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REVIEW ARTICLE

Role of stem cells in regenerative treatment of dry eye disease caused by lacrimal gland dysfunction

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Abstract

An ageing population and increased screen use in younger people have contributed to a rise in incidence of dry eye disease (DED). Quality of life can be significantly affected by DED, with patients experiencing eye dryness, burning, pain and sensitivity to light. If left untreated, DED may progress to cause lasting damage to the delicate cell layers of the ocular surface. The aqueous-deficient form of DED is characterized by decreased tear volume. This can occur through underlying disease or damage to the lacrimal gland (LG), which results in increased inflammation at the ocular surface and decreased tear secretion. Regenerative therapy for treatment of aqueous-deficient DED would ideally restore LG function without causing adverse side effects and be feasible in terms of cost, production and practical application in the clinic. In this review, we evaluate research directed at the development of clinical procedures for regeneration of the LG using various stem cell types and their products. We also discuss work identifying potential therapeutic targets that may alter pathways to effect healing and ameliorate development of DED. Finally, we discuss shortcomings and recommend future avenues for research. These include determination of the best tissue of origin for mesenchymal cells and transference of knowledge gleaned from animal studies to clinical investigations.

KEYWORDS
dry eye disease, lacrimal gland, regeneration, stem cells

Catherine J. Jackson and Maria Naqvi contributed equally to this work.

1 | INTRODUCTION

The primary function of the tear film is to protect and lubricate the ocular surface and underlying ocular tissues including the sclera and cornea (Willcox et al., 2017). Tears are composed of an anterior lipid layer, synthesized by the meibomian glands and an underlying muco-aqueous layer produced mainly by the lacrimal glands (LGs) and conjunctival epithelial cells. Dry eye disease (DED), also known as keratoconjunctivitis sicca, is a chronic multifactorial disease, which is categorized by loss of tear film stability and hyperosmolarity of tears (Villatoro et al., 2017). DED patients experience a wide range of symptoms including pain, discomfort and blurred vision (Bron et al., 2017). Reduced tear secretion can eventually lead to peripheral nerve damage (Belmonte et al., 2017). Risk factors for development of DED include age, sex, ethnicity, environmental humidity levels and computer use (Bron et al., 2017). Although DED is one of the most common ocular surface conditions, no definite curative treatment is available. Treatment options are for the most part palliative and include artificial tears, nutritional supplements and topical steroids (Milner et al., 2017). Prevalence of DED ranges from 5% to 50% (Villatoro et al., 2017), and DED symptoms are among the most common reasons for ophthalmology visits in the United States.

Dry eye disease is classified as evaporative DED or aqueous-deficient DED. However, there is often overlap of these subtypes, termed mixed DED (Craig, Nichols, et al., 2017). Evaporative DED is typically caused by meibomian gland dysfunction, which results in destabilization of the lipid layer leading to excessive evaporation of tears and hyperosmolarity. Diseases such as diabetes, rosacea and thyroid disease can contribute to LG damage and the development of aqueous-deficient DED (ADDE) (Craig, Nelson, et al., 2017). LG damage can also occur though injury, for example, through radiation therapy to the head and neck (Tiwari et al., 2017). ADDE is further classified as Sjogren's and non-Sjogren's dry eye. Aqueous-deficient DED and evaporative DED may be considered as different entry points to the 'vicious cycle of dry eye disease' (Figure 1) (Baudouin et al., 2016; Craig, Nichols, et al., 2017). The vicious cycle of DED often initiates from hyperosmolarity (Bron et al., 2017), which leads to cell damage at the ocular surface. Cells that are damaged include goblet cells that are responsible for mucin secretion into the tear film. Damage to goblet cells may lead to ocular surface stress that can cause nerve stimulation of the LG to secrete more aqueous tear fluid. Prolonged damage to the ocular surface can lead to cytokine and matrix metalloproteinase (MMP) release resulting in inflammation. Over time, the cascade of these events leads to tear film instability and excessive evaporation of the tear film (Baudouin et al., 2013; Bron et al., 2017).

Chronic inflammation is considered a major contributing factor underlying LG dysfunction, eventually affecting LG secretion that leads to ADDE (Zoukhri, 2006). Chronic inflammation may be caused by among other factors, autoimmune diseases (Sjogren's syndrome, diabetes), organ transplantation (chronic graft-versus-host disease) or through ageing. The LG is the main contributor to the aqueous layer component of the tear film. In addition, the LG supplies the ocular surface with numerous proteins and protective factors such as enzymes, antimicrobial factors and immunoglobulins that protect the integrity of the cornea and conjunctiva (Dartt & Willcox, 2013). LG dysfunction therefore significantly contributes to disruption of the tear film and its protective function (Pflugfelder & de Paiva, 2017). Treatment options for ADDE include supplementation with artificial tears and prescription of anti-inflammatory drugs that inhibit the expression of inflammatory mediators and promote the secretion of tears (Hessen & Akpek, 2014). In addition, punctal plugs may be used. However, complications such as spontaneous extrusion have been reported in connection with punctal plug use (Tai et al., 2002).

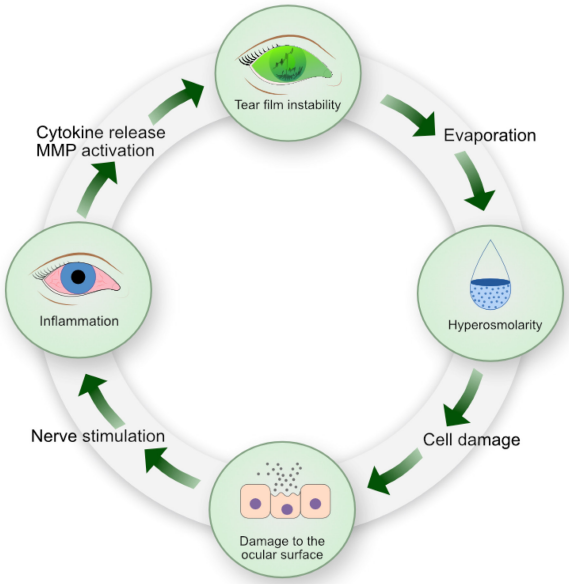

FIGURE 1 Vicious cycle of dry eye disease. Illustration adapted form Baudouin et al. (2013). A vicious cycle of dry eye disease can be initiated by several factors. Chronic exposure to cell damaging conditions can give rise to an inflammatory state resulting in the release of cytokines and matrix metalloproteinases (MMPs) at the ocular surface. This in turn can lead to tear film instability, which contributes to hyperosmolarity and continuation of the vicious cycle. Copyright K. Skårdal/M.

The LG consists of several lobules that are further composed of functional units called acini (Figure 2). Acini are comprised of three main cell types: acinar, ductal and myoepithelial cells. Acinar cells are the most abundant (~80%). They are highly polarized epithelial cells responsible for the synthesis, storage and secretion of proteins, electrolytes and water that are essential for homeostasis of the ocular surface. LG duct cells modify the primary LG fluid derived from acinar cells by secreting electrolytes and water into the lumen of the ducts (Shatos et al., 2012). LG duct cells supply about 30% of the LG fluid (Dartt, 2009). In addition, myoepithelial cells surround the acini and play a contractile function ensuring the secretion of fluid onto the ocular surface (Makarenkova & Dartt, 2015). The LG is an exocrine tubular structure capable of self-regeneration (Shatos et al., 2003).

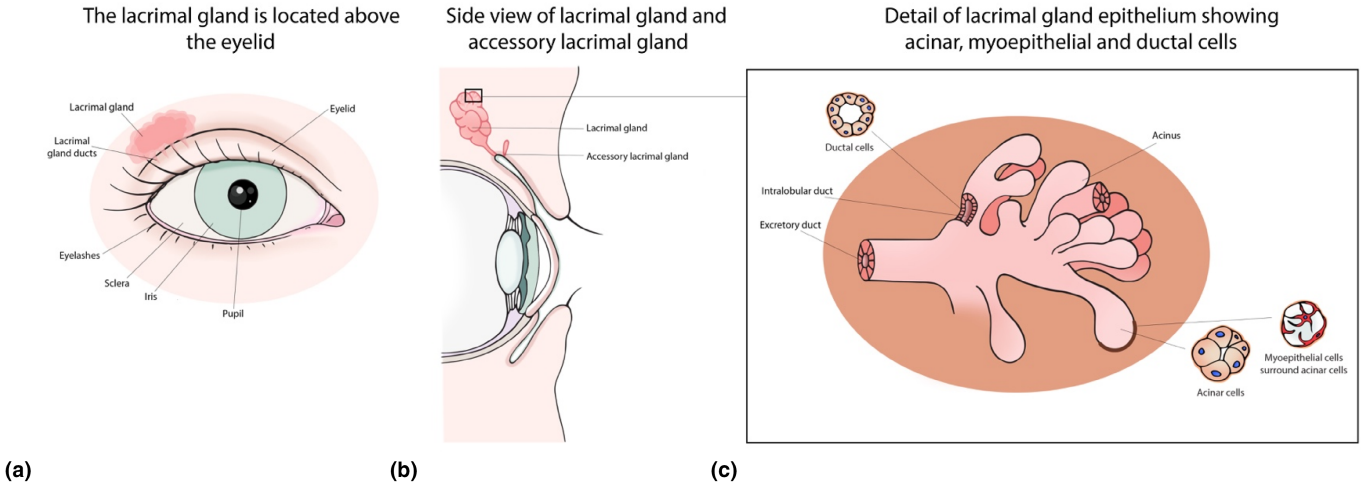

FIGURE 2 The illustration shows the location and structure of the lacrimal gland. Copyright K. Skårdal/M.

Recent developments within the field of regenerative medicine have demonstrated the therapeutic potential of stem cell (SC) transplantation to restore organ/tissue function albeit not to original levels. For instance, limbal SCs have been used to promote regeneration of the corneal epithelium in the eye (Stern et al., 2018). SCs are defined as undifferentiated cells capable of self-replication. They are multipotent, meaning that they have potency to differentiate to other cell types.

Stem cells are primarily divided into two categories; embryonic and adult. Embryonic SCs are pluripotent SCs derived from the inner cell mass of a blastocyst. Their ability to differentiate into all three germ layers that form the whole body make them good candidates for use in regenerative medicine. However, SCs in the adult body that function to maintain cellular turnover and perform repair have been found to retain varying degrees of differentiation potency. Application of adult SCs in regenerative therapy means that ethical and immunological issues associated with use of embryonic SCs can be avoided. It has been shown that adult murine LG stem cells (LGSCs) retain the ability to differentiate to produce acinar, myoepithelial and ductal cells, making them potential candidates for use in the repair of LG damage (You, Tariq, et al., 2011; Zoukhri et al., 2007). Thus, discovery of SCs in the adult LG offers a potential alternative to the use of embryonic SCs in regeneration of the LG. For example, a portion of the LG may be harvested before radiation therapy to ensure supply of SCs for future expansion and transplantation in the event the LG is damaged during therapy (Tiwari et al., 2017). However, alternative sources of adult SCs present in various tissues throughout the body also offer a potential source of autologous cells. Mesenchymal stem cells (MSCs) are particularly important as they do not express immune stimulating markers on the cell surface, thus avoiding initiation of a graft-versus-host response if used as an allogeneic source (Han et al., 2019). Furthermore, MSCs are present in many different tissue types including adipose tissue, skin and bone marrow, which present several easily accessible sites for harvest from the patient for auto-transplantation (Rajabzadeh et al., 2019).

Regenerative therapy for treatment of ADDE will ideally restore LG function without causing adverse side effects and be feasible in terms of cost, production and practical application in the clinic. This review discusses work identifying potential therapeutic targets that may alter pathways to effect healing, ameliorating the development of DED. Additionally, this review focuses on research directed at the development of clinical procedures for regeneration of the LG using various SC types and their products.

2 | SEARCH STRATEGY

Three separate PubMed searches were conducted using the search terms: (1) Lacrimal gland repair AND regeneration AND stem cells OR progenitor cells AND dry eye; (2) progenitor cell* OR stem cell* AND lacrimal gland and; (3) lacrimal gland AND ocular surface AND bioengineering AND dry eye disease (Figure 3).

Of the initial 489 articles discovered, 25 were included in this review (Figure 3). All review articles, studies not available in English and duplicates were excluded. The remaining search results were assessed according to their relevance to the aim of this review based on the title and abstract. The articles were independently reviewed by two of the authors (MN and CJ). Further examination of the 47 remaining studies showed that the majority focused solely on LG cell markers or different laboratory methods that may be used to isolate LGSC. While interesting, these topics were considered too large to include in this review. Studies focused on therapy for patients with chronic graft-versus-host disease were also omitted.

3 | REGENERATION OF THE LACRIMAL GLAND USING STEM CELLS

3.1 | Overview of studies

The remaining 25 studies included in this review investigated the development of new drug targets and therapies or focused on direct LG regeneration using SCs from various sources (Table 1). Studies were published between June 2008 (Zoukhri et al., 2008) and September 2020 (Yu

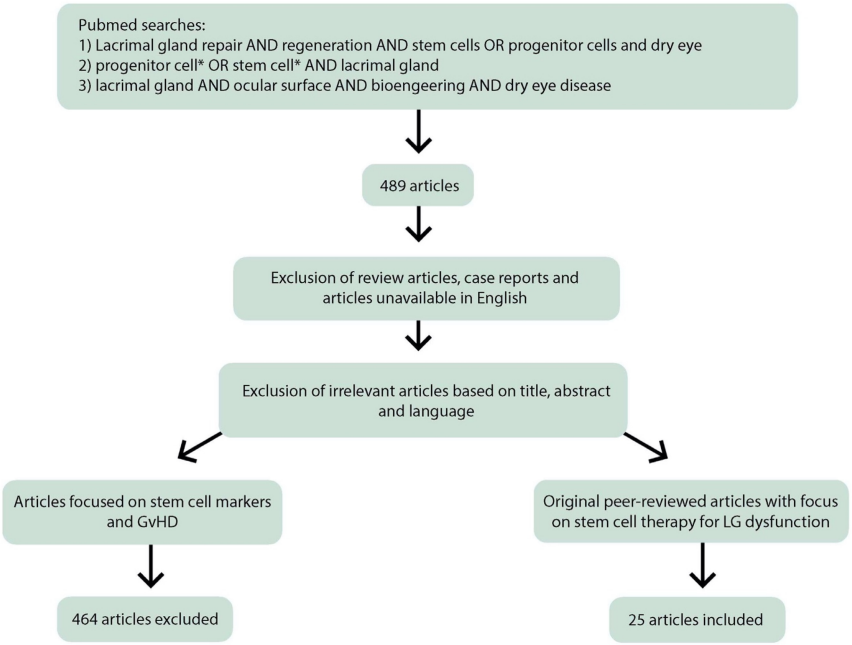

FIGURE 3 Flow chart illustrating the PubMed search, inclusion and exclusion of articles. Copyright K. Skårdal/M.

TABLE 1 Overview of the 25 studies included in this review.

Study	Focus	Outcome measures	Model	Key findings		Clinical relevance
Drug target identification					**Key pathways involved**	
Dietrich, Roth, et al. (2019)	LG epithelial cell survival after ethanol damage	-	Mice/in vitro	The MSC secretome has beneficial effects on the viability of damaged LG epithelial cells	Secreted proteins with binding capacity (STAT1 and RAC1) promote LG epithelial cell survival which may enhance LG regeneration	DT
Yao et al. (2019)	Role of IL-27 in MSC transplantation	-	Mice/in vitro	MSC transplantation elevates IL-27 levels	MSCs mediate IL-27 production in dendric cells resulting in balanced Th17/T regulatory cell ratio	DT
Ali et al. (2017)	LG PC and LG epithelial cell markers in damaged LG	-	Human/ex vivo	Progenitor marker expression decreases with age. LG epithelium marker histatin-1 expression may decrease with DED	Histatin peptides have antimicrobial properties and may reduce ocular inflammation	DV
Roth et al. (2015)	Effect of oxygen concentration on MSC phenotype	-	Mice/in vitro	Low oxygen concentration (5%) is beneficial to MSCs during in vitro expansion	At higher oxygen concentrations, ROS levels increase and lead to upregulation of p53 which may cause apoptosis (cell death)	DT
Zhang et al. (2014)	Radioprotective effect of iPSC-conditioned medium on gamma irradiation-induced LG injury	-	Mice/in vitro	iPSC-conditioned medium has protective effects on radiotherapy-injured LGs. Characterized by an increase in neutrophil influx and production of p38	iPSC-conditioned medium inhibits p38/JNK signalling. High levels of midikine in iPSC-conditioned medium contributes to LG regeneration	DT
You et al. (2012)	Epithelial–mesenchymal transition in LG regeneration	-	Mice/in vitro	Epithelial– mesenchymal transition is induced during LG repair by Snail transcription factor to generate MSCs that migrate to the site of injury	Snail induces vimentin expression. Vimentin and nestin are likely involved in promoting migration of MSCs to the site of injury	DV

TABLE 1 (Continued)

Study	Focus	Outcome measures	Model	Key findings		Clinical relevance
Zoukhri et al. (2008)	Mechanisms involved in LG injury and repair	-	Mice/in vivo	LG contains SC/PC capable of tissue repair after injury	BMP7 pathway is active in SC/PC during regeneration of the LG contributing to tissue remodelling and inhibition of apoptosis	DT
Stem cell transplantation					**Method of application**	
Jeong et al. (2021)	Establish a 3D LG organoid with different cell types	N/A	Mice/in vivo	LG organoids were successfully engrafted into recipient mice and expressed Aquaporin 5	LG organoid transplantation into recipient mice	T
Xiao and Zhang (2020)	Establish culture methods for LGs	TS and lymphatic infiltration	Mice in vivo	Serum-free culture of LG SC is efficient for the transplantation and repair of LG	Allotransplantation of LG SC into diseased LG	T
Moller-Hansen et al. (2020)	Allogeneic adipose-derived MSC transplantation into LG	OSDI, OSS, tear osmolarity, TS and TBUT	Human/in vivo	Injection of allogeneic adipose-derived MSC into the LG is a safe and feasible treatment for severe ADDE	Adipose-derived MSC injected into LG	T
Yu et al. (2020)	Therapeutic efficacy of human adipose-derived MSC-extracellular vesicles on DED	TS, OSS	Mice/in vivo	Human adipose-derived MSC extracellular vesicles eye drops effectively suppress NLRP3 inflammatory response and alleviate ocular surface damage in DED	Topical administration of SC-derived extracellular vesicles	T
Abughanam et al. (2019)	Sjogren's syndrome treatment using MSCs and MSC extract	Lymphocytic infiltration, corneal thickness and TFR	Mice/in vivo	MSCs and MSC extract therapies were successful and comparable in preserving LG function in NOD mice	Injection of MSCs and MSC extract into the tail vein of NOD mice	T
Yao et al. (2019)	MSC transplantation benefits	Lymphatic infiltration, SFR	Mice/in vivo	MSC transplantation reduced Sjogren's syndrome-like symptoms and increased IL-27 in mice	Injection of MSC into the tail vein of NOD mice	T
Dietrich, Ott, et al. (2019)	MSC transplantation in mice with induced ADDE	TS, OSS and corneal thickness	Mice/in vivo	Application of MSCs may induce LG regeneration in ADDE patients	MSC were injected subcutaneously into wild-type mice with surgically induced ADDE	T
Liu (2017)	Therapeutic effects of adipose-derived MSC in autoimmune dacryoadenitis	TS, TBUT and OSS	Rabbits/in vivo	Adipose-derived MSC transplantation reduced T-regulatory cell response and reduced Th17 cytokine gene expression	Adipose derived-MSC were injected intravenously into rabbits	T
Aluri et al. (2017)	Bone marrow-derived MSC transplantation in Sjogren's syndrome mice	TS, lymphocytic infiltration	Mice/in vivo	Transplantation increased TS, reduced lymphocytic infiltration and increased aquaporin 5 expression	Bone marrow-derivedMSC injected intraperitoneally into NOD mice	T
Gromova et al. (2017)	Epithelial PC transplantation for ADDE therapy	TS, LG morphology	Mice/in vivo	Epithelial PC engraftment into acinar and ductal compartments results in improved LG function	LG epithelial PCs were injected into IL-1 injured LG	T
Basova et al. (2017)	PC engraftment	N/A	Mice, human/ in vivo, in vitro	Panx1 and/or Casp4 inhibition enhance donor cell engraftment	Epithelial PCs were transplanted into IL-1-injured LG in wild-type mice	T

(Continues)

TABLE 1 (Continued)

Study	Focus	Outcome measures	Model	Key findings		Clinical relevance
Bittencourt et al. (2016)	MSCs transplantation in dogs	TS, Conjunctival hyperaemia	Dogs/in vivo	MSC transplantation statistically improved TS and clinical symptoms	MSC transplantation into the dorsal LG of dogs with DED	T
Villatoro et al. (2015)	LG transplantation of allogeneic adipose-derived mesenchymal stromal cells	TS, ocular discharge, Hyperemia and corneal changes	Dogs/in vivo	Transplantation reduced clinical signs of DED with a sustained effect during the study period	Adipose-derived MSC transplanted around the main LG of dogs with DED	T
Xie et al. (2015)	Characterize potential candidate cells for constructing a tissue-engineered LDE	N/A	Rabbits/ in vitro, in situ	Primary palpebral and fornical conjunctival epithelial cells are similar to LD epithelial cells and are candidate cells in treatment of LD diseases	N/A	T
Lee et al. (2015)	MSCs transplantation in mice	TS, epithelial integrity	Mice/in vivo	MSCs suppresses T-cell-mediated inflammation and contributes to TS and goblet cell survival	Injection of human or mouse bone marrow-derived MSC into the periorbital space of mice treated with zolazepam–tiletamine immediately prior to transplantation	T
Beyazyildiz et al. (2014)	Topical application of MSC	Schirmer, TBUT and OSS	Rats/in vivo	Treatment with MSC reduced inflammation, increased epithelial recovery, goblet cell density and tear volume	Bone marrow-derived MSC applied topically once daily for 1 week	T
Hirayama et al. (2013)	Transplantation of bioengineered LG	OSS, TS	Mice/in vivo	Bioengineered LG develop in vivo leading to physiological functionality	LG replacement with bioengineered LG into wild-type mice	T
Mishima et al. (2012)	Side population cells in hypofunction of LGs	TS	Mice/in vivo	Endothelial cell-derived clusterin possibly inhibits ROS-induced hypofunctional LGs	Transplantation of side population into irradiated LG	T

ADDE, Aqueous-deficient dry eye; BMP7, Bone morphogenic protein 7; DED, Dry eye disease; IL-27, Interleukin 27; DT, Potential drug target; DV, Diagnostic value; iPSC, Induced pluripotent stem cell; LD, Lacrimal duct; LG, Lacrimal gland; MSC, Mesenchymal stem cells; NOD, Non-obese diabetic; OSDI, Ocular surface disease index; OSS, Ocular surface staining; PC, Progenitor cells; RAC1, ras-GTPase-activating-binding-protein 1; ROS, Reactive oxygen species; SC, Stem cells; SFR, Salivary flow rate; STAT1, signal transducer and activator of transcription 1; T, Transplantation; TBUT, tear film break-up time; TFR, tear flow rate; Th17, T-helper 17 cells; TS, tear secretion.

et al., 2020) in the United States (Ali et al., 2017; Aluri et al., 2017; Basova et al., 2017; Gromova et al., 2017; Lu et al., 2017; You et al., 2012; Zoukhri et al., 2008), China (Liu, 2017; Xiao & Zhang, 2020; Xie et al., 2015; Yu et al., 2020; Zhang et al., 2014), Germany (Dietrich, Ott, et al., 2019; Dietrich, Roth, et al., 2019; Roth et al., 2015), Canada (Abughanam et al., 2019; Yao et al., 2019), Japan (Hirayama et al., 2013; Mishima et al., 2012), Denmark (Moller-Hansen et al., 2020), Korea (Jeong et al., 2021; Lee et al., 2015), Brazil (Bittencourt et al., 2016), Spain (Villatoro et al., 2015) and Turkey (Beyazyildiz et al., 2014) (Figure 4).

3.2 | Stem cell markers employed in studies

Many tissue types in the body are able to self-regenerate after injury and have demonstrated the presence of adult stem cells that exist in specialized protective niches. Stem cells are mobilized to proliferate after injury. There is increasing evidence for the presence of SCs in the mouse LG that have been shown to contribute to reconstruction following injury (You, Kublin, et al., 2011). In addition, alternative cell types originating from other tissues have been studied for their regeneration capability in the LG.

Investigated cell types included MSCs (Dietrich, Roth, et al., 2019; Roth et al., 2015; You et al., 2012), ductal cells (Abughanam et al., 2019), acinar cells (Abughanam et al., 2019; Xiao & Zhang, 2020; Zoukhri et al., 2008), LGSCs (Abughanam et al., 2019; Ali et al., 2017; Xiao & Zhang, 2020; Xie et al., 2015; Zoukhri et al., 2008) and side population cells (Mishima et al., 2012). One study (Jeong et al., 2021) evaluated the SC component in LG organoids cultured in vitro (Tables 2 and 3). MSCs were harvested from interleukin-1β-injured LG from mice

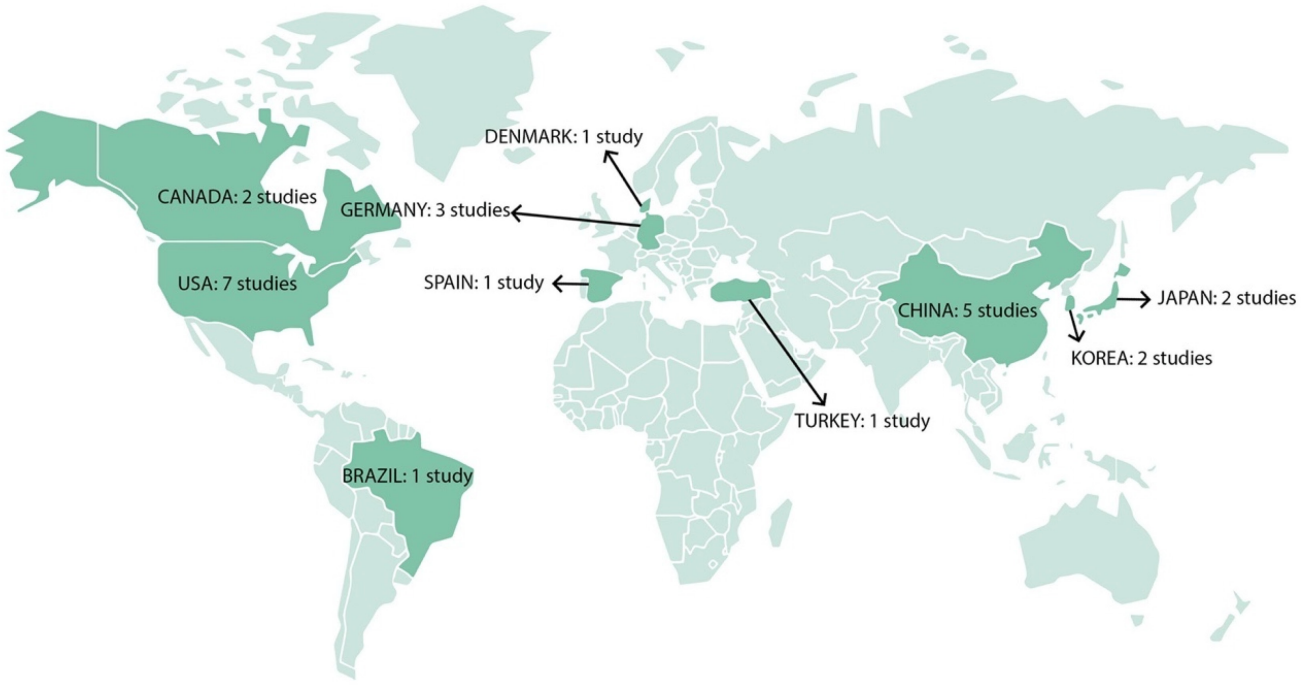

FIGURE 4 World map figure showing the location of the included studies. Copyright K. Skårdal/M.

TABLE 2 Stem cell markers: 10 studies investigated the presence of stem cell markers in various cell types in the lacrimal gland.

		Methods				Proteins investigated								
Study	**Cell type**	**IS**	**PCR**	**WB**	**FC**	**Nestin**	**P63**	**Aqp5**	**α-SMA**	**Lyz**	**Sca-1**	**Ki67**	**Other**	**Species**
Jeong et al. (2021)	LG organoids	+	+					+	+	+			+[a]	Mice
Xiao and Zhang (2020)	Acinar, SC	+	+		+	+	+	+				+	+[b,c]	Mice
Abughanam et al. (2019)	Ductal, acinar and PC	+						+	+	+			+[d,e]	Sjogren's syndrome mice
Dietrich, Roth, et al. (2019)	MSC				+	+					+		+[f]	Mice
Ali et al. (2017)	SC, Epithelial cells	+				+		+					+[f,g]	Human
Roth et al. (2015)	MSC				+						+		+[b]	Mice
Xie et al. (2015)	SC											+		Rabbit
You et al. (2012)	Epithelial cells, MSC	+	+	+		+			+				+[a]	IL1-treated mice
Mishima et al. (2012)	Side population cells		+		+			+			+		+[d]	Mice
Zoukhri et al. (2008)	Acinar, SC	+		+		+			+			+		IL1-treated mice

Abbreviations: Aqp5, Lacrimal tissue marker; C-kit, Marker for stem cells; FC, Flow cytometry; IL1, Interleukin 1; IS, Immunostaining; Ki67, Marker for cell proliferation; Lyz, Marker for tear secretion; MSC, Mesenchymal stem cells; P63, Marker for progenitor cells; PCR, Polymerase chain reaction; SC, Stem cells; Sca-1, Marker for stem cells; WB, Western blot; α-SMA, Marker for myoepithelial cells.

[a]Vimentin (Marker for mesenchymal stem cells),

[b]Krt14 (Marker for stem cells).

[c]Krt5 (Marker for stem cells).

[d]CK5 (Marker for ductal progenitor cells).

[e]C-kit (Marker for stem cells).

[f]CD29 (Marker for precursor cells).

[g]ABCG2 (Marker for precursor cells).

(You et al., 2012) and healthy LG from mice (Dietrich, Roth, et al., 2019; Roth et al., 2015). Techniques such as western blot (You et al., 2012; Zoukhri et al., 2008), flow cytometry (Dietrich, Roth, et al., 2019; Mishima et al., 2012; Roth et al., 2015; Xiao & Zhang, 2020), immunostaining (Abughanam et al., 2019; Ali et al., 2017; Jeong et al., 2021; Xiao & Zhang, 2020; You et al., 2012; Zoukhri et al., 2008) and polymerase chain reaction (Jeong

TABLE 3 Optimization of culture methods.

Study	Cell type	Isolation method	Culture medium	Morphology	Culture observations	Species	Treatment
Xiao and Zhang (2020)	LGSC	Explant and Matrigel	Lacrimal gland stem cell medium	SC from primary culture formed spheres	Spheres expressed E-cadherin, Epcam, Krt14 and Ki67	Mice	LGSC transplantation
Dietrich, Roth, et al. (2019)	MSC	Explant, FACS, and cell strainer	α-minimum essential medium	Spindle-shaped fibroblast-like cells	MSC were able to differentiate into adipocytes and osteoblasts	Mice	Addition of IL-1α and IFNγ to culture medium
Lu et al. (2017)	Acinar cells and conjunctival epithelial cells	Explant and matrigel	Dulbecco's-modified eagle medium	Spheroids with acinus like compartments	Co-culture increased mucin secretion and lactoferrin mRNA increased	Rabbit	IL-1β and dexamethasone
Xie et al. (2015)	LD epithelium	Explant	Dulbecco's-modified eagle medium, foetal bovine serum	Conjunctival epithelial cells with cobblestone morphology	Conjunctival epithelial cells stained positive for CK4 and MUC5AC	Rabbits	N/A
Zhang et al. (2014)	Induced pluripotent stem cells	Explants	Induced pluripotent stem cell-derived conditioned medium	Tubulo-acinar structure	Induced pluripotent stem cell-derived conditioned medium elevated HMGB1 and PAI-1 protein levels	Mice	Gamma irradiation and induced pluripotent stem cell-derived conditioned medium

Note: Five studies focused specifically on culture methods of various cell types.

Abbreviations: CK4, Cytokeratin 4; Epcam, Epithelial cell adhesion molecule; FACS, Fluorescence-activated cell sorting; HMGB1, High mobility group box protein 1; IFN, Interferon; IL-1, Interleukin; Krt14, Keratin 14; LD, Lacrimal duct; LGSC, Lacrimal gland stem cell; MSC, Mesenchymal stem cells; MUC5AC, Mucin 5 AC; PAI-1, Plasminogen activator inhibitor-1; SC, Stem cells.

et al., 2021; Mishima et al., 2012; Xiao & Zhang, 2020; You et al., 2012) were used for identification or isolation of cells.

Overall, most investigators employed well-established SC markers. For instance, nestin, an intermediate filament, was used by many studies to identify LGSCs, which are shown to be upregulated and increase in number following LG injury (Ali et al., 2017; Xiao & Zhang, 2020; Zoukhri et al., 2008). P63, which is a commonly used epithelial SC marker in other tissues, was used to identify LGSCs (Xiao & Zhang, 2020). Aquaporin 5 (Aqp5), an established marker for acinar cells, was employed in many of the studies (Abughanam et al., 2019; Ali et al., 2017; Mishima et al., 2012; Xiao & Zhang, 2020). All of the studies were able to establish the presence of SC markers in the tissue studied, which may indicate the presence of a SC niche in the LG. An alternative hypothesis is the transdifferentiation of existing cells to SCs (You et al., 2012). Further, the studies reported an increase in the number of nestin-positive cells after interleukin-1β injury (You et al., 2012; Zoukhri et al., 2008).

3.3 | Culture methods reported in studies

Culture conditions can influence the quality and character of isolated cells, the phenotype of SCs and control differentiation status. The majority of studies used the explant culture method (Dietrich, Roth, et al., 2019; Lu et al., 2017; Xiao & Zhang, 2020; Xie et al., 2015; Zhang et al., 2014) in combination with flow cytometry (Dietrich, Roth, et al., 2019) cell strainers (Dietrich, Roth, et al., 2019) or Matrigel (Lu et al., 2017; Xiao & Zhang, 2020) for cell isolation. Two studies aimed to optimize the culture method specific for cell type: LGSCs (Xiao & Zhang, 2020) and MSCs (Dietrich, Roth, et al., 2019). Another study identified suitable cells to produce tissue-engineered lacrimal duct epithelium (Xie et al., 2015). Roth et al. (2015) expanded LG MSCs under low oxygen (5%) to mimic the in vivo microenvironment.

Isolated cells were cultured in Dulbecco's modified eagle medium (Lu et al., 2017; Xiao & Zhang, 2020; Xie et al., 2015), α-minimum essential medium (Dietrich, Roth, et al., 2019) or in induced pluripotent stem-cell derived conditioned medium (Zhang et al., 2014). One study explored the potential of using a serum-free medium to maintain LGSCs in 3D culture (Xiao & Zhang, 2020). Cultured cells were classified according to morphology (Dietrich, Roth, et al., 2019; Lu et al., 2017; Xiao & Zhang, 2020; Xie et al., 2015; Zhang et al., 2014) and immunohistochemical staining (Xiao & Zhang, 2020; Xie et al., 2015; Zhang et al., 2014). Some studies identified SCs by their ability to differentiate into various cell types such as adipocytes and osteoblasts (Dietrich, Roth, et al., 2019) or acinar and ductal cells (Xiao & Zhang, 2020). Three studies used 3D culture strategies

to investigate the possibility of lacrisphere transplantation in mice (Jeong et al., 2021; Xiao & Zhang, 2020) and with the aim of defining an in vitro model for the ocular surface (Lu et al., 2017). The authors of the latter article found that co-culturing LG spheres with conjunctival epithelial cells increased tear secretory function. Although the model system does not reflect normal anatomy, it was highlighted as an alternative in vitro model for dry eye as the effects of therapeutics may be studied. Studies investigating LG culture using 3D techniques demonstrate great innovative potential as an alternative to animal models of DED and for allotransplantation.

3.4 | Models of lacrimal gland dysfunction

Five articles included in this review had a particular focus on DED animal models, the mechanism of pathogenesis and the effects of various treatments (Abughanam et al., 2019; Basova et al., 2017; Bittencourt et al., 2016; Dietrich, Ott, et al., 2019; Mishima et al., 2012; Villatoro et al., 2015). Most studies employing animal models used mice (Abughanam et al., 2019; Basova et al., 2017; Dietrich, Ott, et al., 2019; Mishima et al., 2012) to study LG injury (Table 4). Mice were wild-type with genetic modifications such as green fluorescent protein transgene insertion (Dietrich, Ott, et al., 2019; Mishima et al., 2012) or Pax6-labelled (Basova et al., 2017). Other models included non-obese diabetic (NOD) mice that spontaneously develop a DED phenotype (Abughanam et al., 2019) and thrombospondin-1 knockout mice that are normal at birth but progressively develop ocular surface disease characterized by inflammation and secretory dysfunction of the LG (Basova et al., 2017). The two remaining studies used dogs with DED symptoms to study MSC transplantation over 6 months (Villatoro et al., 2015) or 1 year (Bittencourt et al., 2016).

Mishima et al. (2012) induced dry eye in wild-type mice by gamma irradiation, which resulted in inflammation and tissue damage. The authors reported that transplanted side population cells had no ability to reconstitute the damaged LGs. Interestingly, the side population cells did contribute to the recovery of LG hypofunction. Clusterin, a secretory glycoprotein, was identified as a key contributor to this recovery through ROS inhibition, which otherwise can cause cell damage. Comparison of duct ligation with the gamma irradiation method has shown that duct ligation causes more severe tissue damage and only partial regeneration, and therefore, may be a more suitable DED model (Dietrich et al., 2018).

Spheroids and organoids are 3D in vitro model systems that are produced using cells originating in the tissue or organ of interest. They provide an opportunity to extend understanding of the physiology of the organ or tissue in health and disease through finely controlled microenvironments that mimic in vivo conditions (Kang et al., 2021). Lu et al. (2017) developed model of the LG composed of rabbit conjunctival epithelium and lacrimal gland cell spheroids, which produced the aqueous and mucin layers of the tear film. Inflammation was induced to create a model system for DED. Advantages of this approach are a more controlled environment for testing and the ability to alter and measure inputs and outputs accurately. Jeong and colleagues found that organoids generated from mouse LG tissue produce 70% of the original LG protein profile upon stimulation. Transplantation in a mouse model of DED was shown to be successful and organoids remained in place for at least 2 weeks. The authors also generated organoids using LG tissue from Sjogren's syndrome patients. They showed that LG-derived organoids had similar histology to the original LG. These results suggest that transplantation of in vitro 3D-cultured organoids could be a useful strategy for treatment of DED patients.

3.5 | Development of regenerative therapies for the lacrimal gland

So far, there have been two main areas of research aimed at regeneration of the LG: (1) identification of signalling pathways and proteins involved in LG inflammation and promotion of LG regeneration (Ali et al., 2017; Basova et al., 2017; Dietrich, Roth, et al., 2019; Roth et al., 2015; Yao et al., 2019; You et al., 2012; Zhang et al., 2014; Zoukhri et al., 2008), and (2) transplantation of cultured SCs or SC products for direct in situ regeneration of the LG (Abughanam et al., 2019; Basova et al., 2017; Dietrich, Ott, et al., 2019; Gromova et al., 2017; Hirayama et al., 2013; Mishima et al., 2012; Moller-Hansen et al., 2020; Villatoro et al., 2015; Xiao & Zhang, 2020; Xie et al., 2015; Yao et al., 2019; Yu et al., 2020) (Table 1). In addition, some studies found particular proteins may be useful as biomarkers of DED severity and response to treatment (Ali et al., 2017; Yao et al., 2019).

3.5.1 | Potential targets promoting lacrimal gland regeneration

Investigation of regenerative mechanisms in other areas of the body has shown that epithelial-to-mesenchymal transition is an essential part of the process, which enables cell proliferation and instigation of repair. Mobilization of nestin-positive MSCs is an important part of LG regeneration (You et al., 2012). Vimentin and snail are key regulatory proteins that together coordinate epithelial-to-mesenchymal transition (You et al., 2012). Thus, vimentin and snail may offer novel therapeutic targets for promotion of MSC mobilization and LG repair mechanisms.

It has been shown that MSCs secrete therapeutic trophic factors that are involved in tissue repair (Caplan & Correa, 2011). Therefore, Dietrich, Roth, et al. (2019) investigated the LG MSC secretome under inflammatory conditions. Upregulated proteins included lipocalin-2, prosaposin, ras-GTPase-activating-binding-protein-1 and signal transducer-and-activator-of-transcription-1. Importantly, in vitro studies showed that these proteins contribute to the improvement of LG epithelial cell regeneration. Signal transducer-and-activator-of-transcription-1 was also shown to have a beneficial effect on the survival of LG epithelial cells under inflammatory

TABLE 4 Animal models of lacrimal gland dysfunction.

Study	Species	Sex	Age (weeks)	Strain	Characteristics of model animal	Model	Method	Success rate
Abughanam et al. (2019)	Mouse	F	8–24	NOD	Cellular, secretory and immune system disruption	Sjogren's syndrome	Bone marrow cells from wild-type grown in vitro, or bone marrow cells ruptured with liquid nitrogen injected into tail vein	Higher success rate for MSC extract group
Dietrich, Ott, et al. (2019)	Mouse	F M	8–12	ADDE induced in wild-type and enhanced green fluorescent protein	Enhanced green fluorescent protein; ectopic expression of transgene for visualization Wild-type: DL to induce ADDE	ADDE	MSC isolated from enhanced green fluorescent protein mice and injected into LG of wild-type mice with surgically induced ADDE	LG-MSC significantly improved vital acinar structures and LG regeneration
Basova et al. (2017)	Mouse	F	3–5	Pax6-labelled mice and TSP1 knockout	Pax6-labelled mice: allows for visualization of LacZ+ cells TSP-1 knockout: loss of LG secretory function.	Sjogren's syndrome	Epithelial progenitor cell transplantation from Pax6-lacZ mice to recipient wild-type mice injected with IL-1α. injected into LG lobe	Blocking Panx1 significantly increased epithelial progenitor cells engraftment but no significant increase in tear production
Bittencourt et al. (2016)	Dogs	F M	3–11 years	Various breeds	N/A	DED for 1 year with one ocular symptom	MSC transplantation	Significant improvement in DED symptoms over time
Villatoro et al. (2015)	Dogs	F M	4–12 years	Various breeds	N/A	DED for 6 months	MSC transplantation	Reduction in clinical signs of DED
Mishima et al. (2012)	Mouse	M	12	Enhanced green fluorescent protein	Enhanced green fluorescent protein; ectopic expression of transgene for visualization	LG hypofunction	Side population and main population cells injected directly into the LG	Side population do not reconstitute LG function

Note: Six studies investigated animal models of dry eye disease.

Abbreviations: ADDE, Aqueous-deficient dry eye; DED, Dry eye disease; DL, Duct ligation; F, Female; IL, Interleukin; LacZ, Lactose operon product; LG, Lacrimal gland; M, Male; MSC, Mesenchymal stem cells; NOD, Non-obese diabetic; Panx1, Pannexin 1; Pax6, paired box protein 6; TSP1, Thrombospondin 1.

conditions. In a novel approach, Roth et al. (2015) found that culture in physiologically relevant (5% oxygen) conditions during production of MSC-conditioned medium heightened the effect of this medium on migration and proliferation of LG epithelial cells. Further investigation is necessary to determine which factors are responsible for the beneficial effects of hypoxic MSC-conditioned medium on LG epithelial cells.

The bone morphogenic protein-7 pathway has been shown as upregulated in nestin-positive SCs during LG repair and may direct the fate of these cells towards epithelial and mesenchymal components of the LG (Zoukhri et al., 2008). The beneficial effect of bone morphogenic protein-7 in ameliorating the severity of damage through injury has been shown in animal models, for instance, repair of the kidney is promoted through prevention of inflammation and fibrosis (Simic & Vukicevic, 2005). Exogenous addition of bone morphogenic protein-7 to the LG injury site or during transplantation may therefore be beneficial in promoting regeneration by reducing the inflammatory process. Application of induced pluripotent stem cell (iPSC)-conditioned medium to radiation-damaged LG epithelial cells has also been shown to markedly reduce inflammation through inhibition of the p38/JNK pathway (Zhang et al., 2014). Furthermore, the conditioned medium was found to contain midkine, a heparin-binding growth factor, which significantly increased LG epithelial cells migration and proliferation, two important components of the regenerative process (Zhang et al., 2014).

Yao et al. (2019) used a sophisticated study design to reveal that MSCs secrete interferon-β (IFN-β), which promotes dendritic cells to produce interleukin-27. Interleukin-27-deficient mice had exacerbated Sjogren's syndrome symptoms, whereas MSC transplantation alleviated Sjogren's syndrome symptoms by elevating the level of interleukin-27 to restore the T-helper 17 cells/T-regulatory balance. The authors suggested that

interleukin-27 may be a potential target for treatment of Sjogren's syndrome, as elevated interleukin-27 might drive naïve T cells to differentiate into regulatory T cells cells rather than into pro-inflammatory T-helper 17 cells. Serum testing in Sjogren's syndrome patients confirmed that disease severity is correlated with a low level of interleukin-27.

Basova et al. (2017) pursued the hypothesis that preventing the import of extracellular ATP, used as fuel for the inflammatory process, would suppress inflammation and promote epithelial PC engraftment. The authors showed that blocking Pannexin-1 (Panx1), an import channel protein, reduced inflammation, enhanced donor cell engraftment and facilitated LG regeneration.

The ratio of histatin-1-positive acini was shown to decrease in association with symptoms of DED (Ali et al., 2017). Thus, a larger study is needed to assess the value of histatin-1 as an indicator of DED subtype and severity. Though more work is needed, the above studies suggest promising strategies and therapeutic targets for moderating inflammation and promoting LG regeneration.

3.5.2 | Stem cell transplantation

Our search returned 18 studies investigating the direct application of SCs in regeneration of the LG. Of these, only one study used human subject (Moller-Hansen et al., 2020) (Table 1). Several cell types have been investigated for their potential regenerative function in the LG (Figure 5). MSCs were applied in mice (Abughanam et al., 2019; Dietrich, Ott, et al., 2019; Yao et al., 2019), dogs (Bittencourt et al., 2016; Villatoro et al., 2015), rabbit (Liu, 2017) and human subjects (Moller-Hansen et al., 2020). Side population SCs (Mishima et al., 2012), SCs from epithelial and mesenchymal tissue of embryonic murine-LG germs (Hirayama et al., 2013), LG epithelial progenitor cells, (Basova et al., 2017; Gromova et al., 2017) and LGSCs were investigated for LG regeneration in mice (Xiao & Zhang, 2020). Studies investigating the effect of MSC-derived extract (Abughanam et al., 2019) and application of extracellular vesicles derived from human adipose-derived MSCs (Yu et al., 2020) also used a mouse model of DED.

In humans, cells were administered by transconjunctival injections (Moller-Hansen et al., 2020). Animal studies applied cells or cell extract by injection into the tail vein (Abughanam et al., 2019; Yao et al., 2019), LG (Basova et al., 2017; Dietrich, Ott, et al., 2019; Gromova et al., 2017; Mishima et al., 2012; Xiao & Zhang, 2020) or around the LG of subjects (Liu, 2017; Villatoro et al., 2015).

Bioengineered lacrimal gland from embryonic germ cells

Initial work by Hirayama et al. (2013) showed that the transplantation of a functional bioengineered LG is achievable in a mouse model. SCs were harvested from epithelial and mesenchymal tissue of embryonic day 16.5 murine-LG germs. Following organ culture, the bioengineered LG was transplanted into 7-week-old LG-defect mice. Interestingly, further LG development occurred post-transplantation. Histology indicated that the transplant achieved the correct 3D structure and received nerve invasion following transplantation. Dye tracking experiments showed that the LG successfully connected to the excretory duct in the recipient mouse and the major tear protein lactoferrin was secreted in tear fluid upon stimulation of the eye with menthol. The authors reported a significant improvement in the area of impaired corneal epithelium, corneal thickness and ocular surface staining of treated mice compared to LG-defect controls indicating improved LG function and a healthy

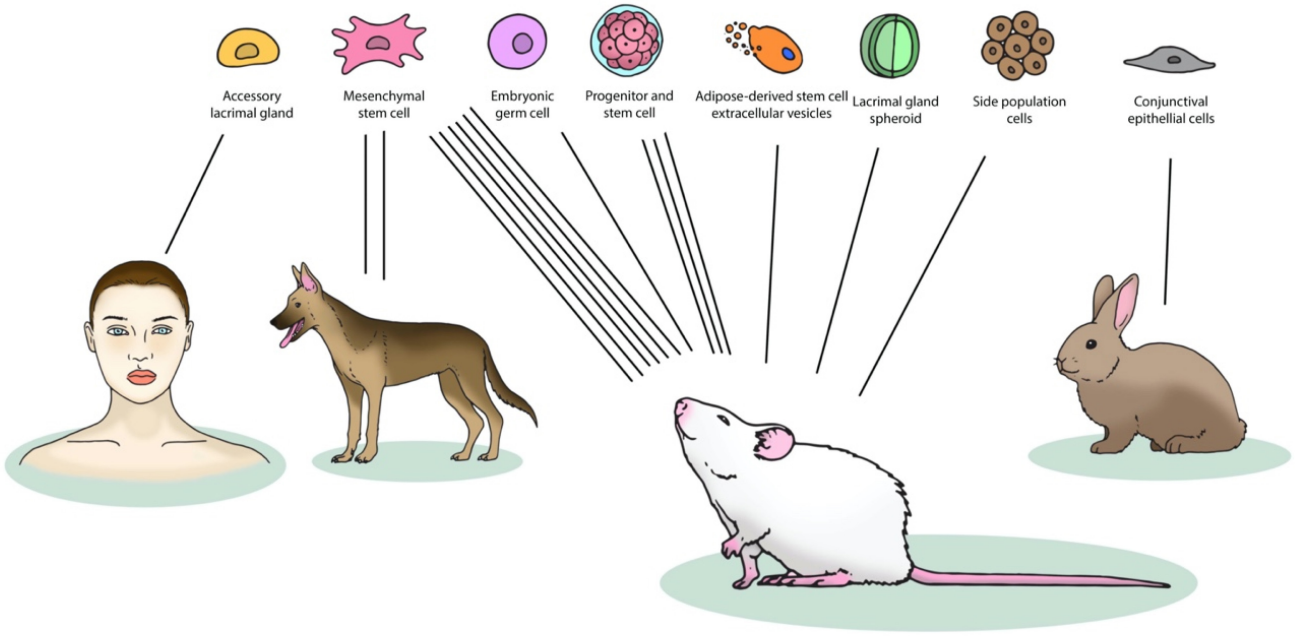

FIGURE 5 Stem cell harvesting and transplantation. The illustration shows the cell types, cell origins and animal models used for transplantation. Copyright K. Skårdal/M.

ocular surface. While these results are encouraging, they rely on harvest of embryonic material, which poses ethical challenges.

Lacrimal gland stem and progenitor cells

Label-retaining experiments suggest the existence of a population of fast-cycling transit cells that do not retain the label long term and a rare population of slow-cycling label-retaining cells that may represent adult LGSCs (You, Tariq, et al., 2011). Experiments showed that the number of BrdU-label retaining cells increases following injury to the LG, suggesting that injury stimulates proliferation of BrdU-label retaining cells to aid in repair. Importantly, the discovery of BrdU-label retaining cells in the LG presents a potential source of adult SCs that may be the most appropriate cells to harvest for production of a bio-engineered LG.

An important aspect of developing SC-based therapy to treat LG injury is ensuring successful engraftment of the transplanted cells. Two studies investigated the engraftment efficacy of LG epithelial PCs in murine LGs (Basova et al., 2017; Gromova et al., 2017). The first study demonstrated that LG epithelial PCs engraft more efficiently at the beginning of the regeneration phase (3 days post-injury) compared to at the initial phase of inflammation (1 day after injury). In addition, the authors examined the engraftment efficiency of LG epithelial PCs in mice with chronic LG inflammation (TSP-1 knockout mouse model) and reported a significant increase in tear production and restoration of LG structure by morphological examination 4–8 weeks after engraftment. Based on these results, the authors suggested that inhibition of chronic inflammation in the LG may further increase the effectiveness of cell engraftment during treatment (Gromova et al., 2017). Support for this idea was demonstrated by improved cell engraftment with inhibition of Panx1, a membrane channel upregulated during LG inflammation (Basova et al., 2017). On the other hand, Xiao and Zhang (2020) showed that successful engraftment of LGSC spheroids in a mouse model of aqueous-deficient DED can significantly improve tear secretion as well as decrease lymphatic infiltration and inflammation.

Accessory lacrimal gland stem cells

The accessory LG is located anterior to the main LG (Figure 2b). It is a key contributor to both basal and reflex tear production (Hunt et al., 1996) and has the benefit of being easily accessible for harness of tissue for cell expansion and regenerative application. Few studies have characterized SCs in the human accessory LG, which could potentially be a reliable source of regenerative LGSCs. Notably, it contains a similar structure to that found in the main LG; it is a compound tubule-acinar gland consisting of acini, ducts, nerves, plasma cells and myoepithelial cells (Dartt, 2009). Ali et al. (2017) showed the presence of cells exhibiting precursor cell markers nestin, ABCG2 and CD90, which suggested they are SC in nature, and found that expression of ABCG2 declined with age.

Side population cells

Stem cells may be selected and sorted using flow cytometry and established cell surface markers. However, where cell surface SC markers are as yet uncharacterized, it is possible to capture them based on their small size and dye exclusion capability (Goodell et al., 1996). SCs appear on flow cytometry readings as a separate side population based on these properties. Side population cells typically have higher expression of ABC transporter proteins at the cell membrane facilitating transport of dye out of the cells (Zhou et al., 2001). The therapeutic potential of side population cells harvested from lacrimal and salivary glands was investigated in irradiation-induced LG hypofunction in a mouse model (Mishima et al., 2012). LG function was restored and tear secretion was significantly increased following transplantation of LG side population cells into the hypo-functioning LG. However, the transplanted cells did not contribute to regeneration of the damaged LG.

Primary palpebral and fornical conjunctival epithelial cells

An ideal source of SCs for regeneration of the LG is the patients' own tissue, limiting the risk of an immune response and transplant rejection. Comparison of primary palpebral and fornical conjunctival epithelial cells, bulbar conjunctival epithelial cells and lacrimal duct epithelial cells found that primary palpebral and fornical conjunctival epithelial cells had superior in vitro growth (Xie et al., 2015). It was also noted that these cells had morphological characteristics, immune phenotypes and proliferation features that were similar to lacrimal duct epithelial cells making them good candidate cells for use in tissue engineering aimed at repairing lacrimal duct damage. Follow-up in vivo studies in an animal model and comparison of these cell types in human tissue are important next steps.

Mesenchymal stem cells and extract

Mesenchymal stem cells are self-renewing stromal cells that can be isolated from various mesenchymal tissues such as bone marrow, adipose and umbilical cord (Pittenger et al., 1999). They have several unique features that facilitate regeneration. For instance, they can differentiate to several different cell types and they can modify the microenvironment via direct cell–cell communication and by secretion of immune-related molecules that promote repair. MSC-based therapy has been investigated in animal models of a variety of immune-related disorders due to MSC immunomodulatory and trophic effects (Galipeau & Sensebe, 2018). However, so far human trials have fallen short of results obtained from animal studies.

Mesenchymal stem cells derived from three different tissue types have been investigated for their therapeutic value in mouse models of DED: the umbilical cord (Yao et al., 2019), LG (Dietrich, Ott, et al., 2019) and bone marrow (Abughanam et al., 2019; Aluri et al., 2017; Lee et al., 2015). MSCs were also harvested from bone marrow in a rat model of DED (Beyazyildiz et al., 2014) and from adipose tissue in a rabbit model of Sjogren's syndrome (Liu, 2017).

Application of LG-derived MSCs resulted in 62% of LG acinar structures regenerated compared to undamaged control by day 21 following injury (Dietrich, Ott, et al., 2019). However, a substantial increase to 50% was also seen in the saline injection control group. Indications of improved immune reaction were observed with decreased TNFα and increased interleukin-6 RNA levels in the experimental group, but these cytokines were not detected at the protein level. No improvement was seen in tear secretion or corneal thickness with LG MSCs compared to saline injection. A longer follow-up time of at least 4 weeks could further distinguish results of LG MSCs compared to saline control.

Eyedrops may be an effective mode of MSC delivery to the eye (Beyazyildiz et al., 2014). Eye drops applied topically once daily for a week resulted in discovery of labelled MSCs in the conjunctival epithelium and Meibomian glands despite the short duration of the treatment. Furthermore, tear film volume and tear stability were improved. Treated rats also had an increased number of secretory granules and goblet cells. Follow-up studies are needed to further test this mode of delivery as it is non-invasive, very accessible and results of the treatment were promising.

Periorbital injection of bone marrow MSCs were found to reduce the infiltration of $CD4^+$ T cells and inflammatory cytokine levels in the intra-orbital gland and ocular surface of a mouse model of inflammation-mediated DED (Lee et al., 2015). The authors reported an increase in aqueous tear production and in the number of conjunctival goblet cells. Use of human cells enabled testing for the presence of the injected cells. Interestingly, MSCs suppressed the immune response and improved symptoms of DED without long-term engraftment. Similarly, injection of bone marrow MSCs were found to increase tear production over a 4-week period in a mouse model of Sjogren's syndrome despite lack of evidence of long-term engraftment of the green fluorescent protein-labelled cells (Aluri et al., 2017). Preliminary investigation of the underlying mechanism revealed that expression of the water channel protein aquaporin 5 was increased, which may explain increased tear production. Further, while the number of lymphocytic foci in the LG did not change, the size of the foci decreased, which suggested inflammation was reduced. However, investigation of markers associated with modulation of inflammation did not reveal any significant changes or highlight any clear pathway to explain the reduction in lymphocytic foci size. The authors therefore suggested that the observed improvement in LG function may not involve modulation of the inflammatory responses. Transcription of Rab genes that are involved in directing vesicular trafficking were shown to increase, but not significantly, and staining for Rab and aquaporin-5 proteins did not reveal any reliable change in expression compared to controls. Further study is needed to resolve the downstream targets associated with bone marrow MSC application that increase tear production.

Treatment with adipose-derived MSCs in a rabbit model of Sjogren's syndrome decreased autoimmune responses and restored secretory function of the LG (Liu, 2017). T-helper 1 and T-helper 17 cell responses were also downregulated, whereas T-Reg function was enhanced. The authors showed that the mechanism involved suppression of the expression of matrix metalloproteinase (MMP)-9, MPP-2, interleukin-1β and interleukin-6, and enhanced the expression of the anti-inflammatory cytokine interleukin-10. Implantation of adipose-derived MSCs around the LGs in dogs with DED showed that the allogeneic cells were well tolerated and improvements were seen in the Schirmer test, other clinical tests and ocular surface integrity (Villatoro et al., 2015). The treatment had a sustained effect during the 9-month follow-up period. Improvements in Schirmer and ocular surface tests were also reported over 12 months in dogs with DED that received adipose-derived MSCs (Bittencourt et al., 2016). In dogs with mild–moderate DED Schirmer test values returned to those of healthy eyes. In more severe cases, an improvement in tear production and other clinical signs was seen. These studies are particularly important as the results are relevant for understanding the safety and efficacy of allogeneic adipose-derived MSC administration in spontaneously occurring DED.

A recent study used transconjunctival injection to introduce allogeneic adipose-derived MSCs in the LG in a small clinical study of seven patients with aqueous-deficient DED (Moller-Hansen et al., 2020). Compared to MSCs originating from other sources, adipose MSCs have the advantage of being the easiest and most abundant MSC type to acquire from adult tissue. A large range of outcome measures were included during the 16-week follow-up period. The primary aim of this study was to determine the safety and feasibility of using adipose-derived MSCs in the treatment of aqueous-deficient DED. Therefore, any adverse events were considered the primary outcome measures. The secondary outcome measures included changes in ocular surface disease index (OSDI) scores, tear break-up time (TBUT), tear osmolarity, Schirmer's I test, corneal staining and development of donor-specific antibodies. Notably, improvement was observed in all of these clinical parameters.

According to a review by Norozi et al. (2016) MSCs have both positive and negative effects on tissue regeneration and tumour survival. Mechanisms involved in anti-tumour effects may include downregulation of signalling pathways such as AKT and Wnt/β-catenin, leading to decreased tumour cell proliferation, suppression of oncogenes and increased tumour cell death. Thus, MSCs may be candidates for use in cancer therapy. On the other hand, MSCs also have invasive, tumour-homing and tumour supporting qualities. A more recent review by Eiro et al. (2021) concludes that the type of activity may depend on the harvest site of MSCs and the type of tumour involved. MSC infusion into patients was first reported in 1995 (Lazarus et al., 1995) and has since shown a good safety record (Pittenger et al., 2019). As of 2019, there were over 950 registered MSC clinical trials listed with the FDA with over 10 000 patients treated in a controlled clinical setting.

Paracrine factors, known as the secretome, are estimated to be responsible for up to 80% of the therapeutic effect of MSCs (Eiro et al., 2021). The use of MSC extract or MSC-conditioned medium instead of direct

transplantation of cells may therefore be a method to harness the therapeutic potential of MSCs. It was shown that tear flow rate and corneal integrity were preserved with transfer of either MSCs or MSC extract in a mouse model of Sjogren's syndrome (Abughanam et al., 2019). Tear flow rate and corneal integrity were preserved with transfer of either MSCs or MSC extract in a mouse model of Sjogren's syndrome. The thickness of the epithelial layer was also maintained and peripheral tolerance of the immune system was re-established. These promising results were maintained for up to 16 weeks follow-up, opening new avenues for further investigation into a safer and more convenient therapy using MSC extract.

Adipose-derived mesenchymal stem cell extracellular vesicles

Adipose-derived MSCs secrete high levels of extracellular vesicles that have immunomodulatory effects (Yu et al., 2020). Administration of human adipose-derived MSC extracellular vesicles topically as eye drops in a mouse model of DED resulted in improved tear synthesis, corneal staining and reduced lymphatic infiltration following application (Yu et al., 2020). This study highlighted the potential therapeutic effect of human adipose-derived MSC extracellular vesicles in suppressing the inflammatory response and alleviating ocular surface damage. Another advantage of this strategy is that it allows topical administration of MSCextracellular vesicles as an alternative to transconjunctival injection, an invasive procedure.

4 | CONCLUSIONS AND FUTURE WORK

The majority of studies investigating SC-based treatments for regeneration of the LG in DED reported promising results. No adverse events were reported in any of the studies, suggesting SC-based therapy could be a feasible therapeutic option in the future. So far, however, only three studies have used human SCs either in animal models or in humans. Of these, only one study was clinical in nature suggesting a large gap in the literature with respect to small clinical studies that investigate therapeutic application of SCs or SC products for LG regenerative purposes.

The single clinical study reported so far evaluated the safety and feasibility of injecting allogeneic adipose-derived MSCs into the LG as a treatment for ADDE and had a follow-up period of 16 weeks. Results showed improvement in clinical tests, tear production and ocular surface integrity with application of MSCs. A larger study by the same group will be completed in 2023 (https://www.clinicaltrials.gov). This Phase II trial involves 40 patients with Sjögren's syndrome and will evaluate the effectiveness of injecting allogeneic adipose-derived MSCs in the LG with the aim of improving the ocular comfort. Another study currently underway in China is evaluating the effectiveness of applying eye drops containing umbilical MSC-derived exosomes on the ocular surface to relieve DED associated with chronic graft-versus-host disease. This study will also be completed in 2023.

Studies with a longer follow-up in animal models are also necessary to resolve concerns over the tumourigenic capacity of MSCs. Most studies had a very short follow-up period, leaving doubt over long-term efficacy and safety. A valuable study in dogs demonstrated that application of adipose-derived MSCs reversed symptoms of spontaneous DED and showed efficacy and safety over a year-long follow-up period (Bittencourt et al., 2016).

While transplantation of spheroids into the damaged LG showed promise in animal studies, the use of embryonic tissue to generate these spheroids poses an ethical challenge for use in humans. This review reports on a number of different adult SC types that can be harvested from the patient or a donor, that have shown good potential for LG regeneration, for example, LD epithelial cells, LGSCs and SCs harvested from the accessory LG. Labelling studies illustrated the slow-cycling nature of the selected SCs and their capability to migrate to the LG, suggesting that use of embryonic material is unnecessary. The accessory LG is an especially interesting SC source, as it is relatively easy to access this tissue. However, one study using accessory LGSCs showed that the number of SCs decline with age, suggesting that studies using young donor material are warranted.

Determination of the best tissue of origin for MSCs could be key. MSCs from various tissues have been used in separate studies and not directly compared. Variation in MSC differentiation status, tumourigenicity and SC capabilities may be revealed in a comparative study selecting MSCs from various tissues of origin. In addition, more studies on MSC products such as MSC-conditioned medium, trophic factors, extracellular vesicles and specific MSC-secreted proteins are needed as application of these products may be preferable to transplantation of the cells themselves. While many of the studies reported success with transplantation, it was also reported that the cells were not present at follow-up, suggesting that secreted factors could be sufficient to facilitate regeneration. Thus, a realistic goal for regeneration of the LG is development of a solution containing cells or therapeutic products for simple administration in the clinic or at home.

CONFLICT OF INTEREST

Catherine Joan Jackson, *Maria Naqvi* and *Kjell Gunnar Gundersen* declare no conflict of interest. *Tor Paaske Utheim* Irrespective of potential conflict of interest, for the sake of transparency: Tor Paaske Utheim is co-founder and co-owner of The Norwegian dry eye clinic and the Clinic of eye health, Oslo, Norway, which delivers talks for and/or receives financial support from the following: ABIGO, Alcon, Allergan, AMWO, Bausch & Lomb, Bayer and European school for advanced studies in ophthalmology, InnZ Medical, Medilens Nordic, Medistim, Novartis, Santen, Specsavers, Shire Pharmaceuticals and Thea Laboratories. He has served on the global scientific advisory board for Novartis and Alcon as well as the European advisory board for Shire Pharmaceuticals. Utheim is the Norwegian Global Ambassador for Tear Film and Ocular Surface

Society (TFOS), a Board Member of the International Ocular Surface Society, a Consultant at the Norwegian Association for the Blind and Partially Sighted and the Editor-in-Chief of Oftalmolog, an eye journal distributed to all eye doctors in the Nordic region since 1980.

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