

Newer approaches to dry eye therapy: Nanotechnology, regenerative medicine, and tissue engineering

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Definitive treatment of dry eye disease (DED), one of the commonest ocular surface disorders, has remained elusive despite several recent advances in better diagnostics and the introduction of newer therapeutic molecules. The current treatment paradigms rely heavily on lubricating eye drops and anti-inflammatory agents that may need to be used long-term and are mainly palliative. Research is ongoing not only for a curative treatment option but also to improve the potency and efficacy of existing drug molecules through better formulations and delivery platforms. In the past two decades, significant advancement has been made in terms of preservative-free formulations, biomaterials such as nanosystems and hydrogels, stem cell therapy, and creation of a bioengineered lacrimal gland. This review comprehensively summarizes the newer approaches to DED treatment, which are biomaterials such as nanosystems, hydrogels, and contact lenses for drug delivery, cell and tissue-based regenerative therapy for damaged lacrimal gland and ocular surface, and tissue engineering for developing artificial lacrimal gland. Also, their potential efficacies in animal models or *in vitro* studies and possible limitations are discussed. The ongoing research looks promising and needs to be supported with clinical efficacy and safety studies for human use.

Key words: Contact lens, dry eye disease, lacrimal gland regeneration, mesenchymal stem cells, nanomaterials

Dry eye disease (DED) is a global health problem affecting millions of individuals approximately 5–50% of the adult population worldwide.^[1] According to the Tear Film and Ocular Surface Society (TFOS) and Dry Eye Workshop II (DEWS II) 2017, DED has been classified mainly into three categories: aqueous deficient dry eye (ADDE), evaporative dry eye (EDE), and mixed types based on distinct signs and symptoms.^[2] Although the pathogenesis of EDE is related to hyperosmolar tissue damage, there is also the induction of inflammation and loss of epithelial and goblet cells. The existing treatment options for DED are lubricants and drugs that are palliative in nature and have inherent limitations of frequent use and preservative toxicity.^[3–5] To address these limitations, different approaches have been explored by various groups in the fields of regenerative medicine, pharmacology, nanotechnology, and biomedical engineering, wherein newer nanosystems, hydrogels, and contact lenses are designed to improve drug

delivery to the ocular surface and application of mesenchymal stem cells (MSC) has immunomodulatory effects and epithelial regenerative changes on the ocular surface [Fig. 1]. Either newer drug molecules or new drug delivery devices are researched to circumvent the limitations of current conventional topical ophthalmic drugs that have reduced bioavailability (due to nasolacrimal drainage, reflex tearing, and blinking), drug dilution with tears, non-compliance (frequent instillations), metabolic degradation, and preservatives with potential surface toxicity.^[6,7]

As the DED spectrum ranges from mild to severe disease, pharmacology-based research on newer drug delivery devices addresses mild to moderate DED but the ADDE due to Sjogren's disease, which can present with more severe disease, needs novel regenerative approaches targeting the lacrimal gland. An alternative curative treatment strategy of regenerating the damaged lacrimal gland, which is responsible for producing the major aqueous component of the tear film, is required [Fig. 1]. The regenerative approach explores either lacrimal gland repair with MSCs or regenerating the lacrimal gland using bioengineering in animal models.^[8–12] The paracrine and autocrine effects of the secretome derived from MSCs

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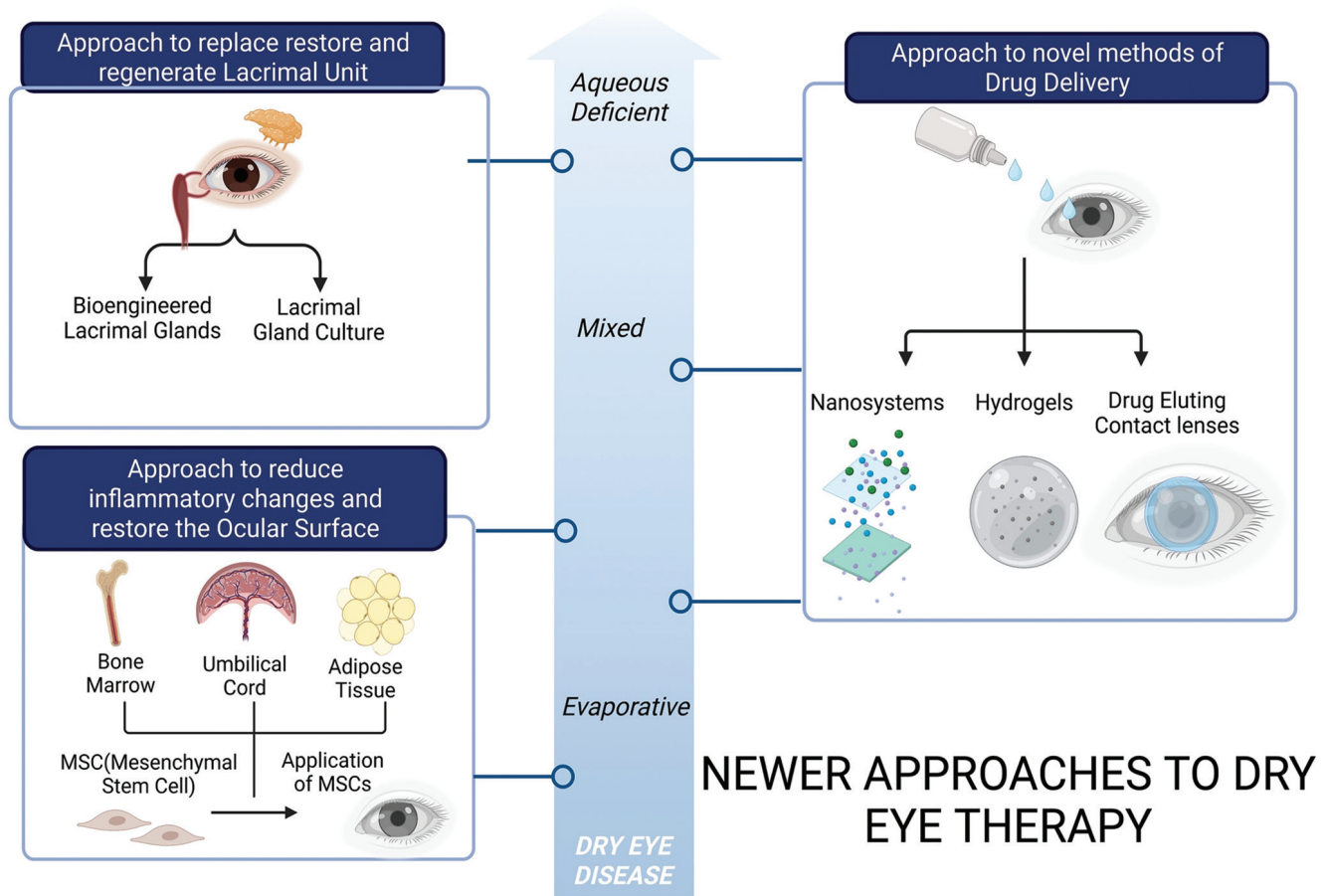


Figure 1: Flow chart to represent various novel strategies to treat Aqueous-Deficient Dry eye Disease (ADDE), Evaporative Dry eye (EDE) and Mixed variant of the disease using nanotechnology, biomedical engineering and regenerative medicine. Approach to newer drug delivery strategies in all variants of the dry eye includes using newer nano drug delivery systems, hydrogels and drug-eluting contact lenses to improve the drug bioavailability at the ocular surface. The approach to improve the ocular surface by immunomodulation, paracrine activity and repair properties of MSC (mesenchymal stem cells) or their secretome is a vast area of research in EDE and the Mixed variant of the disease. Finally approach to regenerate lacrimal glands by bioengineering strategies remains a possible strategy to tackle ADDE

have shown promising long-term immunomodulatory effects on the ocular surface and corneal epithelial repair.^[13-16] This review summarizes the various biomaterials being explored for therapy in DED and the regenerative strategies used for lacrimal gland regeneration as well as ocular surface and their possible therapeutic applications.^[5,17]

Nanotechnology and Biomaterials in Dry eye

Nanosystems

Nanotechnology-driven delivery systems have been explored vastly in pharmacotherapeutics and medicine. In ophthalmology, nanomaterials are used for various anterior segment, glaucoma, and posterior segment diseases.^[18] A nanoparticle can be lipid-based, polymer-based, or can be made of materials such as silica, carbon (carbon nanotubes), and cobalt/nickel (magnetic). Typically, in the initial days, delivering lipophilic drugs with a hydrophobic formulation was a challenge. Various lipid-based nanocarriers were used to increase the drug availability in the posterior segment ciliary body, choroid, and retina. The lipid-based nanoparticles can be

divided based on their core structure having a liquid or vesicle base, which comprises previous colloidal emulsions, such as micelles (central hydrophobic core formed only by the tail ends of a single layer of surfactant molecule), liposomes (liquid core surrounded by double phospholipid layer), and nanoemulsions drop (hydrophobic liquid core oil dispersed in water and stabilized by surfactant with nanoparticles 10 nm–100 nm).^[19,20] The positive charge of liposomes helps them adhere to the negatively charged mucin layer in the tear film and they can be coated with an adhesive polymer or dispersed into an adhesive gel to bind with the cornea. Liposome formulations or lipid-containing eye drops for the treatment of dry eyes available in the USA are Vyseo®, Clarimist®, ActiMist,™ and Tears Again® and can be used for the treatment of patients with mild to moderate evaporative DED. Oil in water nanoemulsions is used as a carrier for lipophilic drugs such as cyclosporin A and Restasis® (nanoemulsion for cyclosporin A). Although these modes of drug delivery improved drug availability, they had certain disadvantages such as physical stability, complicated production method, low percentage entrapment, and cumbersome industrial-scale manufacturing.^[19] However, the invention of newer solid lipid nanoparticles (SLN) (consists

of lipids in a solid state at room temperature core) and NLC (nanostructured lipid carriers consisting of at least 30% triglycerides, which are in a liquid state at room temperature) in recent years have revolutionized the nano drug delivery systems by being more stable with a longer shelf life up to 3 years, better reproducibility, feasible for large scale manufacturing, better carrier for hydrophobic drugs, lesser immune reactions, and extreme site-specific drug delivery.^[21-23] Experimental Cys-NLC formulations of cyclosporin A have shown better drug residence time in rabbits, whereas NLC ophthalmic solutions based on phosphatidylcholine and squalene surfactants have shown to be useful artificial tear products.^[24,25] Their nanoscale size have the ability to enhance penetration through the ocular surface layers, protect the active molecules from degradation, and increase the drug residence time on the ocular surface compared to the eye drops formulation.^[26,27] Nanosystems in the ocular surface exhibit the adhesive property that prevents degradation by the ocular surface defense mechanisms.^[28,29]

Polymeric nanoparticles are composed of either natural (chitosan, gelatin) or synthetic polymers (polylactic acid and poly L-lysine). They exhibit both biocompatibility and biodegradability. These polymers are non-toxic and eliminated by the liver and provide a customizable surface, which can be used to coat nanoparticles. Li *et al.*^[30] demonstrated gold nanoparticles carrying two drugs with complementary actions such as amfenac (anti-inflammatory) and catechin (antioxidant), which reduced ocular surface inflammation and Reactive oxygen species (ROS) levels in mice models after 4 days of treatment as compared to the commercial cyclosporine (CsA)

eye drops. Drug-loaded poly (catechin) shell forms a loose polymeric matrix that helps in loading on gold nanoparticles forming a uniform shell [Fig. 2]. Similarly, cerium oxide has a potential role in controlling ROS activity; therefore, it was encapsulated with chitosan to make it water soluble and be used as an eye drop to alleviate DED. A significant improvement in tear film break-up time (TBUT), ocular surface inflammation, tear volume, and a decrease in intracellular ROS was noted in the rabbit cornea and conjunctiva.^[31] Polymeric nanoparticles (NPs) in the form of cationized gelatin have also been used as a plasmid carrier for delivering genes coding MUC5AC in a mice DED model^[32] [Fig. 2]. A reduction in inflammatory cytokines and CD4 + T cell infiltration was noted in mice. The nanoparticle poly (D, L-lactide)-b-dextran (PLA-b-Dex) containing cyclosporine reduced the dosing schedule to once a week compared to thrice daily administration of Restasis (Allergan Inc., Irvine, CA) in DED mice model.^[33] This study suggests that weekly once administration can maintain the drug's therapeutic level on the ocular surface.

For the delivery of lipid-soluble drugs, nanocapsules are used for better drug loading. Nanocapsules are composed of a polymeric wall and an oily core.^[25,34] CsA was loaded in lipid nanocapsules and better therapeutic effects (increased TBUT, reduced corneal fluorescein score) were observed in the rat DED model compared to Restasis. Dexamethasone-containing nanostructure lipid carriers (NLCs) have also shown prolonged retention time on the rabbit ocular surface with better ocular surface changes in the benzalkonium chloride (BAC)-induced rabbit

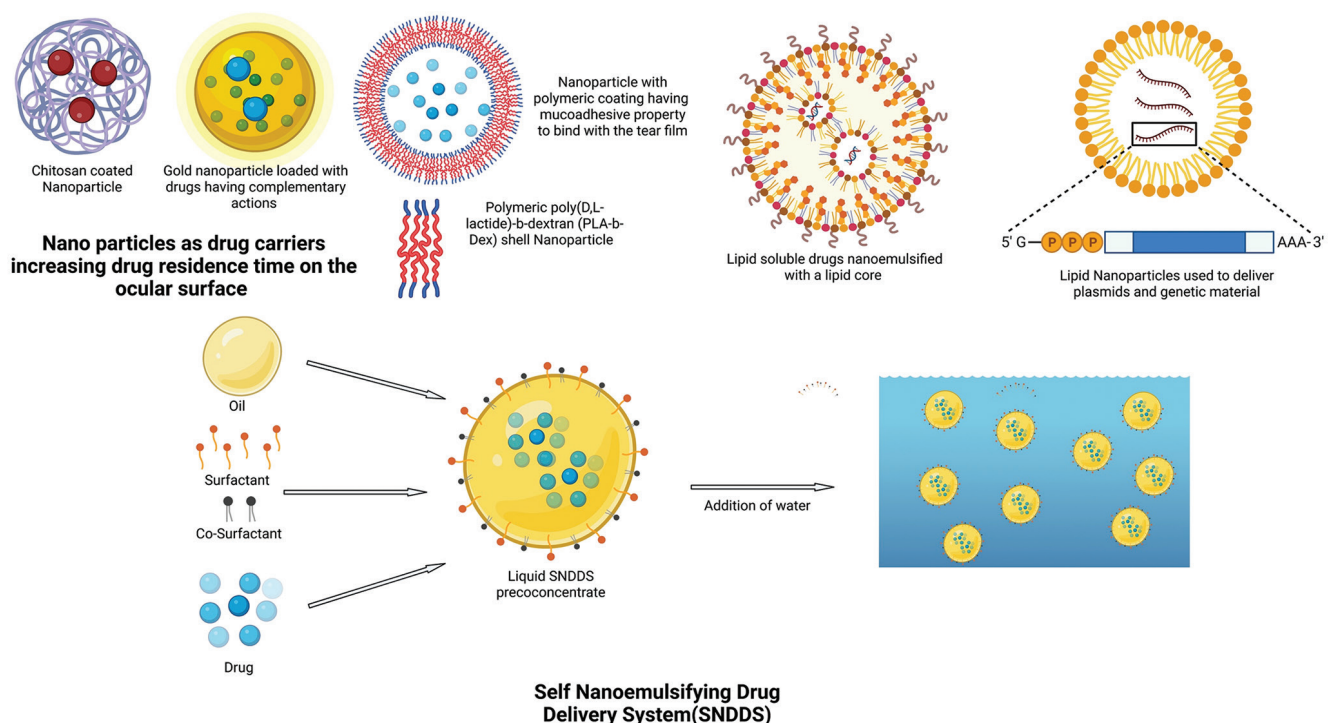


Figure 2: Various Nanoparticle drug delivery systems likely Polymeric nanoparticles with chitosan coating, and gold nanoparticles with mucoadhesive properties are shown to load and deliver the drugs to the ocular surface. Lipid-based nanoparticle drug delivery system for lipid-soluble/hydrophobic drugs in liposome formulation. Plasmids with genetic material are packaged within the lipid core of a nanocapsule by the process of nano-emulsification. Below a mechanism of constituting and working of a self-nano-emulsifying drug delivery system is schematically demonstrated

DED model.^[35] Nano wafers is another novel modality of drug delivery, wherein drug such as dexamethasone was loaded onto wells in a nano wafer composed of carboxymethyl-cellulose used as hydrogel showed improved corneal epithelial healing, smoother corneal surface, and increased tear film in a mouse model of dry eye.^[36] This decreased the frequency of administration of the drug. Restasis is thermodynamically unstable and requires a large amount of energy in its preparation.^[37] Another benefit is reduced burning and stinging sensation associated with Restasis on the ocular surface. Kang *et al.*^[38] showed that cyporin N, a 0.05% CsA nanoemulsion product (Taejoon Pharmaceutical, Seoul, South Korea), had better improvement in TBUT compared to Restasis in primary Sjögren's syndrome (SS) patients. Another two self assembling nano-emulsifying drug delivery systems (SNEDDS)-Cyporin-N and T-sporin have also shown comparable efficacy with Restasis in the BAC-induced DED model [Fig. 2].^[39]

Nano micelles are colloidal carrier systems ranging between 5 nm and 200 nm in size, which are synthesized from block copolymers and consist of different hydrophobic and hydrophilic monomer units and are called polymeric nano micelles, whereas nanosized micelles are composed of amphiphilic molecules with water-loving head groups (anionic and cationic) and hydrophobic tails and are referred to as surfactant nano micelles.^[40] In 2018, the US FDA approved Cequa® (Sun Pharma, Mumbai, India), a preservative-free 0.09% surfactant nano micellar formulation of CsA for DED, which has better stability and cost-effectiveness.^[41] The other nanostructured ophthalmic products currently marketed for the treatment of DED are Cyclokat® (preservative-free 0.1% CsA cationic emulsion), Lacrisek® (Vitamin A, Vitamin E liposomal spray), and Artelac Rebalance® (hyaluronic acid, vitamin B12, and viscoelastic eye drops).^[42,43] Besides, SYSTANE®, an ophthalmic nanoemulsion for dry eye disease has completed a phase IV clinical trial. Lee *et al.*^[43] developed a polyethylene glycol PEG-catechin nano complex, which showed significant improvement in the tear production, anti-inflammatory properties, and downregulation of IL-1b, IL-6, and TNF alpha expression in the lacrimal gland in the DED mice model. For restoring the lipid layer of the tear film, a nanoscale-dispersed ointment containing a mixture of semi-solid lipids (petrolatum and lanolin) and liquid lipids (medium-chain triglycerides) was explored.^[44] A marked decrease in fluorescein staining scores and improved TBUT were found compared to artificial tears.

Hydrogels

Hydrogels are made up of hydrophilic polymer chains that make them absorb a huge quantity of water without getting disintegrated into it and maintaining a three-dimensional (3D) structure. A variety of polymers including natural, semisynthetic, and synthetic can be used to form hydrogels. Hyaluronic acid, chitosan, methylcellulose, poly (vinyl alcohol), and poly (acrylic acid) are some of the ocular lubricating polymers. Hydrogels have tunable physical properties and degradation rates, which provide spatial and temporal control over the environment and the release of encapsulated drugs at the target site of delivery. Their excellent biocompatibility, prolonged drug release action on the ocular surface, good mechanical properties, and the ability to carry and deliver a wide range of drugs from hydrophilic to hydrophobic in its crosslinked matrix make them an emerging drug delivery

platform for the treatment of ocular surface diseases.^[45-47] Various hydrogel products for DED treatment are available in the market, such as Vidisic® gel (Bausch and Lomb, Rochester, NY, USA), Hylo®gel (URSAPHARM, Saarbrücken, Germany), GelTears® (Bausch and Lomb, Rochester, NY, USA), Viscotears® (Novartis, Basel, Switzerland), and Clinitas gel® (Altacor, Reading, UK) and a few under clinical trials including VisuXL® gel (VISUfarmaSpA, Rome, Italy), and bovine basic fibroblast growth factor (bFGF) gel (Zhuhai Yisheng Biological Pharmaceutical Co., Ltd., Zhuhai, China). Besides, recent patents on hydrogel products for DED treatment include multi-arm PEG insert with CsA/Dex, PNIPAAm and butyl acrylate plug, Guar gum, PVA, and boric acid drop containing diquafosol sodium.^[48-50]

The research on hydrogel-based products for DED treatment continues and groups worldwide have studied various materials. Soft hydrogels containing hyaluronic acid (HA) alone have been explored in a few rabbit DED models.^[45,51] Compared to lubricants, cross-linked HA hydrogels significantly improved ocular surface signs and symptoms in canine DED models [Fig. 3].^[51] Further, thermo-responsive hydrogels containing mucoadhesive gelatin and lectin helix pomatia agglutinin are loaded with epigallocatechin gallate (EGCG) or FK506 (immunosuppressive agent) and are used in DED management [Fig. 3].^[52,53] Single use on the DED-induced rabbit ocular surface maintained EGCG bioavailability above the therapeutic level for 14 days.

Hydrogels have also been explored as intracanalicular plugs (hydroxybutyl chitosan [HBC]) for blocking the lacrimal outflow system.^[54] Clinical trials in eight humans showed improvement in TBUT and ocular surface disease index (OSDI) scores but no comparison with available plugs was done. Hydrogels are also under research for use as a mini eye patch with the ability to convert visible light into heat to stimulate lacrimal tear secretion to treat dry eyes.^[55]

Drug-eluting contact lenses

Drug-eluting contact lenses are advantageous for their prolonged drug release, improved bioavailability, and increased patient comfort and compliance in comparison to conventional eye drops. Research on drug delivery through contact lens (CL) picked up its pace in the 2000s with the advancement in material science understanding. Conventional hydrogels and silicone hydrogels are the two major materials used for developing soft contact lenses. The ocular drugs are loaded in the contact lenses by various strategies such as soaking (wherein the CLs are soaked in drug solution), direct incorporation into the CL matrix, nanocarriers, molecular imprinting (wherein a high drug affinity sites are created in the CL matrix), ring implant (which includes the incorporation of the drug-infused ring into the CL polymer matrix), and vitamin E barrier (creating transport diffusion barriers for drugs). Soaking is the simplest method to obtain therapeutic CLs. This method retains its therapeutic effect for a few hours to a few days.^[56,57]

Lubricants and anti-inflammatory drugs are explored for delivery via contact lenses (CL) where a constant release of the drug has been achieved for a longer time, ranging from 48 h to 15 days in *in vitro* studies.^[58-60] Hyaluronic acid-loaded CLs have improved epithelial defect healing in BAC-induced rabbit DED

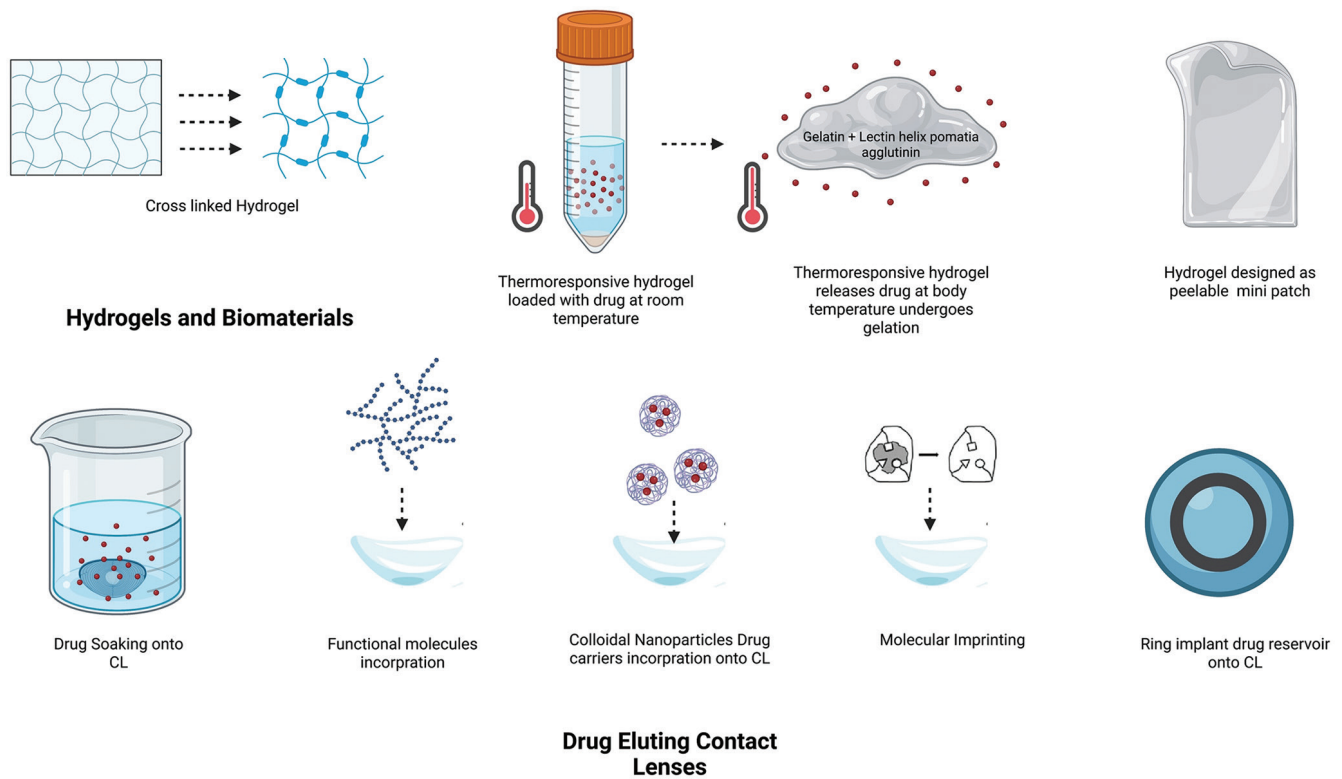


Figure 3: Hydrogels with tunable physical properties can be customised in different shapes and consistency thickness for drug delivery by cross-linking or smart designing with CAD software to make a thin patch for application over the ocular surface. Thermoresponsive hydrogels shrink at higher temperatures at the target site in the organ and allow the release of the drug particles as the degrade. Below various techniques to load the drug over the CL(contact lens) are schematically represented

compared to untreated rabbit eyes; however, a comparison with HA drops without CL would give more information about its efficacy. The use of poly (vinylpyrrolidone) (PVP-K90)-coated CLs has shown a high tear volume in the animal study compared to the uncoated ones and commercial eye drop solution. The techniques used for loading CLs are soaking, molecular imprinting, ring implant, and incorporation of nanocarriers and functional molecules [Fig. 3]. Anti-inflammatory drugs such as cyclosporine A, dexamethasone, and betamethasone have also been incorporated into CLs, with the ability to release the drugs for a long period of up to 14 days.^[61-63] Vitamin E-soaked lenses have been loaded with steroids such as betamethasone and ciprofloxacin and have shown better extended-release. Non-steroidal anti-inflammatory drugs (NSAIDs) such as flurbiprofen sodium, diclofenac sodium, and ketorolac tromethamine have also been studied for extended delivery through vitamin E cationic surfactant-loaded commercial silicone hydrogel (SiH) CLs.^[64]

Approach to reduce inflammatory changes and restore the ocular surface

The field of regenerative medicine has significantly advanced in recent years, and in the scenario of DED, there have been developments in understanding the mechanisms and actions of stem cells, their immunomodulatory effects over the ocular surface, cell-to-cell interaction, regenerative capacity to re-epithelialize corneal defects and re-establish secretory functions of lacrimal glands.

Applications of stem cells in DED and ocular surface inflammation

Dry eye and other ocular surface inflammatory diseases are characterized by increased surface inflammation, tear film, epithelial abnormalities, hyperosmolarity, and oxidative stress. The applications of stem cells derived from various sources (limbal epithelial stem cells, iPSCs, and MSCs) and their paracrine functions have been explored extensively. Induced pluripotent stem cells (iPSCs) are derived from skin or blood cells and reprogrammed back to an embryonic stage of pluripotency, from where they can be grown into any desired cell lineage.^[65] The iPSC-derived RPE cells have recently been used for the treatment of ARMD; however, in DED, these cells can be used to regenerate lacrimal glands though there is no ongoing direct trial to prove the same.^[66] iPSC-conditioned media has been used by a group to regenerate lacrimal glands in a mouse model of DED.^[67] However, currently, high tumorigenic potential, high cost, and regulation involved in culturing and applying iPSCs are limitations of this technique on a larger scale.^[65] Similarly, human amniotic membrane epithelial cells are being investigated for the regeneration of salivary glands and can be used in the future to regenerate lacrimal glands. This has been demonstrated in certain double-chamber systems, wherein the human amniotic membrane epithelial cells trans-differentiate into acinar cells of the salivary gland.^[68]

Typically, allogenic or autologous MSCs can be derived from the bone marrow, adipose cells, umbilical cord, Wharton's jelly,

and dentine pulp. The ability of stem cells to modulate innate and adaptive immune systems, and secrete growth factors and anti-inflammatory factors in response to inflammation make them an attractive therapeutic modality. MSCs are capable of cell-to-cell interaction, secreting immunomodulatory factors such as indoleamine 2,3-dioxygenase, interleukins-1 β , -6, -8, transforming growth factor- β , nitric oxide, ciliary neurotrophic factor, glial cell line-derived neurotrophic factor, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF) in response to pro-inflammatory stimuli such as IFN- γ and various interleukins.^[14,69,70] Their paracrine activity makes them an ideal candidate to be administered systemically. MSCs can be applied topically or injected subconjunctivally. Their paracrine activity makes them an ideal modality to inject systemically. Beyond ophthalmology, MSC application in the field of orthopedics has been seen with the JOINTSTEM phase 2b clinical trial, wherein autologous adipose-derived MSC were injected intra-articularly in patients to promote cartilage growth, and results have shown no side effects, significant improvement in function and pain.^[71] In the field of cardiology, PERISCOPE clinical trials have tested the use of MSCs derived from the allogenic umbilical cord, Wharton's jelly or autologous bone marrow was seen for safety and efficacy in patients with infarcted myocardial tissue and chronic ischemic cardiomyopathy.^[72] Autologous MSCs have also shown short-term benefits with improvement in symptoms in patients with multiple sclerosis and are being tried for other neurological disorders such as stroke, Alzheimer's disease, and amyotrophic lateral sclerosis.^[73-75] MSCs have also shown faster wound healing properties when they were tried on patients with diabetic foot ulcers and amputation stumps.^[76] Recently, MSCs have been tried in various clinical trials in COVID-19 patients to control the cytokine storm and improve outcomes in acute respiratory distress syndrome and multiple organ failure.^[77,78]

Studies by various groups have shown faster healing and corneal epithelial regeneration after the application of MSCs in various animal models of corneal wound healing and chemical burns.^[79-84] Of the two types of dry eye disease, evaporative and aqueous deficiency, stem cells and their role have been studied in the later in the past few years. Xu *et al.*^[85] showed inhibitory effects of intravenously administered MSCs on experimental animal models and clinical SS in patients, wherein it was seen that T cells directed toward Treg and Th2 suppressed Th17 and Tfh responses, and alleviated disease symptoms. Beyazyıldız *et al.*^[86] demonstrated that topical application of MSCs was safe and effective in a BAC-induced dry eye disease model in rats. They showed significant improvement in aqueous tear volume, ocular surface evaluation tests, and engraftment of MSCs within the conjunctival goblet cells and meibomian glands in the rats. This was speculated to be due to homing characteristics of MSCs, paracrine effects, differentiation of topically transferred MSCs to goblet cells or glandular cells, or stimulation of repair mechanisms of damaged goblet cells of conjunctiva. The microvillus structure of the epithelium was maintained and there was no neutrophilic infiltration observed.

Dietrich *et al.*^[8] have demonstrated a surgically induced model of aqueous deficiency dry eye by duct ligation for 3 days in mice, which showed clinical signs of acute ADDE with impaired functional LG tissue and tear physiology with minor impact on the ocular surface. The MSC cell population

used was unique in the way that it belonged to the native tissue of the same species in this case murine LG from eGFP mice. In comparison with direct saline injection within the LG, extrinsic tissue-specific MSCs showed accelerated regeneration with an enhanced amount of vital acinar structures, an increase in the total area of viable lacrimal gland tissue, earlier decline in apoptotic cells, a modulated macrophage invasion, and a lower number of proliferating cells during acute inflammation, a lower expression of TNF α , and an increased expression of IL-6. This approach showed the possible benefits of extrinsic MSCs in severe aqueous deficiency dry eye individuals with poor intrinsic LG regenerative capacity.

Going ahead from the learnings of this animal study, Hansen *et al.*^[10] conducted a first clinical trial wherein the safety and therapeutic efficacy of allogenic adipose-derived MSCs in seven Sjogrens disease patients was studied. The MSC suspension was directly injected into the LG capsule up to 50% of the LG volume as detected on the MRI imaging of the gland, and the outcomes were measured by looking for adverse reactions to the injection, changes in the OSDI score, tear osmolarity, TBUT, Oxford grade, Schirmer's I test, and development of donor-specific anti-HLA antibodies. No adverse events were noted to the injection, and overall there were improvements in all the parameters in the study eye. The changes seen were hypothesized to be due to the anti-inflammatory activity of MSCs and this treatment was more likely to have an effect in patients with early phases of the DED than in later phases with the presence of cicatricial conjunctivitis.

Recently, topical application of exosomes containing bioactive molecules and micro RNA (miR-204) secreted by MSCs was explored in a group of Graft versus Host disease (GVHD) patients, wherein the exosomes act like the secretome of the MSCs and were shown to improve the signs of symptoms of dry eye.^[87] The miR-204 targeted interleukin-6 receptor (IL-6R) to suppress the activation of the IL-6/IL-6R/Stat3 pathway, inducing the inflammatory M1 macrophages to shift toward the immunosuppressive M2 phenotype, which was desirable in GVHD patients. Similarly, topical application of autologous platelet-rich plasma in a prospective non-randomized clinical study showed improvement in dry eye signs in 322/368 patients. In comparison with autologous serum, the platelets secrete various growth factors, which are responsible for ocular surface reconstruction.^[88]

Approach to replace restore and regenerate Ocular Surface and Lacrimal unit

Lacrimal gland regeneration

For patients with severe DED, lacrimal gland function can be restored by either transplanting the artificially grown lacrimal gland having a secretory activity or by transplanting autologous salivary glands or allogenic lacrimal gland transplantation. The first two options have been tried in animal and human eyes, respectively, whereas the third option is still a creative imagination. Salivary gland transplantation for severe dry eye disease (<2 mm) achieved some improvement in tear volume but the dependency on lubricants remains and ocular surface changes did not reverse remarkably. Autologous minor and submandibular salivary glands have been transplanted but hyposmolar tears of the submandibular gland and very small

changes in tear volume with minor salivary gland transplant limit its widespread use. Porcine lacrimal gland shares similar morphology, ductal system, and vascular supply as the human lacrimal gland. The idea of using it as a xenograft was put forth. However, tear composition and immune rejection other than regulatory issues would limit its further exploration. The transplantation of a donor human lacrimal gland could be a possibility in the future as subconjunctival lacrimal gland transplantation has shown good results in the surgical dacryoadenectomy rabbit DED model. However, the transplanted gland was 70 mg in volume and Schirmer values were assessed at 1 month only.

Bioengineered lacrimal glands

The lacrimal gland is derived from the surface ectoderm during the ontogenesis in the embryo.^[89-91] The invagination of surface ectoderm forms an epithelial component and surrounding mesenchyme forms myoepithelial cells.^[92] We can replicate this *in vitro* with the use of epithelial and mesenchymal progenitor cells.^[93] Lacrimal gland revival with the use of stem cells can repair the lacrimal glands, which have some leftover function. In eyes with absolute tear deficiency, the whole lacrimal gland is non-functional and demonstrates no remaining tissue *in vivo*. Hence, we need to develop an *in vitro* technique that can fabricate a 3D fully functional bioengineered organ, which has transplantation potential.^[94] The existing bioengineered tissues and organs are composites of polymers and cells.^[95] Computer-aided design (CAD) and computer-aided manufacturing (CAM) technologies have developed fully functional organs and their substitutes for replacing organs, such as the eyes, heart, liver, and kidneys.^[96,97]

Bioengineering using the organ germ method was used initially for developing tooth and hair follicles.^[98] *In vitro* bioengineered organ germ method involves compartmentalization between epithelial and mesenchymal cells.^[99] The compartmentalized epithelial and mesenchymal cells are cultured at a high cell density and then grown in a type I collagen gel as a bioengineered organ germ.^[92] [Fig. 4]. The collagen drop method and co-culture of mice LG cells and Harderian gland's dissociated epithelial

and mesenchymal cells were employed for lacrimal gland regeneration.^[100] Branching morphogenesis was successfully observed in this culture after 3 days, along with stalk elongation and cleft-like structure formation. The mouse embryonic epithelial and mesenchymal cells were obtained from 16.5 embryonic days and were cultured using the collagen drop method. The cultured cells along with endothelial cells were seeded on matrigel and spheroids with acinar cell-like morphology were seen. The spheroids with duct were transplanted into a mouse model (no extra orbital gland) where the duct was coapted with intraorbital lacrimal gland duct segment [Fig. 4]. There occurred changes in corneal epithelial staining. The histology confirmed the morphological similarity with the lacrimal gland but it needs proof of *in vivo* secretory function and nerve stimulatory response.^[100] Likewise, these 3D bioengineered functional organ regeneration techniques were also employed in several other studies and screened for their potential in lacrimal gland regeneration.^[101,102] The development of a bioengineered LG organ germ offers a successful model for transplantation as it has shown a sufficient level of secretory potential.^[100]

Lacrimal gland culture

Lacrimal gland obtained from mice, rabbits, and humans has been cultured and grown *in vitro* in two- and three-dimension in the form of monolayers, spheroids (3D aggregation of cells), and organoids (3D with central secretory lumen) under appropriate culture media conditions.^[11,12,103] Very few studies have transplanted the cultured spheroids or organoids into the animal models of DED with encouraging short-term results on viability. Their effect on overall ocular surface health and dry eye improvement is yet to be studied. To be able to regenerate the lacrimal gland with transplantation potential, a 3D scaffold could be a better option. Scaffold can be either artificially printed material or decellularized human or animal tissue. Decellularized tissue contains an extracellular matrix and tissue-specific microenvironment without any immunogenic material. Hence, decellularized lacrimal gland or another tissue scaffold can be used. Extracellular matrix offers a better substrate for cell adhesion, growth, and proliferation as seen in many studies. Culturing lacrimal

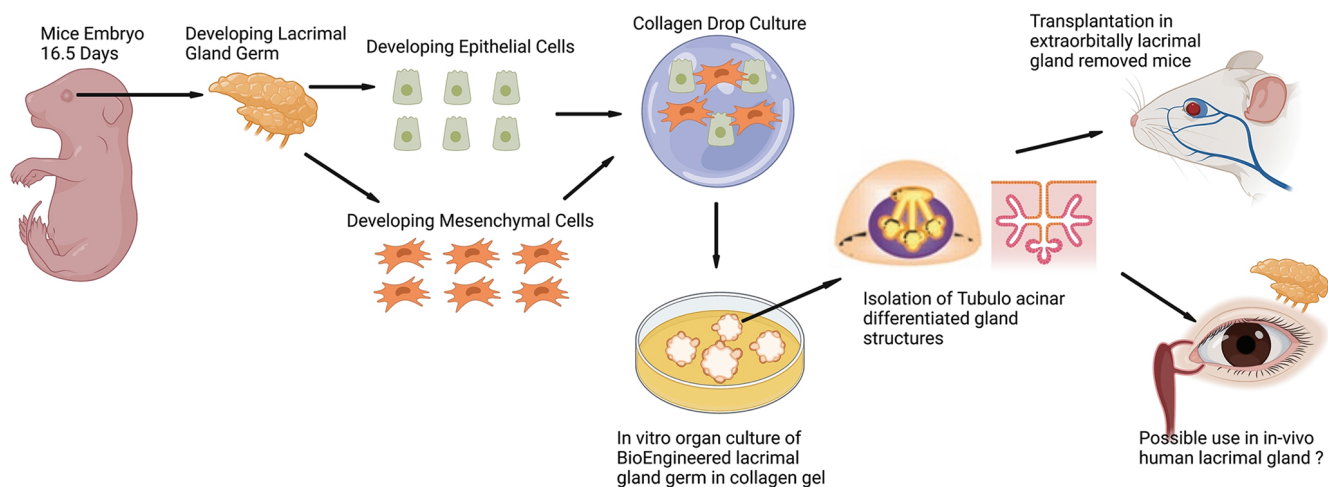


Figure 4: Bioengineered organ germ method to culture Lacrimal gland from the lacrimal gland germ cells of mice embryo 16.5 days old. The epithelial and mesenchymal cells are isolated and co-cultured using a collagen drop method. Over weeks tubule acinar structures are identified and these can be transplanted into the diseased glands

glands in decellularized tissue requires bioreactors. Porcine and rabbit lacrimal glands have been decellularized and repopulated with cultured epithelial cells [Fig. 5]. Acinar-like and ductal structures could be seen in these gland tissues growing over decellularized scaffold [Fig. 5].^[11] Thus, there is a possibility of culturing cells derived from the human lacrimal gland on the decellularized lacrimal gland. However, these artificially engineered lacrimal glands can only be placed into the damaged lacrimal gland as they lack vascular supply for *de-novo* transplantation.

Researchers have been able to create a scaffold with a blood supply. A biological vascularized scaffold (BioVaSc) is a decellularized segment of the porcine intestine along with its arteriovenous blood supply (Mertsching). The decellularized tissue can be used for culturing any tissue-specific cells where vascular channels and intestinal lumen are infused constantly via a bioreactor. The BioVaSc could be reendothelialized with autologous porcine endothelial cells after 3 weeks in a dynamic culture,^[104,105] were successfully implanted with vascular anastomosis to the pig's external iliac artery and vein. BioVaSc re-endothelialized with human autologous peripheral

blood mononuclear cells could be successfully implanted into a patient's upper arm and maintained its integrity without any thrombosis at 1 week.^[106] *In vitro*, BioVaSc, and bioreactor systems are used for creating a liver-like tissue, a fully functional vascularized human skin model, or a human cardiac patch.^[107-109] Porcine intestine is bigger in size for ocular use; hence, murine BioVaSc from rat intestine was generated. The Chinese hamster ovary (CHO) cells embedded in a collagen hydrogel inside the lumen of the mBioVaSc maintained secretory activity until 1 week.^[110] For the lacrimal gland, the luminal part of BioVaSc without vessels, that is, SIS-muc (small intestinal sub-mucosa with preserved mucosa) was used as a scaffold for growing LG epithelial cells under dynamic culture conditions [Fig. 5].^[12] For creating a 3D structure mimicking LG, spheroids containing acinar epithelial cells, mesenchymal stem cells, and endothelial cells were cultured in the lumen [Fig. 5]. The crypts of SIS-muc got seeded well with the spheroids and showed secretory properties.^[111] The bioreactor system provides a pulsatile flow and sufficient nutrient supply for supporting growth, expansion, and functional maturation *in vitro*.^[109,110] With reendothelialized vascular channels and lumen containing

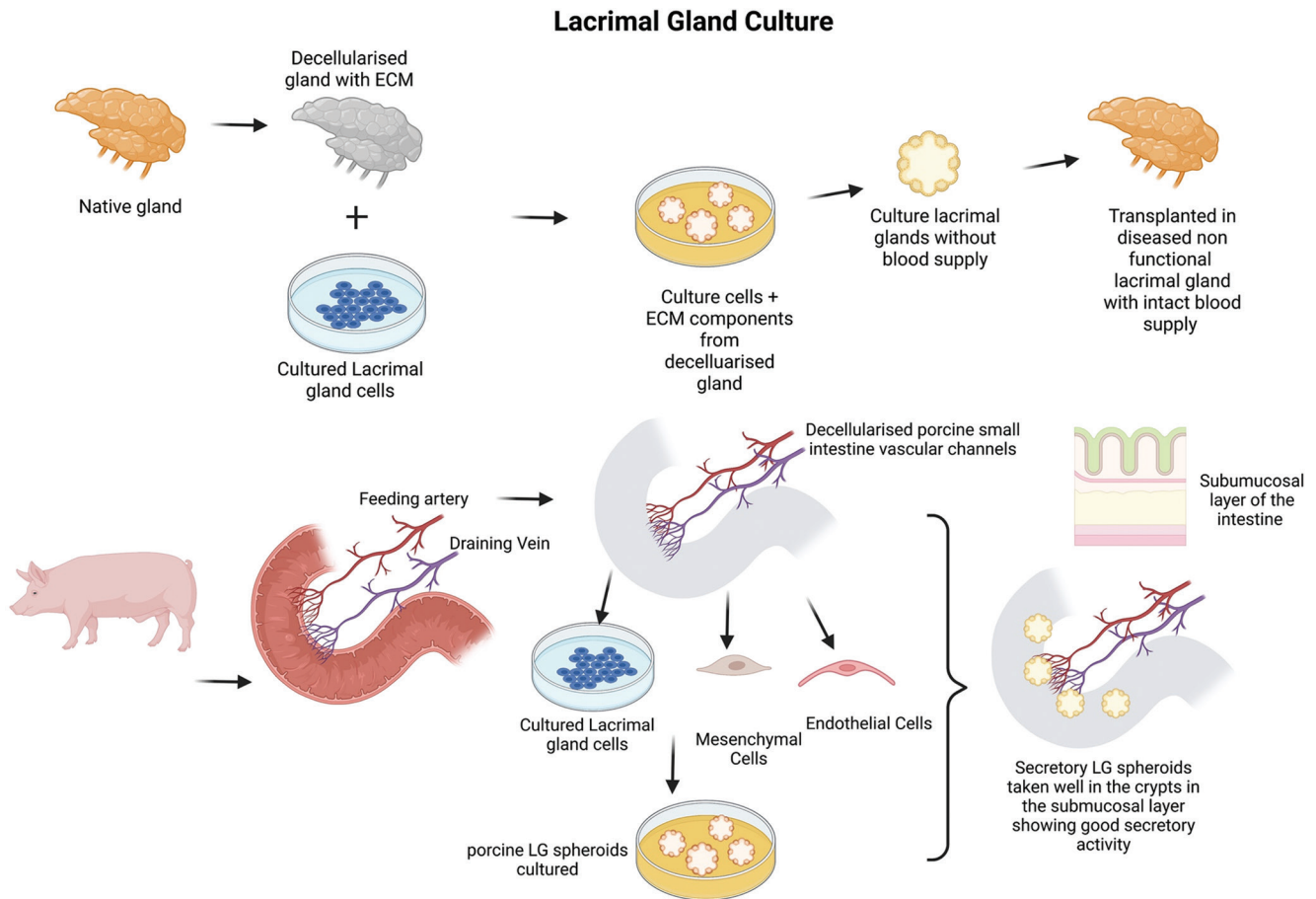


Figure 5: Above is a schematic representation of tissue-based lacrimal gland reconstruction- Extracellular matrix obtained from the decellularised lacrimal gland is repopulated with primary lacrimal gland cells to generate lacrimal gland constructs which are transplanted into the diseased gland with intact blood supply. Below is a schematic representation of developing a vascularised Lacrimal gland construct/spheroid/organoid grown within a segment of the porcine small intestine (jejunum) with intact vascular channels stemming from the iliac artery and veins. The cultured primary lacrimal gland cells along with MSCs and endothelial cells are grown in the submucosal region of the crypts of the intestine. These vascular channels can be connected to a bioreactor with perfusion of culture media mediated through pressure sensors and peristaltic pumps to maintain the nutrition of the constructs and develop viable blood vessels

lacrimal gland organoids, it is possible to transplant them into the eye after establishing vascular anastomosis.

Future Perspectives and Unmet Needs

Research and development breakthrough in biomaterials technology opens up a promising area of research in DED. There is a distinct advantage over conventional administration routes, but there is a scope for further improvement in their design and efficacy of the biomaterials and studying the effect of nanosystems particle size, composition, and surface charge on pharmacokinetic studies.^[112] Further standardization to improve large-scale manufacturing, long-term studies, and clinical trials to determine their ocular toxicity and biocompatibility will help in bringing these therapies to the clinic. Similarly, further studies are needed to understand the *in vivo* behavior and relationship of drugs with hydrogels and drug-eluting contact lenses in human DED patients.

In contrast, the regeneration of the complete lacrimal gland in the future looks promising. Future directions would be to look into the understanding of the genes regulating lacrimal gland development, identifying cell sources, and also important transcription factors, which can induce LG regeneration from induced pluripotent stem cells. MSCs derived from lacrimal gland tissue are multipotent and can support the growth of lacrimal gland acinar and epithelial cells; however, it is difficult to isolate a potent and pure colony of these cells.^[113] Although bioengineered glands have been transplanted successfully in animals, their therapeutic efficacy in humans needs to be tested. Identification of the appropriate progenitor cells in human LG and establishing protocols to isolate, culture, and maintain these cell lines for injection in damaged human lacrimal glands could be the next step in translational research. Detailed *in vivo* studies are essential to study clinical applicability of cultured lacrimal glands.

Most stem cell work has shown short-term benefits in improvement in the secretion of residual acinar cells of salivary glands in animals, the results of which can be extrapolated to the lacrimal glands; however, this needs to be studied separately. Also, it is important to explore various techniques that can evaluate lacrimal gland anatomy and cellular structure and function in patients for the clinical application of therapies.

MSCs through various routes have shown significant advantages in their use to decrease inflammation and immunomodulation of the ocular surface and in the future, establishing standard protocols for isolating appropriate cell sources, types, and manufacturing for commercial use could be a way ahead.

Conclusion

The new emerging therapies discussed in this review have the potential to decrease the burden of disease due to DED and provide a definite advantage over the conventional palliative therapy of applying lubricant eye drops. They also provide a solution to the advanced stages of the disease by addressing the root cause.

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Conflicts of interest

There are no conflicts of interest.

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