine and cevimeline enhance secretion by activating residual parasympathetic pathways and are widely used in Sjögren's syndrome and age-related dry eye.

Physical neurostimulation. An FDA-cleared intranasal neurostimulation device (TrueTear®) engages the trigeminal–parasympathetic reflex arc and produces rapid, clinically meaningful increases in tear production with supportive symptom improvements in randomized trials and a meta-analysis. These effects are predominantly immediate/short term, and evidence for durable structural benefit remains limited; market availability should be verified at the time of use.

Neurotrophic factors. Preclinical studies indicate that delivery of neurotrophic agents such as nerve growth factor (NGF) can promote remodeling of the lacrimal neural network and enhance secretory function [39, 186].

Combining neuromodulatory modalities with hormonal therapy may yield synergistic gains by improving both gland sensitivity and reflex-driven output, thereby addressing the dual liabilities of endocrine imbalance and neural decline characteristic of glandular aging.

In summary, hormone replacement and neuromodulation provide complementary mechanisms to sustain lacrimal gland health in older adults. Androgen supplementation helps preserve glandular architecture and secretory potential, whereas neural stimulation and trophic support enhance dynamic, reflex-mediated tearing. Together, these strategies constitute a

multifaceted and evolving framework for preventing and treating age-related dry eye disease.

7.4 Lacrimal Gland Regenerative Potential and Stem Cells

7.4.1 Lacrimal Gland Stem/Progenitor Cell Subpopulations and Their Characteristics

Recent work indicates that adult lacrimal gland tissue harbors multiple epithelial stem/progenitor subpopulations with regenerative capacity, each defined by distinct molecular markers and functional attributes [10]. Early signals of structural and functional rescue have been reported in small-animal studies (e.g., organ-bud transplantation, MSC-assisted regeneration), but the evidence remains preliminary and scale-limited. Accordingly, these approaches are categorized as experimental/preclinical, and larger, controlled studies with standardized functional endpoints are needed before clinical translation.

KRT14$^+$ ductal basal cells. Basal ductal cells enriched for KRT14, KRT5, and p63 reside along the ductal epithelium [187]. Single-cell sequencing and lineage-tracing studies show that KRT14$^+$ basal cells possess multipotent capacity, generating ducts, acini, and myoepithelial cells [10, 168]. Many co-express c-Kit and exhibit slow-cycling, label-retaining behavior, with injury-induced proliferation and differentiation.

LGR5$^+$ putative stem cells. LGR5, a canonical Wnt target, marks adult stem cells in multiple epithelia. Transcriptomic profiling detects Lgr5 and Axin2 in lacrimal tissue and organoids, implicating a Wnt-responsive LGR5$^+$ compartment analogous to other exocrine glands [77, 168]. Definitive localization and function within the lacrimal gland will require genetic lineage tracing.

SOX9$^+$ progenitor population. SOX9, essential for branching morphogenesis, is expressed in adult ductal epithelium and maintains a progenitor-like state that supports regeneration after injury [10]. Loss of SOX9 impairs branching and regenerative potential, underscoring its role as a ductal progenitor marker [168].

Additional progenitor markers. Other immature epithelial populations in human and rodent glands are identified by ABCG2, Sca-1, CD34, and c-Kit [188]. Flow cytometry has delineated c-Kit^dim/ EpCAM$^+$/Sca-1$^-$ progenitors capable of forming acinar and ductal structures in three-dimensional culture; upon transplantation into injured glands, these cells integrate and support structural and functional recovery [189].

Collectively, the adult lacrimal gland contains several epithelial progenitor pools—regulated by discrete signaling pathways—that confer intrinsic regenerative potential. Defining the signals governing their maintenance, activation, and lineage output will be critical for advancing regenerative strategies for age-related gland dysfunction. While basic and preclinical studies have characterized candidate stem/progenitor subsets and their reparative capacities, early translational efforts are only beginning to leverage these insights for human lacrimal gland disease, as detailed below.

7.4.2 Application of Organoid Technology in Lacrimal Regeneration Research

Organoid technology provides advanced in vitro models and enables translational exploration of lacrimal regeneration [168]. Lacrimal organoids—three-dimensional, self-organized "mini-glands" derived from adult stem/progenitor cells or human induced pluripotent stem cells (iPSCs) under 3D culture—recapitulate key architectural, molecular, and functional attributes of native tissue [168, 190].

Foundational work by Jeong et al. established protocols for generating human lacrimal organoids in Matrigel-based matrices. These organoids robustly express hallmark markers (AQP5, lysozyme, lactoferrin, PIP), resemble native histology, and respond to cholinergic stimulation (e.g., pilocarpine) with Ca^{2+} influx, granule exocytosis, and secretion of tear proteins including lysozyme, lactoferrin, and tear lipocalin [168, 191-194]. Essential determinants of organoid formation and maintenance—such as EpCAM and the transcription factor Pax6—have been identified [168]. Complementary work from Clevers and colleagues shows that human lacrimal organoids preserve a native-like transcriptome and neurotransmitter responsiveness during long-term culture [15]. Researchers have adopted the organoid swelling assay (termed the "crying assay") as a functional readout; however, current literature indicates this method lacks standardized protocols across laboratories and remains untested in aged organoid models [16]. In parallel, a magnetic 3D-bioassembly platform supports higher-throughput lacrimal organoid generation and screening, operationalizing "crying" phenotypes for drug discovery in DED [195]. These advances build directly on the first human lacrimal organoid report demonstrating long-term culture, neurotransmitter-evoked swelling/tear secretion, and orthotopic engraftment capacity [15].

To address limited donor tissue, staged differentiation protocols now derive lacrimal organoids from iPSCs that express characteristic markers (e.g., AQP5) and exhibit core secretory functions [109, 168, 193, 194]. Related innovations—such as magnetic 3D bioprinting—enable rapid assembly of complex, multilineage glandular constructs in salivary systems, foreshadowing analogous progress for lacrimal tissue engineering [195, 196].

The primary translational promise of lacrimal organoids is regenerative replacement. In preclinical studies, transplanted organ buds or human lacrimal organoids survive, integrate with host vasculature and nerves, retain neurotransmitter responsiveness, and ameliorate dry eye phenotypes, suggesting a capacity to enhance tear secretion and improve ocular-surface health [15, 197]. Key barriers to clinical translation remain, including reproducible graft positioning and fixation, mitigation of immune rejection (e.g., patient-specific iPSC-derived organoids), and long-term safety; proposed solutions include incorporation of genetic safety switches [197, 198].

Beyond replacement therapy, organoids serve as versatile platforms for disease modeling, aging simulation, high-throughput drug screening, and optimization of regenerative protocols. Induction of senescence-like states or application of bioprinting and microenvironmental cues accelerates preclinical testing of candidate agents and delivery strategies [198].

In summary, organoid technology is advancing the field from proof-of-concept studies toward functional tissue reconstruction and translational application in lacrimal regeneration. Organoid-derived and organ-bud constructs have demonstrated engraftment and preclinical tear-secretion readouts in rodents; however, validation in larger animals, durability of function, neural/ductal integration, and immune compatibility remain open challenges. Accordingly, these approaches should be regarded as emerging, preclinical strategies under active investigation that require larger, controlled studies with standardized endpoints before clinical adoption.

7.4.3 Application of Stem Cell and Regenerative Medicine Therapies in Lacrimal Gland Repair

Stem cell–based approaches—particularly those employing MSCs—show promise for repairing aged or injured lacrimal glands and restoring tear secretion. Therapeutic activity appears to be mediated predominantly by paracrine mechanisms rather than durable engraftment, with key effects on epithelial and stromal regeneration and on local immune modulation [168, 199].

Regenerative Effects: MSCs upregulate reparative gene programs (e.g., EGF, FGF2, BMP7), stimulate proliferation of residual epithelia, and facilitate stromal remodeling, yielding increased acinar number and improved glandular architecture [168]. Functionally, MSC administration elevates secretory markers (lysozyme, AQP5/AQP4), myoepithelial (α-SMA) and ductal-basal (Krt5) signatures, and Ki-67, consistent with active proliferation and recovery of secretory capacity [200].

Immunomodulatory Actions: MSCs attenuate chronic inflammation by reducing lymphocyte and macrophage infiltration, downregulating proinflammatory cytokines (TNF-α, IL-1β, IFN-γ, IL-17A), and increasing anti-inflammatory IL-10, thereby creating a microenvironment conducive to repair [168, 200]. Increased AQP5 expression may contribute to both fluid secretion and immunoregulation. Cell-tracking studies indicate that transplanted MSCs are largely transient; observed benefits are attributed primarily to secreted bioactive factors [168].

Clinical Translation: MSCs can be sourced from bone marrow, adipose tissue (ADSCs), or the lacrimal gland. ADSCs are attractive for accessibility and ex vivo expansion and have progressed to early clinical evaluation [168]. In a Phase I study, allogeneic ADSC suspensions injected into lacrimal glands of patients with severe dry eye yielded significant improvements in symptoms, tear osmolarity, TBUT, Schirmer scores, and ocular-surface staining without serious adverse events [17, 201]. Pilot trials of topical MSC-derived preparations have likewise reported encouraging signals. A first-in-human study using umbilical-cord-derived MSC eyedrops increased tear secretion, improved tear stability, lowered inflammatory mediators (e.g., IL-6, IL-17A), and enhanced MUC5AC production with effects sustained up to one year [201]. Although autologous lacrimal-derived MSCs avoid alloimmunity, stem-cell function may be compromised in older donors, supporting allogeneic ADSCs as a practical alternative [202].

iPSC-Based Regeneration: platforms enable generation of large numbers of lacrimal epithelial derivatives. Guided differentiation protocols that recapitulate ocular embryogenesis yield organoids containing acinar, ductal, and myoepithelial populations, expressing AQP5 and producing core secretory enzymes [193, 203]. In vivo transplantation of iPSC-derived lacrimal cells remains to be achieved; nonetheless, autologous iPSC strategies represent a future path toward "gland regeneration" with immune compatibility.

In summary, regenerative medicine strategies—MSC paracrine therapy and iPSC-based cell replacement—offer mechanistically informed solutions to cell loss and reparative failure in the aging lacrimal gland. Preclinical and early clinical data are promising, and ongoing advances in bioengineering may shift care from tear supplementation toward restorative approaches that target the underlying gland dysfunction. However, current studies are heterogeneous and small; rigorous randomized trials are required to establish efficacy and durability. An integrated schematic of regenerative modalities for structural and functional rescue of the lacrimal gland is presented in Figure 10.

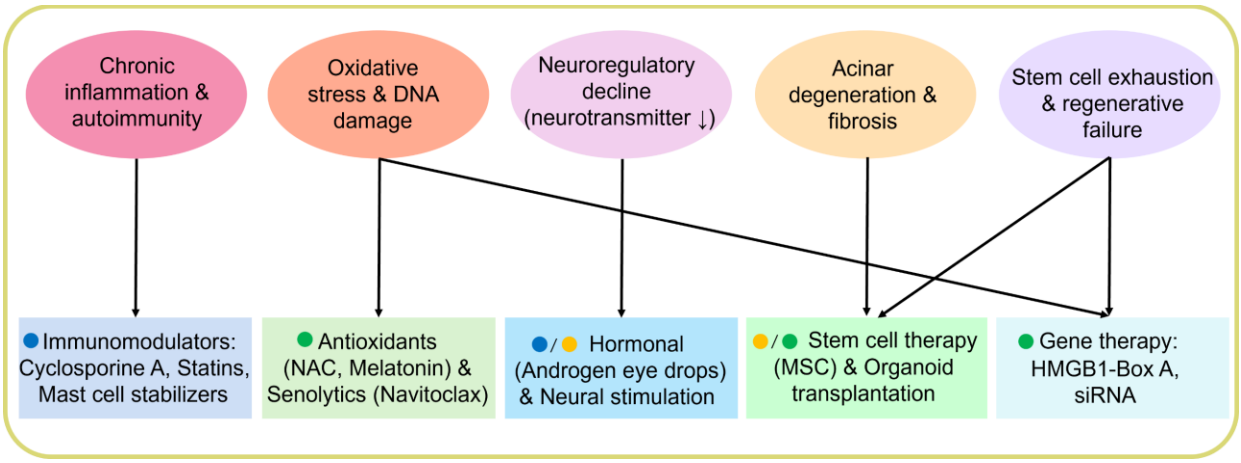

Figure 10. Integrated framework of aging mechanisms and therapeutic targets in the lacrimal gland. This schematic summarizes the multifactorial pathophysiology of lacrimal gland aging and maps corresponding therapeutic strategies to their primary mechanistic targets. At the top of the graph, key aging-related processes include acinar atrophy and fibrosis, chronic inflammation with autoimmune features, oxidative stress and DNA damage, neuroregulatory decline (reduced innervation and neurotransmitter release), and stem cell exhaustion with impaired regenerative capacity. The lower part of the figure displays emerging interventions aimed at these mechanisms: (1) Immunomodulators such as cyclosporine A, statins, and mast cell stabilizers to suppress inflammatory infiltration and cytokine overproduction; (2) Antioxidants (e.g., NAC, melatonin) and senolytics (e.g., navitoclax) to mitigate oxidative damage and eliminate senescent cells; (3) Hormonal and neural stimulation therapies to counteract neuroendocrine decline; (4) Regenerative approaches using stem cells and lacrimal organoids to restore structural integrity and function; and (5) Gene-based therapies (e.g., HMGB1-Box A, siRNA) targeting intracellular stress and senescence pathways. The framework emphasizes a mechanism-guided, multi-modal treatment paradigm for age-related tear dysfunction and dry eye disease. Evidence Stage definitions: ● early-phase clinical (Phase I–II or preliminary clinical use); ● feasibility (human proof-of-concept or advanced preclinical); ● preclinical (animal/in-vitro only).

7.4.4 Strategies for Constructing Bioengineered Lacrimal Glands

The overarching goal of lacrimal bioengineering is to design and assemble transplantable, structurally coherent, and functionally competent glandular tissue for cases of severe atrophy or injury in which endogenous regeneration is inadequate [168, 204]. This objective extends beyond self-organizing organoids or isolated stem-cell transplantation, focusing on engineered constructs that recapitulate native physiology.

Scaffold Material Design and Selection. Biomaterial scaffolds are foundational, providing an extracellular-matrix (ECM)–like niche that supports cell adhesion, proliferation, differentiation, and spatial organization [109, 168, 204, 205]. (1) Natural Polymers (e.g., collagen, fibrin, hyaluronic acid, alginate) offer biocompatibility and biomimicry but can exhibit batch variability, immunogenicity, and limited mechanical robustness. (2) Synthetic polymers (e.g., PLGA, PCL) enable tunable mechanics and degradation kinetics with high porosity for diffusion and vascularization, though they are typically less bioactive than natural matrices. (3) Decellularized ECM (dECM). Tissue-derived dECM—such as small-intestinal submucosa (SIS) or lacrimal tissue itself—retains tissue-specific composition and ultrastructure. Combining SIS dECM with Matrigel enhances spheroid viability and differentiation marker expression (e.g., AQP5), while lacrimal dECM coatings more precisely reproduce the native microenvironment.

Structured Construction Strategies. Bioengineering emphasizes controlled tissue assembly rather than passive self-organization. (1) Organ-bud reassembly: Guided by developmental biology, embryonic epithelial and mesenchymal rudiments are recombined in vitro to generate "organ buds." After orthotopic implantation, these buds have the potential to mature, establish vascular and neural connections, promote tear secretion, and protect the cornea [109, 197]. Constraints include cell sourcing and scalability. (2) Scaffold-mediated 3D assembly: Lacrimal progenitors or stem-cell–derived epithelia are seeded onto pre-designed 3D scaffolds (e.g., porous sponges, fiber meshes, microcarriers). Optimizing cell density, co-culture with MSCs (to promote angiogenesis), and application of defined biophysical/chemical cues guide alignment and self-organization into miniature glands with acinar–ductal architecture [109, 168, 204]. (3) 3D Bioprinting: Computer-aided design with layer-by-layer deposition of cell-laden bioinks enables precise, zoned patterning. Although fully bioprinted lacrimal glands remain rare, analogous salivary models achieving complex multicellular organization and functional regeneration highlight the feasibility of physiologically mimetic lacrimal bioprinting [109, 196].

Challenges and Prospects for In Vivo Integration. Key translational hurdles include [109, 168, 192, 195]: (1) Positioning and Fixation: precise placement and anchoring within the lacrimal fossa, favoring bioresorbable scaffolds that balance initial mechanical support with timely degradation. (2) Neurovascular Integration: rapid perfusion and establishment of autonomic innervation to confer secretory responsiveness; strategies may incorporate genetic cues (e.g., VEGF, NGF expression) or co-transplantation of vascular/neural precursors. (3) Immune compatibility and durability: minimizing rejection (autologous iPSC sources or immunoisolation) and sustaining function in inflamed niches. (4) Functional drainage: engineering patent ductal connections for reliable delivery of tears to the conjunctival sac.

In summary, successful construction of bioengineered lacrimal glands will require coordinated advances in materials science, stem-cell biology, developmental engineering, biofabrication, and gene editing. Through optimized scaffolds, advanced bioprinting, and pro-integration/immune-modulatory strategies, future implants may achieve durable functional rescue. At present, evidence remains preclinical; further optimization of cell sourcing and lineage fidelity, surgical delivery and fixation, neurovascular and ductal integration, long-term durability, and safety is needed before rigorously controlled clinical trials can establish curative potential.

7.5 Gene Editing and Expression Modulation Strategies

7.5.1 Gene Therapy: HMGB1-Box A as an Anti-Aging Factor

Preclinical studies identify HMGB1-Box A—the N-terminal domain of high-mobility group box 1—as an antagonist of HMGB1-driven proinflammatory signaling and a candidate senescence-modulating transgene. In lacrimal organoids, plasmid-mediated Box A expression attenuated DNA-damage–induced senescence and preserved more youthful transcriptomic, proteomic, and metabolomic profiles [198, 206]. In aged rodent models, administration of HMGB1-Box A plasmids partially re-established youthful molecular features, further supporting target plausibility [198]. Mechanistically, Box A has been proposed to induce "Youth-DNA-GAPs"—transient DNA nicks that activate repair pathways and could contribute to genome stability—although the generality and safety of this mechanism require independent validation [198, 206]. At present, evidence is limited to organoid and rodent systems; no human clinical data are available.

Key translational hurdles include vector selection and design (nonviral plasmids versus viral vectors), efficient and cell-type–specific targeting of acinar, ductal, and myoepithelial compartments, route and dosing (e.g., intraglandular or ductal delivery), control of expression level and duration, biodistribution and off-target exposure, immunogenicity, and long-term safety/oncogenicity. Overall, HMGB1-Box A has shown senescence-attenuating activity in lacrimal organoids and aged rodents, but advances in vector engineering, targeting specificity, and safety gating are prerequisites to human translation [198, 206].

7.5.2 RNA Interference (siRNA/ASO) for Modulating Senescence Pathways

Small interfering RNAs (siRNAs) and antisense oligonucleotides (ASOs) enable sequence-specific silencing of pro-aging and profibrotic drivers in lacrimal tissue. In cGVHD mouse models, intraglandular delivery of anti-HSP47 siRNA using retinoid-conjugated lipid nanoparticles significantly diminished collagen deposition and structural injury, indicating effective antifibrotic action in vivo [207, 208]. Beyond matrix targets, suppressing core senescence and inflammatory nodes—such as NF-κB, p16^INK4a, and p21^Cip1—seeks to reduce the senescent-cell burden, attenuate SASP output, and improve the health of the glandular microenvironment.

The principal obstacle to translation is efficient, cell-type–directed delivery within the lacrimal gland. Approaches under evaluation include nanocarrier systems for intraglandular administration and ductal cannulation to achieve localized, high-concentration exposure while limiting off-target distribution [209]. Continued advances in ophthalmic drug-delivery platforms—together with optimization of dosing, durability, and safety—will be critical for moving siRNA/ASO therapeutics toward clinical application.

7.5.3 CRISPR-Based Gene Editing

Although direct application to lacrimal gland aging has not yet been reported, CRISPR-based platforms offer a rational means to correct or modulate pathogenic programs. Candidate strategies include editing epithelial or immune compartments to attenuate proinflammatory signaling (e.g., CRISPRi/CRISPRa of cytokine and SASP nodes), repairing degeneration-linked mutations, or disabling senescence-promoting pathways via targeted gene knockout. Precision editors—such as base and prime editors—could refine these interventions by minimizing double-strand breaks and reducing the risk of insertion–deletion mutations.

Given the technical constraints of in vivo editing, an ex vivo paradigm is likely the most tractable near-term approach: patient-derived lacrimal epithelial cells, progenitors, or iPSC-derived organoids can be edited, expanded, and re-introduced as an autologous "cell drug" with enhanced secretory, regenerative, or anti-inflammatory properties. This framework parallels adoptive cell therapies and allows rigorous quality control (on-/off-target profiling, clonality, and potency assays) prior to implantation. For eventual in vivo translation, tissue-directed delivery (e.g., ductal cannulation, hydrogel-mediated localization) and safer editors (high-fidelity Cas variants, inducible systems, self-limiting expression) will be essential, alongside embedded safety gates (suicide switches) to mitigate long-term risk.

7.5.4 Integrative Strategies and Future Directions

Gene-based interventions—whether by augmentation (e.g., HMGB1-Box A), silencing (siRNA/ASO), or editing (CRISPR)—are likely to achieve greatest impact when combined with cell and tissue-engineering modalities. Lacrimal organoids provide a scalable preclinical platform to nominate, screen, and validate genetic targets; optimize vector choice and delivery route; and quantify functional rescue before progression to animal or clinical studies [198, 206, 208]. With continued advances in gene-therapy vectors (e.g., AAV and refined nonviral systems) and safety features (tissue-restricted expression, inducible control, and improved off-target profiling), gene-directed therapeutics for lacrimal gland aging represent promising candidates for future early clinical evaluation as components of integrated, multimodal treatment strategies. Robust dose finding, biodistribution assessment, immunogenicity monitoring, and long-term follow-up will be essential to translate these approaches responsibly.

7.6 Translational Perspectives

Regenerative approaches have advanced to early human evaluation, with some of the most clinically progressed efforts centered on MSC-based therapies. In a pilot study, patients with non-Sjögren and Sjögren aqueous deficient dry eye received twice-daily umbilical cord–derived MSC eyedrops for two weeks. This treatment was associated with significant improvements in tear production, tear film stability, and symptoms. These improvements were accompanied by reductions in tear IL-6 and IL-17A and increased MUC5AC. No serious adverse events were reported [168, 201, 210].

Early phase clinical trials (e.g., MESADDE, AMASS) employing adipose derived MSCs delivered by intraglandular or conjunctival routes in Sjögren syndrome have shown good tolerability and signals of enhanced tear production, although efficacy requires confirmation in larger randomized controlled studies [210, 211].

Beyond cell therapy, novel pharmacotherapeutics are advancing. Lacripep, a synthetic lacritin mimetic, has demonstrated safety and clinical benefit in primary Sjögren-associated dry eye, improving ocular-surface signs and symptoms and supporting epithelial and neural repair potential [212]. Reproxalap, a small-molecule scavenger of reactive aldehyde species, recently met endpoints in a phase III chamber study, significantly reducing symptom burden and supporting regulatory progression [212].

Key challenges include the scarcity of RCT-level evidence, variability in MSC sourcing, manufacturing, and delivery, uncertainties surrounding long-term safety, and the paucity of robust biomarkers. To address these gaps, future efforts should prioritize multicenter pivotal RCTs; standardized MSC production, release criteria, and dosing; mechanism-oriented multi-omics profiling before and after treatment; rational combination regimens that integrate regenerative and anti-inflammatory agents; and development of tear-based biomarkers for patient stratification, pharmacodynamic monitoring, and mechanistic insight.

Summary

Emerging interventions for lacrimal gland aging are increasingly anchored in mechanistic insight and translational methodology. Anti-inflammatory therapies such as cyclosporine A and lifitegrast are clinically supported by preliminary data for reducing chronic immune-mediated gland damage. Mesenchymal stem cell–based approaches, now in phase I–II evaluation, report improvements in tear secretion and reductions in inflammatory indices through paracrine and regenerative effects. Gene- and senescence-targeted strategies—including senolytics and nucleic-acid–based therapies directed at fibrosis and persistent inflammation—demonstrate robust activity in preclinical models, while CRISPR-based editing is an emerging avenue for durable pathway modification.

Hormone replacement, particularly androgen supplementation, addresses endocrine contributions to lacrimal dysfunction and has shown benefit in selected trials. Organoid transplantation and gland-on-a-chip platforms provide tractable systems for tissue replacement and drug discovery, whereas neural stimulation devices and neurotrophic factors enhance secretion by engaging and restoring reflex circuitry.

Collectively, these modalities represent a concerted effort to shift from symptomatic care toward

mechanism-directed, multi-level regeneration with the goal of achieving disease modification. Successful clinical adoption will require sustained translational progress, standardized platform validation, and development of patient-specific treatment strategies.

The integrated framework linking aging mechanisms, therapeutic targets, and interventions is shown in Figure 10. Table 7 synthesizes current and emerging options across anti-inflammatory drugs, cellular and genetic therapies, tissue engineering, and neuromodulation. In combination with advances in organoid and stem-cell engineering, these approaches may advance care beyond palliation toward disease-modifying, and potentially restorative, therapies for age-related lacrimal gland dysfunction.

8. Conclusions and Future Perspectives

Age-related lacrimal gland failure arises from the convergence of acinar attrition, persistent low-grade inflammation, neurosecretory decline, oxidative injury, and progenitor exhaustion. These processes collectively diminish tear volume and alter composition, precipitating aqueous-deficient dry eye. Evidence from human biobank specimens and murine, rabbit, and nonhuman primate studies indicate a conserved aging axis centered on cellular senescence, metabolic reprogramming, and sustained immune activation; translation of these insights into effective therapies remains limited.

Multi-omics resolution of cellular crosstalk. Single-cell and spatial multi-omics resolve transcriptomic, epigenetic, and metabolomic heterogeneity across epithelial, immune, neural, and stem/progenitor compartments at subcellular resolution, revealing previously unappreciated drivers of decline and clarifying the temporal order of degenerative events. Coupled with in situ lineage tracing, these datasets should refine therapeutic windows and cell-specific targets for intervention.

Next-generation modeling platforms. Patient-derived organoids integrated with organ-on-a-chip microfluidics and perfused bioreactors recapitulate human lacrimal gland architecture, extracellular-matrix context, and mechano-osmotic stress. These hybrid systems support high-throughput pharmacologic and CRISPR screens with physiologically relevant readouts—secretion flux, barrier integrity, and neuro-immune signaling—thereby narrowing the preclinical-to-clinical gap. The crying assay offers a potential quantitative endpoint for secretagogue testing; however, cross-laboratory protocol harmonization and validation in aged organoid models remain critical unmet needs [16].

Regenerative and genome-editing strategies. Advances in xeno-free media, GMP-grade iPSCs, and nonviral CRISPR base editing are accelerating production of transplantable acinar organoids and enabling in situ correction of age-exacerbated mitochondrial or secretory-pathway defects. Immediate priorities include harmonized release criteria, immune-evasive scaffolds to support durable engraftment, and adequately powered early-phase trials.

Emerging focal vistas. (i) Immune–circadian coupling: phase-shifted clock-gene oscillations in aging immune subsets heighten NF-κB activity and exacerbate nocturnal tear instability; resetting rhythmicity through time-restricted feeding, controlled light exposure, or REV-ERBα agonists has the potential to improve acinar bioenergetics and may inform chronotherapy-guided dosing of secretagogues or anti-inflammatory agents. (ii) Senolytic-guided precision therapy: a tear-detectable SASP signature (MMP-3, CXCL8, HMGB1) could support liquid-biopsy–based stratification for targeted senolytics; intermittent clearance of p16^INK4a-positive cells represents a promising strategy to ameliorate neuro-immune crosstalk and potentially enhance basal tear flow, motivating biomarker-driven adaptive trials. (iii) Smart bio-scaffolds: 4D-printed, enzymatically degradable hydrogels that release NGF and VEGF in response to mechano-osmotic cues promote re-innervation, angiogenesis, and organoid engraftment; integration of patient-specific iPSC-derived organoids could enable "gland patches" with durable functional rescue.

Together, integrating high-resolution multi-omics, biomimetic modeling, targeted senolytics, and responsive biofabrication may enable durable restoration of lacrimal gland function and tear-film homeostasis, with the potential to improve vision-related quality of life in older adults.

Authors' Contributions

Z.L. conceived and designed the study. X.P. conducted a comprehensive literature review and primarily drafted the manuscript. Z.L. meticulously edited the final manuscript for linguistic and scientific accuracy. All authors rigorously reviewed and approved the final manuscript for publication.

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Competing interests

The authors declare that they have no competing interests.

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