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

Albert Y. Wu¹,²,³, Michael G. Daniel¹,⁴,⁵

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.

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).

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.

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.
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. 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. 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. In glaucoma, SCT can be used to protect and support these damaged cells through the secretion of neurotrophic factors. They can also be used to replace lost or damaged retinal ganglion or trabecular meshwork cells. Corneal epithelial cell transplantation remains as the second most common type of SCT behind hematopoietic stem cell transplant. Corneal limbal stem cells expanded ex vivo provide a potential source of cells for the treatment of corneal associated disorders through transplantation. 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. 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.

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. 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. 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. ASCs used to reconstruct these wounds have been shown to avoid scar formation and increase the rate of proper wound closure. These cells can also effectively boost healing in radiation-induced wounds. 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 | Therapeutic                                                                 | Current System |
|----------------------------|-------------------------------------|------------------------|--------|----------------------------------------------------------------------------|----------------|
| Reconstruction             | 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 | In vivo transplants |
| Aesthetics (age)           | Erratic pigmentation, Wrinkling Burns, chemical exposure | ASCs                  | N/A    | ASC injection, treatment with ASC conditioned media                          | In vivo transplants |
| Aesthetics (burns)         | Periorbital skin, other locations on body | Epidermal stem cells, keratinocytes | N/A    | Cultured epithelial autografts, stem cell application to wound bed             | In vivo transplants |
| Cancer                     | 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             | Dry Eye Disease                     | Lacrimal gland resident stem cells, in vitro expanded cells | Ki67, Nestin, ALDH1, c-Kit, ABCG2 | Identifying reservoir of resident stem cells, in vitro bioengineering with the hopes of whole organ transplant | In mouse studies, in vitro 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.[19] 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.[20] ASC culture media have been used to induce the migration of dermal fibroblasts and type 1 collagen secretion.[17] 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.[21] 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.[22]

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.[23] 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.[24] 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,[25] conjunctival squamous cell carcinoma,[26] and basal cell carcinoma.[27] Often times, surgery is the best method these cancers, but many reasons keep this choice from being the optimal treatment.[28] It is for this reason that several nonsurgical treatment modalities exist, such as cryotherapy, radiotherapy, and 5-fluorouracil.[29] 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.[30] In uveal melanoma, studies are ongoing to understand the mechanisms driving dormant stem cells and their eventual activation that leads to excessive cell proliferation.[31] 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.[32] The lacrimal gland shares developmental history with other ectodermal organs and forms by interactions between epithelium and mesenchyme.[33] 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.[34][35] In the human, putative-resident stem cells of the lacrimal gland have been identified that are triple positive for ALDH1, c-KIT, and ABCG2.[36] 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.\textsuperscript{36} 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.\textsuperscript{37,38} 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 oculoplastics 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 oculoplastics, 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 oculoplastics, 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 oculoplastics further hinders the rapid application of stem cell biology to this field. Regardless of these obstacles, iPSC technology in oculoplastics 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.

**References**

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145-7.

2. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663-76.

3. Takahashi K, Okita K, Nakagawa M, Yamanaka S. Induction of pluripotent stem cells from fibroblast cultures. Nat Protoc 2007;2:3081-9.

4. Singh VK, Kalsan M, Kumar N, Saini A, Chandra R. Induced pluripotent stem cells: Applications in regenerative medicine, disease modeling, and drug discovery. Front Cell Dev Biol 2015;3:2.

5. Blenkinsop TA, Comeo B, Temple S, Stern JH. Ophthalmologic stem cell transplantation therapies. Regen Med 2012;7:6Suppl:32-9.

6. Lauuri M, Chen E, Looman M, Gallagher M. The burden of disease of retinal vein occlusion: Review of the literature. Eye (Lond) 2011;25:981-8.

7. Li N, Li XR, Yuan JQ. Effects of bone marrow mesenchymal stem cells transplanted into vitreous cavity of rat injured by ischemia/reperfusion. Graefes Arch Clin Exp Ophthalmol 2009;247:503-14.

8. Wein FB, Levin LA. Current understanding of neuroprotection in glaucoma. Curr Opin Ophthalmol 2002;13:61-7.

9. Sun Y, Williams A, Waisbourd M, Iacovitti L, Katz LJ. Stem cell therapy for glaucoma: Science or snake oil? Surv Ophthalmol 2015;60:93-105.

10. Dhmodaran K, Subramani M, Ponnalagu M, Shetty R, Das D. Ocular stem cells: A status update. Stem Cell Res Ther 2014;5:56.

11. Kenyon KR, Tseng SC. Limbal autograft transplantation for ocular surface disorders. Ophthalmology 1989;96:709-22.

12. Kruse FE. Stem cells and corneal epithelial regeneration. Eye (Lond) 1998;8(Pt 1):170-83.

13. Brzeszczynska J, Samuel K, Greenhough S, Ramaesh K, Dhillon B, Hay DC, et al. Differentiation and molecular profiling of human embryonic stem cell-derived corneal epithelial cells. Int J Mol Med 2014;33:1597-606.

14. Tsuji W, Rubin JP, Marra KG. Adipose-derived stem cells: Implications in tissue regeneration. World J Stem Cells 2014;6:312-21.

15. Korn BS, Kikkawa DO, Hickoc KC. Identification and characterization of adult stem cells from human orbital adipose tissue. Ophthal Plast Reconstr Surg 2009;25:27-32.

16. Isherubino M, Rubin JP, Miljkovic N, Kelmendi-Doko A, Marra KG. Adipose-derived stem cells for wound healing applications. Ann Plast Surg 2011;66:210-5.

17. Kim WS, Park BS, Sung JH, Yang JM, Park SB, Kwak SJ, et al. Wound healing effect of adipose-derived stem cells: A critical role of secretory factors on human dermal fibroblasts. J Dermatol Sci 2007;48:15-24.

18. Rigotti G, Marchi A, Galie M, Baroni G, Benati D, Krampera M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: A healing process mediated by adipose-derived adult stem cells. Plast Reconstr Surg 2007;119:1409-22.

19. McCord C, Nahai FR, Codner MA, Nahai F, Hester TR. Use of porcine acellular dermal matrix (Enduragen) grafts in eyelids: A review of 69 patients and 129 eyelids. Plast Reconstr Surg 2008;122:1206-13.

20. Kim WS, Park BS, Park SH, Kim HK, Sung JH. Antiwrinkle effect of adipose-derived stem cell: Activation of dermal fibroblast by secretory factors. J Dermatol Sci 2009;53:96-102.

21. Kim WS, Park BS, Sung JH. Protective role of adipose-derived stem cells and their soluble factors in photoaging. Arch Dermatol Res 2009;301:329-36.

22. Park BS, Jang KA, Sung JH, Park JS, Kwon YH, Kim KJ.
et al. Adipose‑derived stem cells and their secretory factors as a promising therapy for skin aging. Dermatol Surg 2008;34:1323‑6.
23. Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: Eleven years of clinical experience. Burns 2006;32:538‑44.
24. Rasulov MF, Vasilchenkov AV, Onishchenko NA, Krasheninnikov ME, Kravchenko VI, Gorshenin TL, et al. First experience of the use bone marrow mesenchymal stem cells for the treatment of a patient with deep skin burns. Bull Exp Biol Med 2005;139:141‑4.
25. Wali UK, Al‑Mujaini A. Sebaceous gland carcinoma of the eyelid. Oman J Ophthalmol 2010;3:117‑21.
26. McKelvie PA, Daniell M, McNab A, Loughnan M, Santamaria JD. Squamous cell carcinoma of the conjunctiva: A series of 26 cases. Br J Ophthalmol 2002;86:168‑73.
27. Margo CE, Waltz K. Basal cell carcinoma of the eyelid and periocular skin. Surv Ophthalmol 1993;38:169‑92.
28. Murchison AP, Walrath JD, Washington CV. Non‑surgical treatments of primary, non‑melanoma eyelid malignancies: A review. Clin Exp Ophthalmol 2011;39:65‑83.
29. Rene C. Oculoplastic aspects of ocular oncology. Eye (Lond) 2013;27:199‑207.
30. Lin TT, Zhu LM, He YJ, Zhang H. Analysis of expression of cancer stem cell‑related markers in orbital adenoid cystic carcinoma.

Zhonghua Yan Ke Za Zhi 2011;47:703‑8.
31. Kalirai H, Damato BE, Coupland SE. Uveal melanoma cell lines contain stem‑like cells that self‑renew, produce differentiated progeny, and survive chemotherapy. Invest Ophthalmol Vis Sci 2011;52:8458‑66.
32. Gadaria‑Rathod N, Lee KL, Asbell PA. Emerging drugs for the treatment of dry eye disease. Expert Opin Emerg Drugs 2013;18:121‑36.
33. Pispa J, Thesleff I. Mechanisms of ectodermal organogenesis. Dev Biol 2003;262:195‑205.
34. Zoukhri D. Mechanisms involved in injury and repair of the murine lacrimal gland: Role of programmed cell death and mesenchymal stem cells. Ocul Surf 2010;8:60‑9.
35. Kobayashi S, Kawakita T, Kawashima M, Okada N, Mishima K, Saito I, et al. Characterization of cultivated murine lacrimal gland epithelial cells. Mol Vis 2012;18:1271‑7.
36. Tiwari S, Ali MJ, Balia MM, Naik MN, Honavar SG, Reddy VA, et al. Establishing human lacrimal gland cultures with secretory function. PLoS One 2012;7:e29458.
37. Hirayama M, Ogawa M, Oshima M, Sekine Y, Ishida K, Yamashita K, et al. Functional lacrimal gland regeneration by transplantation of a bioengineered organ germ. Nat Commun 2013;4:2497.
38. Hirayama M, Tsubota K, Tsuji T. Bioengineered lacrimal gland organ regeneration in vivo. J Funct Biomater 2015;6:634‑49.