Cultivated Autologous Limbal Epithelial Cells (CALEC) for the Treatment of Limbal Stem Cell Deficiency

Milena Di Meo and Myriam Armant  
Center for Human Cell Therapy, Boston Children’s Hospital  
Boston, MA USA

The limbus, which is anatomically located on the outer edge of the cornea, (Figure 1) normally contains 5-15% of progenitor cells, called Limbal Stem Cells (LSC) that, under steady state, contribute to the corneal epithelium homeostasis (Yoon et al., 2014).

**Figure 1**: The limbus as a source of stem cells involved in corneal homeostasis

Corneal damages caused by a variety of insults (eye injuries, genetic disorder, autoimmunity, and inflammation, ocular surface diseases) are often associated with Limbal Stem Cell Deficiency (LSCD). This condition results in scarring, conjunctivalization and vascularization of the cornea ultimately leading to progressive vision loss. Corneal transplantation is not an option in those cases as grafts fail due to lack of host stem and progenitor cells, which are needed to replenish the surface of the donor graft.

In recent years, cellular therapy such as ex vivo limbal epithelial cell transplantation has shown promising results as a treatment of unilateral LSCD (Kolli et al., 2010; Marchini et al., 2012; Nakamura et al., 2004; Rama et al., 2010; Sangwan et al., 2006; Tsai et al., 2000;
Zakaria et al., 2014). However, these clinical trials have not been available in the United States where LSCD remains an unmet clinical need.

In collaboration with Dr. Ula Jurkanas from the Massachusetts Eye and Ear Infirmary (MEEI) who will lead the clinical trial, our group at the Center for Human Cell Therapy (CHCT) developed new methods to yield Cultivated Autologous Limbal Epithelial Cells (CALEC) for the treatment of unilateral LSCD. The Production Assistance for Cellular Therapies (PACT) supported this translational project where we developed and validated a 2-stage manufacturing process (Figure 2). First, enzymatically-isolated limbal epithelial cells are expanded (primary culture) and then a fraction of the expanded cells (P0) is seeded onto a transplantable substrate (secondary culture). Among several scaffold options, we opted for AmnioGraft®, which received tissue designation by the Food and Drug Administration (FDA) and has anti-microbial, anti-angiogenic and anti-inflammatory properties.

**Figure 2: CALEC process overview**

1. Harvest biopsy
2. Transport to lab
3. Enzymatic digestion
4. Plating & expansion
5. Harvest & QC
6. Seed cells on AmnioGraft®
7. Final product QC & formulation
8. Transport to clinical site

- Counts & viability
- Phenotype (identity=epithelial)
- Colony Forming Efficiency assay
- Proliferative potential assay

End of Primary Culture

Unaffected eye

CALEC ready for transplant on the affected eye
Some of the key changes made to the manufacturing included removing the murine feeder cell line and switching to a serum-free media formulation. In order to mimic as much as possible the actual clinical trial, we developed an in vitro model of limbal biopsy using cadaveric corneas. The established manufacturing process is reproducible with a 97.1% success rate (n=70 products), with an average duration of 18±4 days.

Cellular grafts present unique challenges when it comes to final product testing and release criteria. Unlike suspension cell-based therapies, samples of the graft cannot be taken for testing without compromising the integrity of the graft. Our approach was twofold: 1) validate every steps of the process and 2) develop and validate surrogate assays. We satisfied all regulatory requirements including defining cell counts and viability. To determine the cell dose for each graft, we established a method to count cells in situ using a cell-imaging platform. On the other end, cell viability was determined by validating lactate dehydrogenase (LDH) release as a surrogate marker. LDH is a cytosolic enzyme that is released in the media when the plasma membrane is damaged and it is a well-established indicator of cellular toxicity/lysis. We were able to validate the assay and show a strong correlation (r=0.99) between the amount of LDH release by cells in the culture supernatant and the percentage of dead cells as determined by the LIVE/DEAD® assay.

We completed the process and product development phase by working out the post-manufacturing logistics, including final formulation, stability and transport of the cellular graft back to the clinical site.

The safety and efficacy of CALEC will soon be tested in patients with unilateral LSCD in a Phase I/II trial as the study (BB-IND-16102) recently cleared the Food and Drug Administration (FDA).

References


