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Human Lacrimal Gland Regeneration - Present Status

Vimal Kishor Singh[1], Uttkarsh Kumar Sharma Vaksh[2], Pallavi Sharma[3]

[1]Associate Professor, Dept. of Biomedical Engineering, Amity school of Engineering and Technology, Amity University, Haryana, India

[2]Research Assistant/PhD scholar, Tissue Engineering and Regenerative Medicine Research Lab, Dept. of Biomedical Engineering, Amity School of Engineering and Technology, Amity University, Haryana, India

[3]PhD Scholar, Tissue Engineering and Regenerative Medicine Research Lab, Dept. of Biomedical Engineering, Amity School of Engineering and Technology, Amity University, Haryana, India

Corresponding author Email: *vksingh[at]ggn.amity.edu*,
vim_kissor[at]yahoo.co.in
vimalkishorsingh[at]gmail.com

Abstract: ***The human lacrimal gland is an integral part of the lacrimal functional unit. It is an established component of the secretory immune system. Any changes/interruption in LG function results in the manifestation of an ocular surface disease such as dry eye disease. Despite a high prevalence, the current therapy for dry eye is not promising and lifelong. Advanced treatments have shown to be of limited success. This review gives an overview of the lacrimal gland anatomy, physiology and its role in establishing homeostasis. It also summarizes the current research and various treatments modalities in lacrimal gland regeneration and/or reconstruction.***

Keywords: lacrimal gland, dry eye, regeneration, ocular surface

1. Introduction

The ocular surface of the human eye is the outermost portion of the eye that is in direct touch with the external environment. The ocular surface comprises the cornea's outer layer, the conjunctiva, the tears, and the eyelid edge. Due to its direct contact with the external environment, this eye area is the most frequently injured and leads to ocular disease. A fragile layer of tear film protects the ocular surface from external environmental exposures, keeps the surface wet, and maintains ocular epithelial surface homeostasis.

Tears are essential for ocular surface health and maintenance of various ocular surface components and visibility. The human lacrimal functional unit (LFU) consists of the lacrimal gland, the ocular surface composed of (the cornea, conjunctiva, and meibomian gland), and the associated sensory with motor nerves. The LFU is responsible for the secretion of the major constituents of the tear film and thus is overall responsible for maintaining the stability of the tear film, cornea transparency and image quality (Lin et al., 2003). The tear film comprises three distinct layers - lipid, aqueous and mucin layers; these three layers work together to maintain the eyes' health and fight any infection.

Several factors may cause LFU dysfunction, and any disruptions with severe inflammation in the tear film can lead to dry eye syndrome. (Shubha Tiwari et al., 2014)

The International Dry Eye Workshop (2007) has defined "*Dry eyes to be a multifactorial syndrome of tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface particularly*". The current prevalence/occurrence of dry eye worldwide is estimated to be 11% to 22% (Fairweather D et al.2008). In the Indian context, these numbers are estimated to be around 18.4 to 20% (Jana Dietrich et al.2019), (Schaumberg DA et al.2003) (Johnny L Gayton et al.2009).

In clinical practices, Dry eye is categorized into two groups, namely, (1) aqueous production deficient dry eye syndrome; in which there is less production of tears (2) evaporative dry eye syndrome, in which water is evaporated, resulting in hypoosmolarity. Inadequate tears cause damage to the internal pebral ocular surface and are associated with symptoms of discomfort. In addition, it is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface (S. R. Durkin et al.2007).

Anatomy and physiology of the human lacrimal system

The human lacrimal gland is an essential component of the lacrimal functional unit (LFU) which comprises the lacrimal gland, the ocular surface (cornea, conjunctiva and the meibomian gland) and the associated sensory and motor nerves. The LFU controls the secretion of the major components of the tear film and is overall responsible for maintaining the stability of the tear film, transparency of the cornea and the quality of the image projected onto the retina. The tear film composition is altered due to the dysfunction of either the lacrimal gland or the meibomian glands. The lacrimal gland is a mainly serous, tubule - acinar gland. Histologically, it is composed of acinar cells arranged in individual functional units (the acini) and surrounded by ductal and myoepithelial cells.

Lacrimal gland structure, function and purpose

The main lacrimal gland in humans and other mammals is an orbital organ and lies underneath the upper temporal compartment of the orbital embed DES in the lacrimal fossa (Figure 1). In humans, the lacrimal gland fluid is directly

conducted onto the ocular surface by various short ducts. The mainly serous tubule acinar lacrimal gland, supported by accessory Krause and Wolfring glands, is to secrete lacrimal fluid - the major component of the tear film. The parenchyma of the lacrimal gland is structurally composed of three cell types: acinar, duct, and myoepithelial. Acinar and duct cells form distinctive tubules surrounding DES by associated myoepithelial cells. (Schaumberg DA et al.2003) Acinar cells represent the primary cell type with a proportion of approximately 80%, while duct cells comprise 10%–12% of the cell population. Acinar cells are the primary producer of the lacrimal gland. Between the parenchymal parts lies the interstitial space containing several different cell types such as plasma cells, macrophages, lymphocytes, helper and suppressor T cells, B cells, dendritic cells, and mast cells. In the classic model of the tear film, the lacrimal gland fluid is the mid - layer of the tear film, encompassed by two other layers: the internal mucous layer mainly secreted by the goblet cells of the conjunctiva; and the external lipid layer, which is mainly derived from the meibomian glands. In its sum, the tear film fulfils the following functions:

- Smoothing the corneal epithelium and improving its optical properties
- Moistening the corneal - and conjunctival epithelium
- Contributing to ocular surface metabolism
- Removing dust and other debris from the ocular surface
- Protection against pathogens

Causes & Symptoms of DES

The main symptom includes burning, foreign body sensation, redness, and pain. It can also cause a stringy discharge and blurred eyesight. Symptoms increase in dry weather. (R. W. Yee et al.2007, V. D. Wagh et al.2014, W. E. Shine et al.1998)

Studies have reported that various causing agents of DED are due to the dysfunction of LFU, including less tear production, more tear evaporation, and changes in the amount of mucus or lipids in the tear layer. (E. Peters and K. Colby et al. .2006, M. A. Lemp et al.1995 M. E. Johnson and P. J. Murphy et al.2004), ageing, postmenopausal women, and patients with autoimmune syndromes.

Production of fewer amounts of tears may elicit lymphocyte infiltration, causing the establishment of inflammatory cells, T lymphocytes, which produce cytokines and trigger an inflammatory response, causing various clinical manifestations (J. Y. Niederkorn and H. J. Kaplan et al.2007). An increase in osmolarity of the aqueous layer is a general characteristic of DES.

The risk of dry eye syndrome increases as age increases. (L. Tong et al.2010, D. A. Schaumberg et al.2009) (M. Uchino et al.2014, L. Tong et al.2010). Middle - aged and older adults are the most frequently affected group because of the high frequency of contact lens usage, autoimmune syndromes, systemic drug effects, and refractive surgeries in these groups. The study has shown that DES incidence is between 6% and >30% in various age groups across different countries and worldwide (D. A. Schaumberg et al.2009, J. A. Smith et al.2007, A. Sharma et al.2014). DES is common in women and does not restrict to any particular race/population. (D. A. Schaumberg et al.2001). Studies demonstrate a gender bias among various age groups of dry eye patterns worldwide. Women are more frequently affected than male patients, as indicated by the prevalence of the disease, which is 5 - 30%. In women, at the age of 50, around menopause, there is an imbalance between androgens and estrogens, which leads to hormonal imbalance and affects vital organs or eyes. Studies demonstrate increased inflammation in the lacrimal gland and ocular surface, disturbing the routine homeostatic maintenance of the lacrimal gland and ocular surface. About one - quarter of people with rheumatoid generally arthritis have DES (D. A. Schaumberg et al.2002, M. Fujita). Other individuals who are likely to be affected include patients with *Helicobacter pylori*, computer users, and long - term contact lens wearers (S. C. Sacca et al.2006, C. Blehm et al.2005). Research shows that chronic dry eye develops in over half of the patients who underwent ocular malignancies.

There are two scenarios in which treatment is required (a) the lacrimal gland is entirely nonfunctional, or (b) the lacrimal gland is partially damaged with an insufficient number of or partially functional cells. There are plenty of treatment approaches available, which are classified into two classes/categories. In the first category, where no regenerative potential remains, in vitro manufacture of lacrimal gland tissue that could be transplanted into the patient to restore function may be an appropriate treatment. However, in the second category, triggering the regeneration of existing tissue is preferable since this is less invasive. Ultimately the cells of the host likely hold an increased propensity for regeneration compared with anything engineered in vitro using current techniques.

Clinical management

The current treatment modalities are largely palliative and need long term dependability (Table 1). Some of the treatment includes closure of the puncta lacrimal and the treatment of ocular surface inflammation. Other treatments such as salivary gland transplantation and Punctal plugs have shown limited success. The cell - based therapy is a promising approach and is being explored for many immune disorders, including DES. Stem cells have been shown for their restoration property in both in - vivo and in - vitro.

Innovative treatment options such as salivary gland transplantation are performed (Borrelli M et al.2010, Qin J et al.2013). However, this therapy does not address the underlying path physiology of ADDE and thus can only be considered encouraging. (George D Kymionis et al.2008)

Recent advances have led to the successful regeneration of the lacrimal gland by epithelial - mesenchymal interaction in the mouse model (Hirayama et al.2013), (Hirayama M, Kawakita et al.2016). Therefore, bioengineered LGs with ducts are to be transplanted into mice leading to lacrimal gland recovery.

Transplantation of the salivary gland from the lip to the subconjunctival area leads to the wet ability of the area. Induced pluripotent stem cells (iPSC) can also be used as a

cellular source for tissue - specific stem cells. Overexpression of some transcription factors can show LG epithelial cell phenotype. (Hirayama M, Ko SBH, Kawakita T et al.2017)

Human lacrimal gland regeneration, bioengineered and other recent developments

Various approaches to DES Management

The main aim for approaches for DES Management includes Restoration of tear volume in its correct composition, having anti - inflammatory properties since quantitative DES is often associated with inflammation, non - immunogenic, delivered in a single application without the need for repeated intervention, Good Manufacturing Practice (GMP) - compliant, which is more feasible when the production is simple and quick, cost - effective, especially since the prevalence of quantitative DES is high (5.5%–33.7% depending on nationality), efficient (e. g. if cells are to be administered, they should have the good homing capacity or be available in large numbers), not result in adverse side effects. (J. Y. Niederkorn et al.2007)

The damaged tissue can usually retain its regenerative capacity throughout its lifetime. It has a known self - renewal capability by tissue - resident stem cells and progenitor cells. Three stages are necessary for complete tissue regeneration, i. e., inflammation, new tissue formation, and tissue remodeling (Jana Dietrich et al., 2019).

Inflammation

Inflammation is the first stage that occurs immediately after damage and is generally characterized by an infiltration of neutrophils and macrophages, (neutrophil) granulocyte and/or monocyte and macrophage infiltrates.

The aim is to remove apoptotic cells, and a response against pathogens and common leukocyte marker CD45 and T cells is found (Dietrich J et al.2018, Gao Y et al.2015, Hawley D et al.2017, Zoukhri D et al.2008) in the lacrimal gland after IL - 1 injection, duct ligation, and scopolamine injection. (Cotroneo E et al.2010)

The lacrimal gland shows the common signs of both an early immune response and acute damage necessary for tissue regeneration.

Tissue regeneration

New tissue formation is the second stage and is characterized by proliferation and migration of different cell types, proteins and genes associated with tissue development, epithelial plasticity/epithelial - mesenchymal transition and cell cycle (Zoukhri D et al.2008), proteins such as Ki67 and PCNA, and bone morphogenetic protein 7 (BMP7) pathway are increased in this stage, which is known to be essential. Epithelial - mesenchymal transition is also seen to be involved and has shown to have an increased expression for the genes like snail family members and vimentin; after IL - 1 and Duct ligation induced ADDE (Hawley D et al.2017). Cells showing common stem cell markers such as nestin, Musashi, Nanog, CD133, Oct4 and sox2 are seen in healthy human lacrimal glands. It is also found that nestin cells increased after in vitro induced ADDE by duct ligation and IL - 1 injection (You S et al.2012).

Tissue remodeling

This stage is characterized by going back to its normal function. During lacrimal gland development, it is activated after IL - 1 induced ADDE. Cells expressing standard stems cell markers such as Nestin, Musashi, Nanog, Sox2, Oct4, and CD133 are found in healthy rodents, rabbits and human lacrimal glands; these genes showed expression stem cell properties in the lacrimal gland (J. I. Prydal et al.2006 - 2011).

In general, re - organising the extracellular matrix (ECM) is essential for tissue remodelling. ECM remodelling is marked by an increased synthesis of collagen chains and associated processing enzymes in regenerating the lacrimal gland. In addition, the expression of MMPs and extracellular glycoproteins are shown to be increased during the late phase of lacrimal gland repair after IL - 1 induced DES.

Tissue remodelling is the third stage seen by the end of the activation as in stages one and two, and the tissue returns to its standard. Stage three is known to last for months. The central part is reorganising the extracellular matrix, which is essential for tissue remodelling and is seen by an increase n collagen. The expression of extracellular glycoprotein and MMPs are shown to be up - regulated during the late phase lacrimal gland repair after IL - 1 induced DES. (Zoukhri D et al.2008) In some conditions, such as autoimmune inflammation and age - dependent degradation, the intrinsic regenerative capacity May be impaired. Therefore, it is a promising field to enhance lacrimal gland regeneration.

MSCs based treatment strategies

hMSCs are hematopoietic stem cells and multipotent that can differentiate into the mesodermal lineage. [Hawley D et al.2017] [Uccelli A et al.2008, Gao F et al.2016] They are non - immunogenic, which makes them more promising and potential use in a broad range of regenerative medicine applications. The International Society for Cellular Therapy, MSCs are the plastic adherent cells with cell surface expressions of the cluster of differentiation CD73+, CD90+, cd105+, and no expression of CD34, CD45, CD14, CD11b and HLA - DR. (Hasan Mansoor et al., 2019) HLA class I and HLA class II molecules have low to medium expression levels in undifferentiated mesenchymal stem cells; this is to hide/avoid the recognition by immune cells. HLA class I molecules are present in MSCs at detectable levels, and similarly, expression of HLA class II molecules can be induced by the interferon γ (INF - γ). This ability of HLA molecules to hide their expression from the immune system is called stealth ability. (Matthew B Murphy et al., 2013) This is one of the main reasons and advantages of MSC in allogeneic MSC transplantation therapy to be undetectable by the host immune system.

Studies have shown that after the isolation of these lacrimal gland - derived MSCs, expression of transcription factors (C - Myc and Kruppel - like factor - 4) and pluripotency markers (Nanog, nestin and Sox2) persisted at the mRNA level to passage number 30. Moreover, the expression of early lineage markers for endodermal (GATA4, GATA6),

ectodermal (Pax6), and mesodermal (bone morphogenic proteins - 4 and - 7) development is also demonstrated.

Samantha You and her colleagues isolated the MSCs from the murine lacrimal gland. They have injected IL - 1 to induce inflammation so the lacrimal gland can be injured. They examine the isolated MSCs by expressing nestin - positive cells, ABCG2 and Sca - 1. They state that murine lacrimal glands have MSCs that play a pivotal role in tissue wound injury. (Samantha You et al., 2011)

Driss Zoukhri and his team reviewed murine models to study the role of MSCs in lacrimal gland injury and its repair. (Driss Zoukhri, 2010) To investigate the presence of resident progenitor cells and their regenerative potential, Hui Lin et al. induced duct ligation injury in a rabbit model of the lacrimal gland's central excretory duct. The ligation - injured lacrimal glands temporarily decreased in weight and had impaired tear secretion. The aftermath of the injury in this study indicates that lacrimal glands can tissue repair after duct ligation - induced injury, likely involving resident stem/progenitor cells and epithelial - mesenchymal transitions. Lacrimal gland progenitor cells isolated from ligated tissue can differentiate in 3 - D culture. These stem cells have the potential to treat severe cases of tear deficiency. Hui Lin et al. (2017)

MScs are site - specific and migrate to the site of injury and damage tissue. This ability of mesenchymal stem cells is called homing/migration. (Xiaoxiao Lu et al., 2017) the migration of MSCs induced due to the chemokine activation at the injury site. (Chavakis E, 2008; .) The axis of stromal cell - derived factor 1α (SDF - 1α) and its receptor CXCR4 is an essential biological axis that promotes MSC homing to damaged tissue. (Wang G, 2016)

Jana Dietrich et al. studied the mouse model of surgically induced dry eye disease through duct ligation. MSC transplantation significantly improved LG regeneration, as the amount of vital acinar structures were significantly increased above the intrinsic regeneration capacity of control.

Adult acinar cells
A recent study has shown that adult acinar cells of the salivary gland itself can proliferate and refill damaged cells in - vivo. Marker p63+ cells are detected that cells function as multipotent stem/progenitor cells that contribute to and maintain all epithelial cells during morphogenesis and continuation of the salivary gland. Differentiation acinar cells of the lacrimal glands also actively proliferate and remain yet to be investigated. In this instance, however, there could be a way to induce this proliferation in syndrome stages where this natural process has become settled down to induce lacrimal gland regeneration. [Aure et al.2015]

Side population cells
After two months, SP cells are transplanted into irradiation damaged lacrimal glands and could restore tear secretion, comparable to that of healthy mice. Furthermore, it is known that clustering contributes to the therapeutic effects, as the expression of clustering is significantly higher in transplanted glands.

Epithelial progenitor cells
Epithelial progenitor cells (EPCP) isolated from the murine lacrimal gland exhibit stem cell properties. EPCP engraftment in IL - 1 damaged lacrimal glands could be further increased even during acute inflammation when blocking Panx1 and thus reducing inflammation. The TSP - 1−/− mice represent a model with chronic DES and share standard features of Sjögren's syndrome. Transplantation of EPCP into manifested, chronic DES of TSP - 1−/− mice are performed, and cells can be detected in ductal cells and differentiated, regenerating acinar cells. [Aure MH et al. 2015]

Recent studies suggest the potential isolation of markers such as c - kit+dim /Sca - 1− cells from mice using cell sorting (FACS) resulted in an assumed lacrimal gland epithelial cells progenitor population. [Shatos MA et al. 2012] These cells are known to express Oct4, and Runx1, Pax6 as conventional stem cell markers and are and differentiate into ductal and secretory compartments in the 3D cultures. A detailed description of the isolated cells has shown that the stem/progenitor cells seem to be of myoepithelial, epithelial and mesenchymal cell origin.

2. Discussion

There are multiple concepts to be investigated for lacrimal gland regeneration, such as therapeutic proteins (BMP7 and PACAP), mesenchymal stem cells, salivary gland transplant, lyophilized cell extracts and also the use of adult acinar cells. The use of MSC to improve *in - situ* lacrimal gland regeneration, like their properties, is very favorable for clinical applications. MSC modulates the immune system, has low immunogenicity, promotes tissue regeneration and is involved during lacrimal gland regeneration in mice. LG's, accompanied by ducts, could be transplanted into LG excision mouse models with functional innervations, which will lead to lacrimal functional unit recovery in a mouse transplant model. Induced pluripotent stem cells could be used as a source for tissue - specific stem cells. Over - expression of specific markers could induce LG epithelial cell phenotype.

One of the future challenges is that the structure and location of the lacrimal gland differ between mammals.

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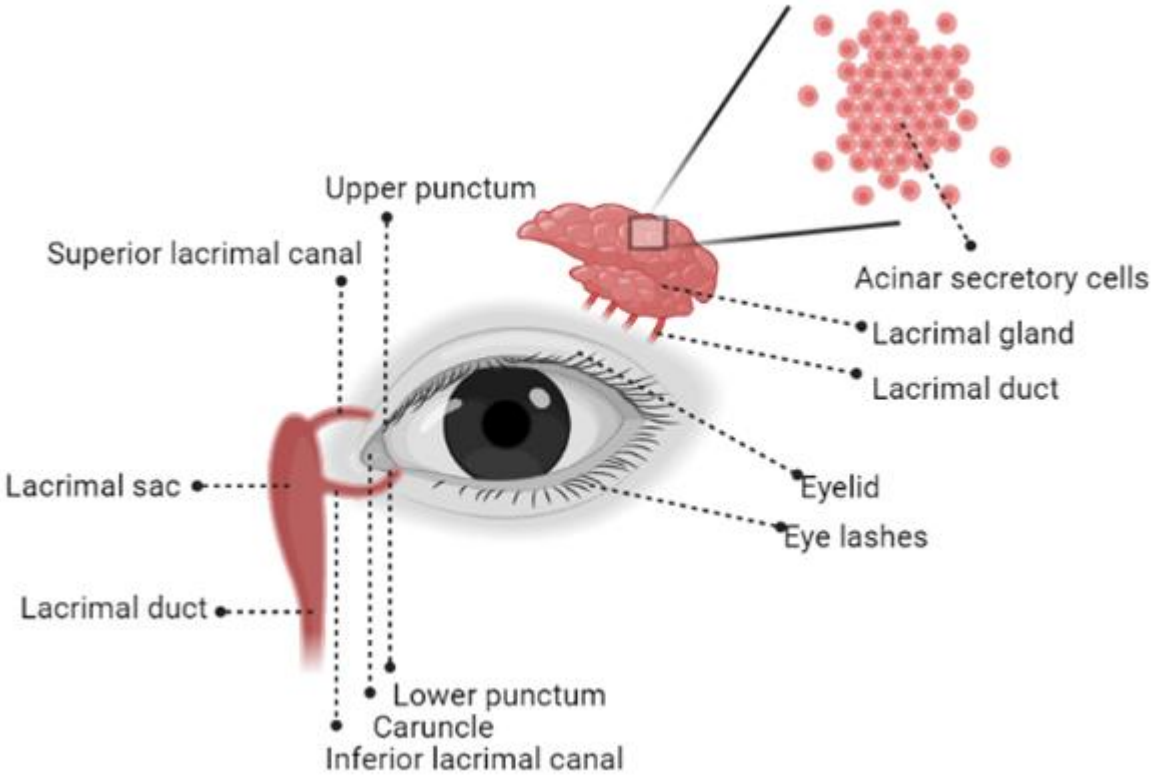

Figure 1: Anatomy of lacrimal gland

Table 1: Treatment Modalities of Dry Eye Syndrome

	Tear replacement strategies	Lubrication substitutes	Tear supplementation/ lubricants	Viscosity enhancing agents • Carboxymethyl cellulose (0.25% - 1%) • Hydroxypropyl methylcellulose (HMC) • Hyaluronic acid (HA) • Hydroxypropyl - guar • Hydroxypropyl cellulose Osmo - protectants • OTC drops Inactive agents • Buffers • Electrolytes (bicarbonate, potassium) Preservatives • Sodium perborate • Polyquad • Sodium chlorite
		Biological tear substitutes	Autologous serum Adult allogeneic serum Umbilical cord serum	
		Other agents	Mucolytics	TRPV1 receptor antagonist
	Tear retention approaches	Punctal occlusion	Punctal plugs (absorbable) Surgical punctal occlusion (non - absorbable)	
		Moisture chamber spectacles Therapeutic Contact lens		
	Tear stimulation strategies	Topical secretagogues	Aqueous secretagogues.	Diquafosol tetrasodium
			Mucin secretagogues	
		Lipid stimulation Oral secretagogues Nasal neuro - stimulation		
Eyelid disorders	Anterior blephxaritis	Lid hygiene/lid scrubs	Bacterial over colonization	Topical antibiotics • Tetracyclines
			Demodex infestation	Tea tree oil Ivermectin
		Meibomian gland dysfunction	Ocular lubricants	Eye drops
			Warm compresses	Blephanteam MGDRx EyeBag EyeGiene mask

				Infrared warm compression device
			Physical treatments	Forceful expression LipiFlow (electronic heating device) intense pulsed light Intraductal meibomian gland probing Debridement of the lid margin
		Blinking abnormalities and ocular exposure	Treatment for corneal exposure	
			Entropion and ectropion	
			Contact lenses	Therapeutic soft contact lenses (bandage lenses) Rigid gas permeable sclera lenses
	Glucocorticoids	Topical cortecosteroids	Methylprednisolone (1%) 17 - beta - oestradiol solution	
	Non - glucocorticoid immunomodulators	Immunosuppressant	Cyclosporine A	Restasis® (0.05%)
		Tacrolimus (0.03%)	Pimecrolimus	
		Non - steroidal anti – inflammatory Drugs	Flubiprofen Allergen Irvine	
		Biologics	Recombinant human nerve growth factor	
			Tumor necrosis factor α - stimulated gene/ protein - 6	
			Interleukin - 1 receptor antagonist	
			Anti - tumor necrosis factor - α therapy	
			Anti interleukin - 17 therapy	
	Surgical treatments	Tarsorrhaphy/lid malposition		
		Treatment for conjunctivochalasis	Cautery approaches Argon laser Glue/ incisinal approaches	
		Lid corrections	Dermatochalasis surgery	
			Blepharoptosis	
			Lower lid blepharoplasty	
		Conjunctival surgery and amniotic membrane grafts	Conjunctival flap	
		Mechanical pump dacryo - reservoirs		
	Nutritional therapy	General hydration state Fatty acid Lactoferrin Other (beta - carotene, vitamins, zinc) Antioxidants		
	Micro - environmental considerations	Chronic topical medications Increase blink rate Decrease desiccating conditions and pollutants Contact lens wear		
	Alternative medicines	Herbal and natural products Honey Milk Acupuncture		

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