Bioengineering Endothelialized Neo-Corneas

J. Koudy Williams[1,3], Jin San Choi[1], Margaret Greven [1], Keith Walter[2], Patrick Laber[2], Anthony Atala[1], Shay Soker[1].

Wake Forest Institute for Regenerative Medicine[1], the Department of Ophthalmology [2], and the Department of Pathology[3], Wake Forest University Health Sciences, Winston-Salem, NC, USA

Introduction

The inner layer of the corneal is a single layer of neural-crest derived cells that form a barrier between the cornea and the aqueous humor. These corneal endothelial cells (CECs) are essential for transport of water from the corneal stroma. Damage or decompensation of the CEC pump function results in corneal edema and loss of vision. CECs do not divide during adult life. Any loss or damage to the CECs is repaired by the spreading of existing cells. When cell density is reduced to a minimum functional level, the cells can no longer adequately function and edema ensues. CEC loss is most well documented as a result of accidental damage during cataract surgery or in an inherited condition known as Fuch's dystrophy. Fuch's pathology presents during later life and has been documented as present in a number of older patients. Additionally, blunt trauma to the eye, as well as concussive head trauma, produce irreversible, blinding damage to the endothelium both in military and civilian situations. Traditional therapy for CEC loss or damage has been penetrating keratoplasty PK. However, a procedure known as DSEK (Descemet's Stripping and Endothelial Keratectomy) is rapidly replacing PK in cases where the corneal stroma is not scarred. In this procedure, CECs and their underlying basement membrane (Descemet's membrane) are physically removed from the recipient and a very thin layer of stroma-containing intact CECs from a donor cadaveric cornea is transferred to the recipient eye. This procedure produces a much more stable eye and restores accurate vision to the recipient much more rapidly and with fewer complications than traditional PK. However, there is an increasing gap between the number of donated corneas and the demand for corneas for transplantation. Bioengineering an endothelialized neo-cornea using expandable populations of human corneal endothelial cells (hCECs) seeded at a high density on decellularized corneal stroma would address these issues by creating a greater supply of transplantable tissue of uniform quality to be used in partial-thickness transplant procedures.

Methods

hCEC were obtained from discarded Descemet's membranes of various aged eye donors by digestion in collagenase II (2 mg/mL D-PBS, 5 mL/cornea) for 1 hour at 37° C. The cells were cultured in EGM-2 (10% FBS) for 28 days and then characterized by morphology and immunostaining for Connexin-43, ZO-1, CD31, and VE-Cadherin. Corneal scaffolds were made using a microtome to cut whole corneas into 125 micron-thick slices. These corneal slices were placed in decellularization solution (2% Triton X100, 0.1% NH_4OH) for 3 days at 4° C. Approximately 300,000 cells were seeded on the decellularized scaffold. The construct was placed in EGM-2 (10%FBS) media for seven days, and then cross-sections were stained with H&E, DAPI, and Connexin-43.

Results

EFFECTS OF DONOR AGE ON CELL GROWTH: The age of the cell donors ranged from 14 to 72 years of age. Cells were more readily obtained from younger donors (below 30 year of age). Representative growth of cells from a younger and older donor are shown in **Figure 1**.

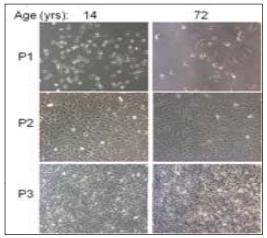

Figure 1. Cell cultures at passage 1, 2, and 3 from donor corneas of a 14 (left panel) and 72 year-old (right panel) patient. Cells were grown on TCP dishes in EGM-2 media. Similar to other divergent age cultures, more viable cells were obtained from the younger corneas (range 14-30 years of age) vs. the older corneas (50-80 years-old).

EFFECTS OF MATRIX ON CELL GROWTH: Underlying matrix has been shown to affect the growth rate of cells. Type IV collagen is a major component of the corneal stroma and fibronectin is commons used to coat endothelial seeded culture plates. Thus, we grew the cells from the 14 year old donor on uncoated tissue culture, plates, plates coated with fibronectin, or plates coated with type IV collagen and measure their growth rates **(Figure 2).**

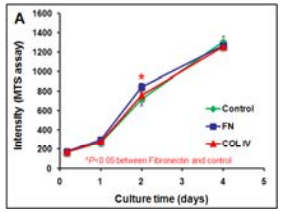

Figure 2. Growth rate (as assayed by MTS intensity) of CECs gown on uncoated culture plates (Control), fibronectin (FN), or type IV collage (Col IV) at 1, 2, 3, 4, and 5 days post seeding.

CHARACTERIZATION OF CULTURED CELLS: Seeded corneal stroma was analyzed for presence of cells (H&E, DAPI), a marker expressed when endothelial cells form tight junctions (ZO-1) and a cell marker expressed when cells have the abilty to pump from the corneal stroma (Na+/K+ ATPase) (**Figure 3**).

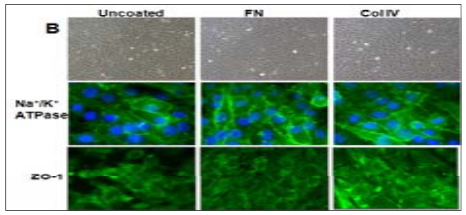

Figure 3. Expression on Na+/K+ ATPase and zona occludens-1 (ZO-1) in CECs coated on tissue culture plates or plates coated with fibronectin, or type IV collagen. CECs also expressed Connexin-43 and VE-cadherin protein (data not shown), but di not express CD31.

DECELLULARIZATION OF CORNEAL STROMA: Once cells were ready for seeding, cross-sections of corneal stroma were decellularized as described. A comparison was made between native and decellularized cornea **(Figure 4).**

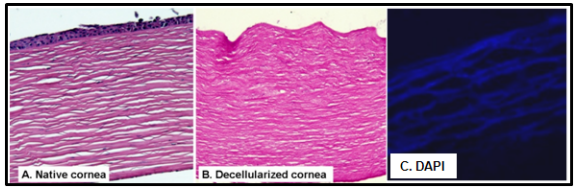

Figure 3. H&E stained full thickness cross-sections of a normal cornea (A) and a de-cellularized cornea (B) (10X). Decellularization removed cells from the stroma, epithelial cells from the anterior cornea (C) DAPI staining of the de-cellularized cornea showing no nuclear material (20X).

SEEDING DECELLULARIZED CORNEAL STROMA: Seeded stroma was analyzed for presence of cells (H&E, DAPI), tight junction protein (Connexin 43) and (Na^+/K^+ ATPase) (**Figure 5**).

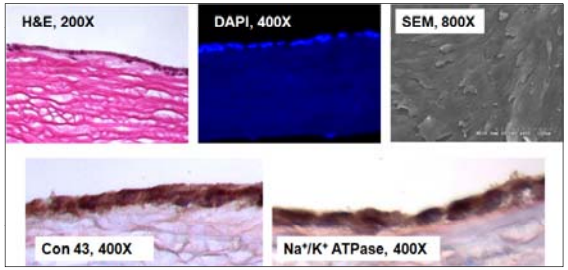

Figure 5. Cross sections of seeded corneal stroma were stained with H&E, or DAPI, prepared for scanning electron microscopic views of endothelial cells, and stained for Connexin 43 (Con 43), or Na^+/K^+ ATPase.

Conclusions

Human corneal endothelial cells can be isolated from scleral rims remaining after the central cornea has been removed for transplantation. These cells express markers typical of corneal endothelial cells. hCECs from one scleral rim can be expanded to the numbers needed to re-endothelialize several neo-corneal constructs. Additionally, corneal scaffolds can be made from corneal tissue unfit for transplantation due to low cell counts. Construction of neo-corneas from tissue and cells not normally used in corneal transplant procedures creates a new potential source of high quality corneal tissue for transplantation.

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