Bioengineering Eodothelialized Neo-Corneas

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Statement of Purpose: The inner layer of the cornea is a single layer of neural-crest derived cells that form a barrier between the cornea and the aqueous humor. These corneal endothelial cells (CEC) are essential for transport of water from the corneal stroma. Damage or decompensation of the CEC pump results in corneal edema and loss of vision. CEC loss is most well documented as a result of accidental damage during cataract surgery or in an inherited condition known as Fuchs' dystrophy. Bioengineering neo-corneas, using an expandable population of endothelial cells derived from unused donor corneas, would address the shortage of corneas needed for corneal repair procedures. The objectives of this study are to establish human CEC isolation and culture and to investigate feasibility of bioengineering cornea using decellularized corneal stroma (hDCS) coated with CEC for transplantation.

Methods: Human CEC (hCEC) were obtained from discarded of various aged eye donors by digestion in collagenase II, expanded and evaluated by IF. hCEC were cultured on collagen IV-coated, fibronectin-coated, and uncoated tissue culture plates to compare their adhesion, proliferation, and cellular morphology. Corneal scaffolds were sliced using a microtome with 110 µm-headblade. These corneal slices were decellularized with 2% Triton X-100/0.1% NH_4OH for 3 days at 4°C. The mechanical properties and ECM components of native and decellularized corneal stroma were examined. Approximately 130 cells/mm^2 of hCEC were seeded on the decellularized tissue. The construct was placed in growth medium for 14 days, and investigated by scanning electron microscopy (SEM), hematoxylin and eosin (H&E) staining, and immunofluorescence staining.

Results: In the present study we explored clinically relevant sources of hCEC. Cell doubling times from young donors (e.g., 14 years) were less than those from older donors (e.g., 70 years) (Table 1) and grew best on fibronectin-coated plates (Figure 1). Confluent hCEC expressed connexin-43, ZO-1, VE-Cad and Na^+/K^+ ATPase, but not CD31. Human DCS were prepared by removing all cellular components chemically and leaving behind the intact ECM proteins. The optimal seeding density to establish a confluent layer of hCEC on hDCS was 130 cells/mm^2. Confluent cells expressed connexin-43, ZO-1, and Na^+/K^+ ATPase (Figure 2).

Table 1. Corneal endothelial cell isolation and culture.

Age (years old)	*n*	No. of successful cultures	Days from cornea collection to cell processing	Doubling time (hr)
Donors ≤ 30	3	3	9 ± 4.6	22.8 ± 3.6
30 < Donors ≤ 60	18	9	7.6 ± 1.5	23.5 ± 2.3
60 < Donors	16	5	6.4 ± 2.8	39.6 ± 12.8

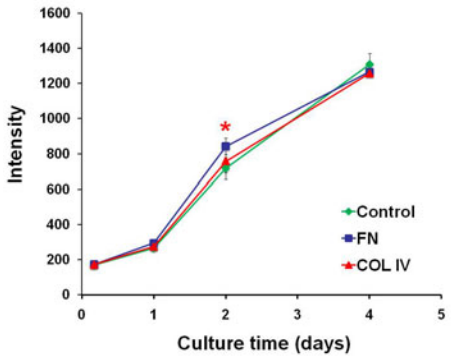

Figure 1. (A) Growth rate of hCEC grown on uncoated culture plates (control), fibronectin (FN), and type IV collagen (Col IV) at 1, 2, and 4 days post seeding. *P<0.05 between FN and control.

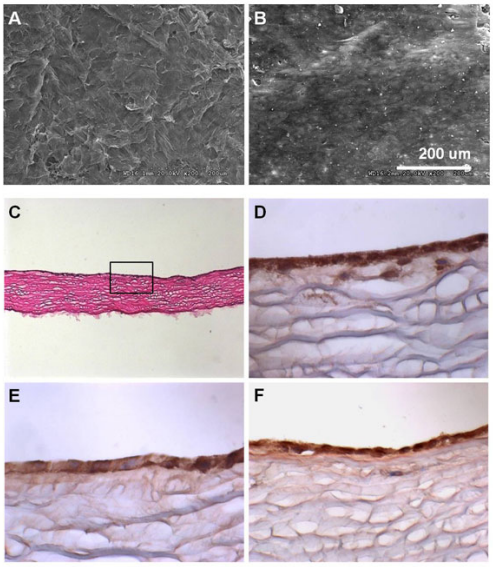

Figure 2. SEM images of hCEC seeded decellularized stroma tissue; (A) unseeded and (B) hCEC seeded (magnification, ×200). Scale bar indicates 200 µm. Cross sections of seeded corneal stroma were stained with (C) H&E (magnification, ×100), (D) ZO-1, (E) connexin 43, and (F) Na^+/K^+ ATPase (magnification, ×630).

Conclusions: Human CECs can be isolated from sclera rims remaining after the central cornea has been removed for transplantation. These cells express markers typical of corneal endothelial cells. hCECs from one donor can be expanded to re-endothelialize several neo-cornea constructs. Corneal scaffolds can be obtained from tissue unfit for transplantation because of flow cell counts. Construction of neo-corneas creates a new source of high quality corneal tissue for transplantation.

References:

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