

Proceedings of the Annual Symposium on Regenerative Medicine(PASRM)

A Rabbit Model of Ex Vivo Cultivation and Transplantation of Autologous Limbal Epithelial Cells Grown in a Thermo-reversible Gelation Polymer (Mebiol Gel)

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Published online on 26 Dec 2006

Purpose:

To evaluate the efficacy of autologous expanded corneal epithelial cell transplants derived from harvested limbal epithelial stem cells cultured on a thermo-reversible polymer (Mebiol Gel) for the management of unilateral limbal stem cell disease (LSCD). Mebiol Gel is a copolymer composed of the thermo responsive polymer block [poly (N-isopropylacrylamide-co-n-butyl methacrylate) (poly NIPAAm-co-BMA)] and the hydrophilic polymer block [polyethylene glycol (PEG)].

Materials and Methods:

In 12 rabbits, a limbal biopsy was taken and thereafter ocular surface damage was created on the same eye by removal of corneal and limbal epithelium. The limbal biopsies were cultured on the Mebiol Gel as explant cultures for 3 weeks to produce confluent epithelial cells, harvested non-enzymatically by reducing the temperature to 4⁰C. Three weeks later, vascularised pannus was removed and expanded autologous limbal epithelial cells

were placed in a drop fashion to cover the entire damaged corneal surface. The rabbits were sacrificed at the end of the study and the damaged corneas were harvested for histological and RT-PCR study for the limbal phenotype (p63 & ABCG2), transient amplifying cell markers (Integrin β 1 & Connexin 43) and corneal phenotype (K3 & K12) markers. Outcome measures such as corneal vascularization (V), corneal haze (H), fluorescein staining (F) and histopathology (P) were evaluated between 6-8 months. Each outcome was scored based on the severity of each variable as judged on photography and histopathology. Two rabbits (controls) underwent transplantation with Mebiol gel alone without cultured cells.

Results:

Reparative surgery was a total success (score, 8-10) in 7 (58.3%), partial success (score, 6-7) in 2 (16.7%) and failure (score, \leq 5) in 3 (25%). Histological and RT PCR study documented successful growth of corneal epithelium onto the recipient surface. RT PCR

studies showed the limbal phenotype in the limbus and cornea phenotype in the central portion. Transient amplifying cell marker is present in both limbus and cornea. Reparative surgery was a failure in controls.

Conclusion:

The results of the rabbit model suggest that autologous limbal epithelial cells grown in thermo-reversible gel polymer may restore a nearly normal ocular epithelial surface in eyes with unilateral LSCD.

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