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# Using stem cell biology to study and treat ophthalmologic and oculoplastic diseases

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## Abstract:

With the rapid growth of the stem cell biology field, the prospect of regenerative medicine across multiple tissue types comes closer to reality. Several groundbreaking steps paved the way for applying stem cell biology to the several subfields within ophthalmology and oculoplastic surgery. These steps include the use of stem cell transplants as well as studies of various ophthalmologic pathologies at the molecular level. The necessity of stem cell transplant is readily apparent, having already been used for several studies such as artificial lacrimal gland design and eyelid reconstruction. Investigating the stem cell biology behind oncological diseases of the eye has also developed recently, such as with the identification of specific markers to label cancer stem cells in orbital adenoid cystic carcinoma. The advent of induced pluripotent stem cells led to a burst of productivity in the field of regenerative medicine, making it possible to take a patient's own cells, reprogram them, and use them to either study patient-specific pathology *in vitro* or use them for eventual patient specific therapeutics. Patient-specific adipose-derived stem cells (ASCs) have been used for a variety of treatments, such as wound healing and burn therapies. As the fields of stem cell biology and regenerative medicine continue to progress, its use will become a mainstay of patient-specific cell therapies in the future.

## Keywords:

Oculoplastic surgery, regeneration, regenerative medicine, stem cells, translational medicine

## Introduction

Although embryonic stem cells (ESCs) can self-renew as well as differentiate into all three germ layers, their use in scientific studies and clinical applications has been challenged due to ethical concerns.<sup>[1]</sup> Takahashi *et al.* identified four transcription factors – OCT4, SOX2, KLF4, and cMYC – that, when overexpressed in fibroblasts, can reprogram them into induced pluripotent stem cells (iPSCs).<sup>[2,3]</sup> These cells highly resemble ESCs and can also self-renew and differentiate into each of the three germ layers. This discovery not only allows evasion of any ethical concern but also constitutes a profound transformation in the field

of stem cell biology. With iPSCs, the concept of personalized regenerative medicine became a reality. In the context of ophthalmology and oculoplastic surgery, these cells can be used to repair or replace ocular tissue, either with transplant of patient-specific stem cells or the products of their differentiation. This technology benefits from the fact that the cells can be abundantly replenished, they can be used to study disease models *in vitro*, and they do not result in immunological complications upon transplant.<sup>[4]</sup> Problems still exist with these cells, however, in the form of incomplete differentiation protocols and low efficiency of reprogramming. On solving these issues, this technology will continue to evolve strengthening both regenerative and personalized medicine.

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In ophthalmology, the use of stem cell biology and transplant of either stem cells or differentiation products is easier than other tissues due to the immune-privileged status of the eye. Direct injection of these cells into and around the eye should thus not result in any immunological complications.<sup>[5]</sup> Stem cell biology has been applied to several subfields in ophthalmology, such as retina, glaucoma, and cornea. In the retina, stem cell therapy (SCT) has been used in an attempt to treat conditions such as retinopathy, optic neuropathy, and macular degeneration.<sup>[6]</sup> As the fields of stem cell biology and regenerative medicine continue to progress, its use will become a mainstay of patient-specific cell therapies in the future.<sup>[7]</sup> In glaucoma, SCT can be used to protect and support these damaged cells through the secretion of neurotrophic factors.<sup>[8]</sup> They can also be used to replace lost or damaged retinal ganglion or trabecular meshwork cells.<sup>[9]</sup> Corneal epithelial cell transplantation remains as the second most common type of SCT behind hematopoietic stem cell transplant.<sup>[10]</sup> Corneal limbal stem cells expanded *ex vivo* provide a potential source of cells for the treatment of cornea associated disorders through transplantation.<sup>[11]</sup> These cells can be found endogenously in the basal limbal epithelium. Damage to these cells through insults such as burns or chemical exposure can result in corneal conjunctivalization, scarring, and opacification.<sup>[12]</sup> As with the aforementioned techniques, other strategies are being taken to repair or replace these corneal cells, such as directed differentiation of human ESCs into corneal-like cells.<sup>[13]</sup>

As ophthalmology has benefitted from stem cell biology, the field of oculoplastics has also seen great potential with these new technologies. This includes the use of stem cells and their differentiation products in eyelid reconstruction

and lacrimal gland development. In the context of oculoplastics, patient-specific stem cell biology will provide sources of replenishable cells for transplant therapy, platforms to model diseases *in vitro*, and opportunities to construct three-dimensional (3D) organ systems *in vitro* with the intent of organ replacement [Table 1]. Using patient-specific stem cell biology in oculoplastics will continue to transform therapeutics for the eye and orbit in a personalized manner.

## Body of Review

### Periocular and eyelid reconstruction

The need for reconstruction of the periocular and eyelid area can occur through a variety of reasons such as cancer resection, burns, and chemical exposure. Adipose-derived stem cells (ASCs) have recently become an exciting and vast supply for reconstructive needs.<sup>[14]</sup> These cells, which can be obtained through periocular fat, are readily available and easy to access. The orbital adipose, the source of ASCs most applicable for periocular and eyelid reconstruction, is derived from the neural crest instead of the mesenchyme, where the majority of other adipose reserves are derived from.<sup>[15]</sup> As a result of this developmental derivation, these cells are postulated to have greater potential for the treatment of many eye disorders due to their closer lineage than cells of other origins.

Several processes, both physiological and pathological, affect wound healing. These include advanced age and infections.<sup>[16]</sup> ASCs used to reconstruct these wounds have been shown to avoid scar formation and increase the rate of proper wound closure.<sup>[17]</sup> These cells can also effectively boost healing in radiation-induced wounds.<sup>[18]</sup> Using cells not derived from the patient, graft rejection can possibly

**Table 1: Use of stem cells in ophthalmologic and oculoplastic surgery**

	Locations	Diseases/condition	Cells	Marker Panel	Therapeutic	Current System
Reconstruction	Periocular and eyelid area	Cancer Resection, Burns, Chemical Exposure	ASCs (from peri-ocular fat)	N/A	ASC transplants that avoid scar formation and increase rate of proper wound closure	<i>In vivo</i> transplants
Aesthetics (age)	Periorbital skin	Erratic pigmentation, Wrinkling	ASCs	N/A	ASC injection, treatment with ASC conditioned media	<i>In vivo</i> transplants
Aesthetics (burns)	Periorbital skin, other locations on body	Burns, chemical exposure	Epidermal stem cells, keratinocytes	N/A	Cultured epithelial autografts, stem cell application to wound bed	<i>In vivo</i> transplants
Cancer	Eye, orbit	Sebaceous gland carcinoma, basal cell carcinoma, conjunctival squamous cell carcinoma	Cancer cells and tissues	ABCG2, CD44, CD133	Cancer resection which brings need for reconstruction and further understanding of eye oncology	Currently under study
Lacrimal Gland	Lacrimal gland	Dry Eye Disease	Lacrimal gland resident stem cells, <i>in vitro</i> expanded cells	Ki67, Nestin, ALDH1, c-Kit, ABCG2	Identifying reservoir of resident stem cells, <i>in vitro</i> bioengineering with the hopes of whole organ transplant	In mouse studies, <i>in vitro</i> tissue bioengineering

ASC = Adipose-derived stem cells, ALDH = Aldehyde dehydrogenase

occur. Due to these immunological issues, periocular reconstruction has encountered several limitations. To avoid graft versus host disease, bioengineered materials have been combined with porcine-derived dermal collagen or human acellular dermis for appropriate grafts.<sup>[19]</sup> These constructs, however, have met with increased fibrosis of the grafts and a limited range of function. iPSC technology could help avert immunological complications and thus be clinically applicable in wound healing and reconstruction in concert with other traditional methods of reconstruction.

### Esthetics

Physiologically, several changes throughout the body can occur during typical aging. These include erratic pigmentation of the skin as well as loss of elasticity. These changes can be exacerbated by other external risk factors such as ultraviolet (UV) exposure and tobacco use. Within the field of oculoplastics, several facets of stem cell biology are already being applied and continue to expand. One such example of this is through the subcutaneous injection of ASCs in UV light-induced wrinkling. The injection of ASCs is thought to thicken the dermis and subsequently result in significantly less wrinkling.<sup>[20]</sup> ASC culture media have been used to induce the migration of dermal fibroblasts and type 1 collagen secretion.<sup>[17]</sup> This functions through the release of growth factors and cytokines from the cultured ASCs stimulating these cells. This parallels the function of mesenchymal stem cells and how their secreted factors appear to reduce the progressive wrinkling of aging skin.<sup>[21]</sup> In humans, we have also observed that injection of ASCs into photoaged periorbital skin increased the thickness of the dermis by 10% and reduced wrinkling.<sup>[22]</sup>

Burn management has benefited from the use of stem cell biology through cultured epithelial autografts (CEAs). These constructs were effectively used for successful epidermal regeneration due to the epidermal stem cells and keratinocytes contained within them.<sup>[23]</sup> CEAs, however, suffer from potential immune rejection, cost, and the time it takes to generate them. These issues have thus reduced their immediate clinical application. Application of stem cell biology can address these problems. This would directly apply to improvements of the wound bed that the CEAs are engrafted into. The quality of the wound bed is directly correlated to the quality of the graft. Previous reports have shown improved growth factor production, wound closure, and neovascularization of the wound bed when supplemented with stem cells.<sup>[24]</sup> Thus, stem cells in combination with CEAs could greatly improve the quality of these grafts.

As with other areas of oculoplastic surgery, the use of iPSCs can improve integration of these esthetic and

reconstructive technologies to the clinic. Patient-specific grafts would reduce the risk of immunological complications as well as improve the general use of grafts for cosmetic surgery and other applications.

### Cancer

Oncological issues of the eye and orbit present as frequent obstacles in oculoplastic surgery. Several major cancers can occur in these areas, such as sebaceous gland carcinoma,<sup>[25]</sup> conjunctival squamous cell carcinoma,<sup>[26]</sup> and basal cell carcinoma.<sup>[27]</sup> Often times, surgery is the best method these cancers, but many reasons keep this choice from being the optimal treatment.<sup>[28]</sup> It is for this reason that several nonsurgical treatment modalities exist, such as cryotherapy, radiotherapy, and 5-fluorouracil.<sup>[29]</sup>

Should resection be chosen as the method of treatment for tumor treatment, surgery leaves defects that must be repaired by reconstructive surgeons. To this end, stem cell biology can be applied in a variety of ways. Autologous grafts using patient-specific cells can be generated that can engraft with less concern for immunological rejection. Understanding the biology behind cancers of the eye can also shed light on future stem cell treatment. In adenoid cystic carcinoma, a population of cells expressing cancer stem cell markers such as ABCG2, CD44, and CD133 has been identified.<sup>[30]</sup> In uveal melanoma, studies are ongoing to understand the mechanisms driving dormant stem cells and their eventual activation that leads to excessive cell proliferation.<sup>[31]</sup> While many oncogenic pathways have been identified, greater promise lies in understanding the overriding networks that orchestrate tumor formation, growth, and spread.

### Lacrimal gland

Through tear secretion, the lacrimal gland actively maintains the ocular surface. When its function is disrupted, patients suffer from dry eye disease, a very common disorder of the eye. This disease can result in epithelial damage of the ocular surface, leading to visual disturbance and ocular discomfort.<sup>[32]</sup> The lacrimal gland shares developmental history with other ectodermal organs and forms by interactions between epithelium and mesenchyme.<sup>[33]</sup> Through further understanding of its development, we can harness the stem cell biology driving its growth and function for therapeutic intervention.

The mouse lacrimal gland possesses a reservoir of resident stem cells that are double positive for Ki67 and nestin that repair the lacrimal glands damaged through interleukin-1-mediated inflammation.<sup>[34,35]</sup> In the human, putative-resident stem cells of the lacrimal gland have been identified that are triple positive for ALDH1, c-KIT, and ABCG2.<sup>[36]</sup> Although these cells

have been potentially identified, they are not yet fully understood. A full understanding of these cells would shed light on their mechanisms to generate this tissue and how the cell types in this organ function together. To this end, some success has been achieved through *in vitro* human lacrimal gland cultures, where these cells continue to retain their function.<sup>[36]</sup> Other *in vitro* studies involve the generation of murine lacrimal gland organs from epithelial and mesenchymal components of the E16.5 mouse lacrimal gland germ. These bioengineered tissue structures successfully adopt the morphology of lacrimal glands.<sup>[37,38]</sup> With these successful *in vitro* studies, the potential opportunity to generate disease modeling platforms as well as replenishable sources for cell and organ transplants is at hand.

The ultimate goal in applying stem cells to oculo-plastics is to use discoveries in developmental and stem cell biology to generate grafts and other constructs required within this field. Using iPSC technology to generate patient-specific cell products, grafts to replace damaged lacrimal gland tissue can be generated. This could greatly benefit patients with several disorders such as dry eye disease to repair their lacrimal glands and restore proper tear function.

## Conclusion

Stem cell biology, particularly upon the advent of iPSCs, has revolutionized the ideas of regenerative and personalized medicine. In ophthalmology and oculo-plastics, we see its relevance in the form of tissue and organ grafts for reconstruction and transplants. Although this field is still growing, sources of transplantable cells and platforms for both disease modeling and 3D organ generation have already been developed and continue to become more clinically relevant.

Specifically, in oculo-plastics, further study is required to more efficiently generate tissue types of interest through either reprogramming or directed differentiation strategies. Similarly, application of these technologies still faces hardship in the context of disease modeling and generation of tissue grafts due to human genetic variation. Furthermore, the complexity of different disorders addressed in oculo-plastics further hinders the rapid application of stem cell biology to this field. Regardless of these obstacles, iPSC technology in oculo-plastics and several other fields will continue to develop until it becomes a regular feature of modern personalized medicine.

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## Conflicts of interest

The authors have no any conflicts of interest to declare.

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