REVIEW ARTICLE

Limbal stem cell disease: Treatment and advances in technology

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Abstract Anterior segment stem cell technology, due to its already well-defined corneal limbal stem cells with greater ease of evaluation, has been at the forefront of ophthalmic stem cell treatment and technology since 1997. This paper provides an overview of the current standard of care for treatment of limbal stem-cell deficient conditions and reviews recent treatment technologies using ex vivo expansion of cultivated limbal stem cells of the cornea.

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1. Introduction

Stem cell technology is an exciting treatment in medicine wherein ophthalmology is leading the way. The visibility of the eye with our anatomic and histopathologic knowledge allows for ease of assessment of stem cell treatments compared to other neurological and visceral organs of the human body. Most of the ophthalmic stem cell headlines come from treatment for posterior segment conditions, but there is still much to learn about the complexities of retinal cell differentiation, their safety, and the proper integration of these cells into the highly complex neuro-retinal circuitry (Azuara-Blanco, 2000). Slit-lamp examination shows recurrent keratinization of the entire ocular surface, ulceration, melting, neoplasia, and microbial keratitis (Dua and Azuara-Blanco, 2000).

1.2. The evolution of limbal stem cell transplantation

In 1965, Barraquer reported the first stem cell autograft using conjunctival-limbal-corneal epithelium harvested from the healthy fellow eye of patients with unilateral chemical burns (Barraquer, 1965). Keratoepithioplasty was reported by Thoft in 1984 using corneal lenticules from whole globes (Thoft, 1982). The first, modern LSC transplant was reported in 1989 by Kenyon and Tseng on patients with unilateral LSC disease with ocular surface stabilization in 19 of 20 eyes (Kenyon and Tseng, 1989). Further refinements to this technique evolved to maximize the harvesting and transplantation of larger amounts of conjunctiva and LSC from the donor to the recipient ocular surface (Holland and Schwartz, 1996; Croasdale et al., 1999; Holland et al., 2002). In 1997, Pellegrini et al. were the first to report on the successful use of autologous cultivated corneal epithelium for restoring the ocular surface in two patients with severe unilateral alkali burns (Pellegrini et al., 1997). This has led to the most recent advances in LSC technology involving the ex vivo expansion of cultivated limbal epithelial LSC for transplantation.

2. Current management of limbal stem cell disease

Partial LSC deficiency is defined as LSC deficiency less than one-half of the entire LSC population. Subtotal LSC deficiency is greater than one-half of the entire LSC population, while total LSC deficiency is defined as loss of the entire LSC population. Mild partial LSC deficiency, where the central visual axis is not involved and there is good visual acuity, needs only observation. Conservative management such as lubrication and removal of any potential inciting causes (i.e., contact lens wear) should be addressed.

Partial LSC deficiency that is symptomatic with irritation, reduction in vision, and signs of conjunctivalization of the cornea, requires mechanical debridement of the conjunctival epithelium from the corneal surface with frequent follow-up (Dua et al., 1994). This allows the corneal epithelium to heal into the debrided area.

In severe LSC deficient states, there are various surgical procedures to transplant LSC to form a new source of corneal epithelium once the host’s irregular epithelium and surface neovascularization have been debrided. In 1996, Holland et al. developed a classification system for the surgical management of severe ocular surface disease based on the source of tissue used; whether the source is an autograft or allograft;
and if using an allograft, whether it is derived from a living relative or a cadaveric donor (Holland and Schwartz, 1996). The LSC transplant also requires carrier tissue as it is not possible to transplant LSC alone, hence the need for conjunctival tissue (conjunctival limbal graft), corneal tissue (keratolimbal graft), or both as a carrier for LSC (Dua and Azuara-Blanco, 2000). The following four sections describe each group within this classification system.

2.1. Conjunctival limbal autograft (CLAU)

In CLAU, limbal tissue with a conjunctival carrier is harvested from the healthy contralateral eye and transplanted to the LSC deficient eye. Since this is an autograft, there is no need for systemic immunosuppression: a tremendous advantage over allograft transplantation. CLAU can only be used for cases of unilateral LSC deficiency such as unilateral burns. Caution must be exercised due to the risks to the healthy donor eye that iatrogenically becomes LSC deficient secondary to harvesting of its LSC for transplantation (Jenkins et al., 1993). CLAU can only be used when the contralateral donor eye has a healthy ocular surface with no risk of future LSC deficiency (e.g., asymmetric OCP or SJS, prior history of ocular surface trauma).

Two trapezoid-shaped CLAUs are harvested, each approximately 6 mm at the limbus, extending 5–8 mm posteriorly in the bulbar conjunctiva, with anterior extension into the cornea anterior to the palisades of Vogt. They are usually harvested from the 12 and 6 o’clock meridians of the donor eye. Attention is directed toward the recipient eye. A 360° peritomy is performed with excision of the bulbar conjunctiva that is extended posteriorly in the superior and inferior quadrants to allow for placement of the two trapezoidal CLAUs. Superficial keratectomy of the entire corneal surface ensues, debriding irregular epithelium and pannus. The CLAUs are secured into position using 10-0 nylon interrupted sutures (Holland et al., 2005; Biber et al., 2010).

2.2. Living-related conjunctival limbal allograft (LR-CLAL)

In LR-CLAL, healthy limbal tissue with a conjunctival carrier is harvested from a living relative and transplanted to the patient. Surgically, the technique is similar to the CLAU, but two separate surgeries are required. Compared to keratolimbal allografts (KLAL), LR-CLAL transplants conjunctival tissue that would be beneficial for patients with compromised conjunctiva. The advantage in using LR-CLAL over CLAU is that it can be used in patients with bilateral LSC deficiency, such as SJS and OCP (Daya et al., 2001; Kwitko et al., 1995). However, unlike autografts, allografts are at risk for rejection, and thus require both topical and systemic immunosuppression (Rao et al., 1999).

Surgeons should be conservative when selecting recipient patients and donors. The recipient must be medically fit to withstand systemic, and most likely, lifelong immunosuppression. The patient must be compliant with medications and postoperative appointments with the transplant surgeon and transplant physicians. The patient must be able to comply with the rigorous monitoring and bloodwork schedules postoperatively. Donors must be screened for potential risk of iatrogenic LSC deficiency in the future. Only a limited amount of LSC can be harvested from the donor, thus fewer LSC can be transplanted so patients with limited LSC deficiency are better suited for LR-CLAL compared to patients with complete LSC deficiency (Holland et al., 2005; Biber et al., 2010).

2.3. Keratolimbal allograft (KLAL)

In KLAL, the recipient receives limbal tissue harvested from cadaveric eyes using corneal tissue as a carrier. This allows for a greater quantity of LSC transplanted to the recipient eye. KLAL is ideal for severe bilateral LSC deficiency, patients with unilateral LSC deficiency unwilling to risk LSC deficiency in their better eye with a CLAU, or in patients who are unable to obtain a willing and living relative for a LR-CLAL. Conditions such as aniridia and iatrogenic LSC deficiency with mild to moderate conjunctival involvement (Schwartz and Holland, 1998) are ideal for KLAL. However, KLAL alone should not be used in recipients with inadequate tear film (Shimazaki et al., 2000), significant active inflammation, and/or severe conjunctival involvement with keratinization of the ocular surface secondary to loss of both LSC and conjunctival epithelial stem cells (Tsubota and Shimazaki, 1999). As mentioned previously, systemic immunosuppression is required with any allograft. The surgical technique for this procedure has been described in detail (Holland et al., 2005; Biber et al., 2010; Lim et al., 2008).

2.4. Combined conjunctival and keratolimbal limbal allograft (C-KLAL)

In C-KLAL, the recipient receives transplantation of KLAL as well as LR-CLAL. This is the preferred procedure in cases with cicatizing ocular surface disease, such as SJS, OCP, and severe chemical burns. The conjunctival transplantation provides additional functional goblet cells to improve the production of mucin in the tear layer. These patients require reconstruction of the conjunctival fornixes and lids. Collaboration with an ocular plastiques surgeon and possibly an otolaryngologist (for harvesting of nasal mucosa from the inferior turbinates) is required (Holland et al., 2005; Biber et al., 2010). Systemic immunosuppression is required.

3. Stem cell technology and corneal epithelial therapy

There are three types of stem cell lines: human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC), and tissue-stem cells. The original stem cells, the hESC, come from human blastocysts and are pluripotent; however, these cells carry tumorigenesis and immunologic rejection issues. The iPSC are man blastocysts and are pluripotent; however, these cells carry tumorigenesis and immunologic rejection issues. The iPSC are derived from the hESC; however, cultivation of these cells has shown low yield with inconsistent tissue lines. Tissue-stem cells are further differentiated stem cells and are unipotent, progenitor cells with minimal tumorigenicity and immunologic reaction.

The corneal LSC are an example of tissue-stem cells that have been successfully identified and used to repair ocular surface disease. The LSC provide the source for corneal epithelial renewal. They are made up of non-keratinizing stratified squamous epithelium located at the basal layer of the epithelium in the transition zone between corneal and conjunctival epithelial cells—the LSC niche. Confocal microscopy has shown how injury to the limbus has affected the LSC niche (Shortt et al., 2007a).
3.1. Ex vivo corneal epithelial limbal stem cell expansion

Penetrating keratoplasty has a poor prognosis in patients with severe LSC deficiency. LSC transplantation can optimize the success rate; however, there are associated risks of inducing LSC deficiency in the donor eye, as well as the need for systemic immunosuppression if the donor is an allograft—either from a living related or cadaveric donor.

In 1997, Pellegrini et al. were the first to successfully treat two patients with total LSC deficiency by cultivating autologous corneal epithelium (Pellegrini et al., 1997). A 1 mm² LSC donor biopsy was taken from the patients’ healthy contralateral eye. The biopsy was then minced, treated with trypsin, and the LSC were isolated and expanded on culture plastic using lethally irradiated mouse 3t3 fibroblast feeder cells. The cultivated epithelial progenitor cells were then successfully transplanted to the recipient eye using a soft contact lens as a carrier. With follow-up of greater than two years, both patients had achieved re-epithelialization of the entire cornea. This landmark study introduced a new perspective in the treatment of LSC deficiency by reducing the risk of morbidity in the donor and maintaining an autologous source.

In cases of bilateral LSC deficiency such as aniridia and SJS, an allogeneic LSC donor source, either living-related or cadaveric, is required. Reports are supportive of using ex vivo expanded LSC allograft, but long-term success has yet to be established (Shortt et al., 2007b; Daya et al., 2005).

3.2. Amniotic membrane and other carrier substrates

Human amniotic membrane, the inner wall of the membranous sac surrounding the embryo during gestation, is the most common carrier substrate used for LSC culture and transplantation. Amniotic membrane provides the cultured LSC with a surrogate niche to assist with survival and function. Amniotic membrane has inherent anti-scarring, anti-angiogenic and anti-inflammatory mediators that enhance re-epithelialization of the ocular surface (Tseng et al., 1998; Schwab et al., 2000; Shimazaki et al., 2002; Grueterich et al., 2002; Tsai et al., 2004). The use of amniotic membrane simplifies manipulation and suturing, while reducing the risk of potential infection associated with using the mouse 3t3 fibroblast feeder cells. Amniotic membrane also acts as a basement membrane enabling cell migration (Dua et al., 2004).

Amniotic membrane is a substrate that allows the LSC to survive and proliferate; however, it requires costly donor screening and there is a potential for transmission of viral disease. Amniotic membrane is also expensive, not readily available, and opaque which may affect vision postoperatively. Processing methods of amniotic membrane are variable and may affect the ability of the amnio to act as a suitable substrate for LSC cultivation. The use of glycerol as a cryoprotectant when processing amniotic membrane has been shown to impair LSC expansion when compared to simple frozen amniotic membrane (Shortt et al., 2009).

Various alternative substrates have been used for corneal epithelial transplantation. These include: fibrin substrate with good results at up to 10 years of follow-up (Rama et al., 2001, 2010); a novel cell-sheet manipulation technology using temperature-responsive culture dishes (Nishida et al., 2004); acrylic acid plasma polymerization to coat the inner surface of a bandage contact lens used to cultivate, transport, and immobilize the tissue (Notara et al., 2007); carrier-free sheets using commercially available fibrin sealant (Higa and Shimazaki, 2008); and autologous serum incorporated into growth media with an FDA-approved soft contact lens as the substrate, carrier, and bandage to protect the eye during transplantation and healing (Di Girolamo et al., 2009).

3.3. Alternate sources of corneal epithelial cells for transplantation

Autologous LSC transplantation and ex vivo expansion of cultured autologous corneal epithelial LSC do not require systemic immunosuppression. However, in cases of bilateral, total LSC deficiency where autologous corneal epithelial LSC tissue is not available for harvesting and expansion, living-related or cadaveric donor allograft with long-term systemic immunosuppression are required. Immunosuppression carries a high risk of serious ocular and systemic complications.

Alternate sources of autologous epithelial cells have been studied in order to avoid the need for systemic immunosuppression in patients with severe bilateral LSC deficiency. Oral mucosal epithelial cells (Notara et al., 2010; Nakamura et al., 2004; Nishida et al., 2004; Inatomi et al., 2006), mesenchymal stem cells (Ye et al., 2006; De Miguel et al., 2010; Joe and Gregory-Evans, 2010), and hair follicle stem cells (Blazewska et al., 2009) may be possible alternative sources.

The in vitro replication of pluripotent hESC derived from blastocysts has been successfully achieved (Ahmad et al., 2007); however, the translation to human therapeutic use must overcome problems with functionality, rejection, and ethical concerns. iPSC have been generated (Takahashi et al., 2007; Park et al., 2008), but challenges still exist with tumorigenicity, immune rejection, refining a specific population, and defining an appropriate model for preclinical studies. The translation of hESC and iPSC technology to human therapeutics is an exciting field that will continue to evolve and develop in future.

3.4. Challenges in using corneal limbal stem cell technology and therapy

There are many challenges in LSC technology and therapy. Most methods of ex vivo LSC expansion require the use of animal products, foreign human tissue/serum, and/or non-approved biomaterials, all increasing the potential risk of xenobiotic infection. Ethical considerations are at the forefront in using hESC as they are harvested directly from blastocysts generated through in vitro fertilization. hESC and iPSC still have complexities with tumorigenicity, immune rejection, refinement to a specific population, and defining appropriate models for preclinical studies.

It is difficult to interpret and compare the efficacy of the numerous ex vivo expansion studies published because of variation among the studies. The main variables are: culture techniques employed, selection of recipient patients for treatment (degree of LSC deficiency in the recipients), evaluation of treatment efficacy (clinical observation vs. impression cytology), lack of outcomes data (visual acuity not reported in some series), variation in follow-up, and the combined use of autol-
ogous and allogeneic transplants in some studies (Shortt et al., 2007b; Ahmad et al., 2010). Despite these challenges, the results are favorable. A recent review summarized the results of 15 studies utilizing autografts showing an 84% success rate in 292 transplants: in nine studies with allografts, there was a 75% success rate in 48 transplants (Ahmad et al., 2010). Further studies with standardized variables will allow for easier interpretation of technology and therapeutic outcomes.

4. Conclusions

Penetrating keratoplasty has a poor prognosis in patients with severe LSC deficiency. LSC transplantation optimizes the success rate; however, there are associated risks of inducing LSC deficiency in the donor eye, as well as the need for systemic immunosuppression if the donor is an allograft—either from a living related or cadaveric donor. The clinical use of ex vivo expansion of cultivated autologous LSC was first described in 1997 (Pellegrini et al., 1997). Modification of this technique and expansion using amniotic membrane and other carrier substrates has enhanced the translation to clinical therapy.

The recent landmark phase 1 clinical study using tissue engineering to produce a biosynthetic cornea has garnered much media interest toward penetrating keratoplasty technology (Fagerholm et al., 2010). A biosynthetic cornea minimizes the risk of rejection, but still requires healthy endothelium and LSC in the recipient, thus highlighting the importance of LSC technology. As LSC technology and tissue engineering continue to evolve, ophthalmologists will have alternatives to manage severe, bilateral LSC deficient conditions, such as chemical burns, SJS, and OCP without the need for systemic immunosuppression and donated cadaveric tissue.

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