

Review Article

Corneal surface reconstruction - a short review

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Cornea is the clear, dome-shaped surface that covers the front of the eye and when damage due to burns or injury and several other diseases, stem cells residing in its rim called "limbus" are stimulated to multiply to support growth of new epithelial cells over its surface. If this ready source of stem cells is damaged or destroyed the natural repair is not possible and such a condition is known as corneal limbal stem cell deficiency (CLSCD) disease. Stem cell transplant helps such persons to regenerate the corneal surface. Human corneal limbal stem cell transplantation is at present an established procedure with reasonable good clinical outcome particularly when autologous limbal epithelial tissue from a fellow unaffected eye is used.^{1, 2} A major concern related to the autograft is the possibility of CLSCD at the donor site, 3 techniques that allowed the expansion of a small limbal biopsy in the laboratory using cell cultures that could be then transplanted to the affected eye have been developed,^{4, 5}

Human amniotic membrane (HAM) is used as a scaffold for both culturing the human limbal epithelial cells and for ocular surface reconstruction with the cultured limbal epithelial cells.⁴⁻⁷ However, researchers have used alternative scaffolds like collagen⁸,

fibrin gel⁹ and cross-linked gel of fibronectin and fibrin.¹⁰ All these are biological materials and also need for animal 3T3 feeder layer for stem cell cultures.

The properties of HAM are unique including antiadhesive effects, bacteriostatic effects, wound protection, pain reduction, and improvement of epithelialization and characteristically lacking immunogenicity. The use of amniotic membrane transplantation (AMT) to treat ocular surface abnormalities was first reported by Graziella Pellegrini, chief of stem cell laboratory at Giovanni Paolo Hospital in Venice, Italy, who was the first to demonstrate the limbal stem cell transplant in 1997. Amniotic membrane has been successfully used in patients with persistent epithelial defects, pterygium, symblepharon, and for ocular surface reconstruction. The role of AMT in ocular disorders has been recently re-evaluated by Schwab and coworkers.¹¹ They carefully examined the protocols used in the manufacture of the bio-engineered construct to assess the risks and reviewed 20 published reports of human trials conducted between 1996 and 2005 in a report suggesting that the currently used transplant procedures carry potential health risks not only to individuals but also to "the wider community" because they "rely on the use of materials

from animal and human donors". Their review revealed that most protocols used animal-derived products including fetal calf serum (FCS) with a potential for transmissible spongiform encephalopathy (TSE) infection (of the brain) or allergic reactions and further state that the use of commercially available fibrin tissue "adds to the risk of microbial or prion contamination". Since no investigations have been done, the use of AMT can potentially induce "disease transmission through contamination with bacteria, viruses, or other infectious agents", they also stated that with 3T3 cells being commonly used (that come from mice) possibilities of "xenozoonosis", or animal-to-human disease transmission are a concern.

Several studies have been undertaken using oral mucosal epithelial cells cultivated on amniotic membrane for useful tissue engineering of damaged corneal surface. Higa and Shimazaki have carried out a study of transplantation in cultivated oral mucosal epithelial which has been useful in achieving a stable ocular surface. However, in addition to using epithelial sheets with AM, they developed a technique for generating carrier-free sheets using fibrin sealants. These sheets seem to contain more differentiated epithelium than those obtained with AM while retaining similar levels of colony-forming progenitor cells. In terms of isolation and cultivation of corneal epithelial stem/progenitor cells, we found that single murine limbal cells exhibited clonal growth and generated stratified epithelial sheets.¹² Dogru et al determined the barrier function and cytologic features of ocular surface epithelium after autologous cultivated oral mucosal epithelial transplantation in a prospective observational study. Cultivated oral mucosal epithelial cells were observed to survive for more than 1 year after transplantation, with gradual replacement by conjunctival epithelium in some cases. Decreased barrier function of the transplanted epithelium may have prognostic implications, suggesting the presence of oral mucosal epithelium long after surgery.¹³ Oral epithelial sheets cultivated in autologous serum (AS) and fetal bovine serum-supplemented media

were similar in morphology, and both formed basement membrane assembly proteins important for maintaining graft integrity. Complete corneal epithelialization was achieved within 2 to 5 days postoperatively. The successful use of an AS-derived oral epithelial equivalent to treat severe ocular surface disease represents an important advance in the pursuit of completely autologous xenobiotic-free bioengineered ocular equivalents for clinical transplantation¹⁴.

Since most of these studies used heterologous carrier for growth of corneal limbal cells with attendant likely complications, we developed a novel method of cultivation of human limbal cells in a synthetic material as a culture substrate and the material we chose was Mebiol Gel (Provided by Mebiol Inc., Japan through Nichi-In Bio Sciences PVT Ltd, Chennai, India). Mebiol Gel is a copolymer composed of thermo responsive polymer block [poly (Nisopropylacrylamide-co-n-butyl methacrylate) (poly NIPAAm-co-BMA)] and the hydrophilic polymer block [polyethylene glycol (PEG)]. This polymer block is hydrophilic at temperatures below 20°C and hydrophobic at temperatures above 20°C forming cross-linking points and homogenous three dimensional (3-D) network of Mebiol Gel in water. Cells or tissues can be embedded in a liquid Mebiol gel solution at lower than 20°C and cultured three dimensionally in a hydrogel state at 37°C. The sol-gel transition temperature can be controlled by altering chemical composition of thermo-reversible gelation polymer (TGP). Mebiol Gel prevents the growth of the fibroblasts and it is not toxic to cells. In our earlier study, continuous culture cell lines¹⁸ and we demonstrated that Mebiol Gel supported the growth of corneal limbal epithelial cells and these cells expressed both limbal and corneal phenotype, suggesting that limbal epithelial cells cultivated in Mebiol Gel would be a promising material for corneal surface tissue engineering.

With this in view, we developed a novel method of cultivation of human limbal cells in a synthetic scaffold made of a thermo

reversible polymer, that allows the limbal epithelial cells to survive, proliferate and differentiate into corneal epithelial cells and demonstrated the effectiveness of this polymer for corneal tissue engineering using an experimentally induced limbal deficiency in a rabbit model. In this report, the detailed procedure of the animal model and the results are discussed. We evaluated the efficacy of autologous expanded corneal epithelial cell transplants derived from harvested limbal biopsy cultured on a thermo-reversible polymer (Mebiol Gel) for the management of unilateral limbal stem cell disease (LSCD). Corneal limbal biopsies from ¹² rabbits were cultured on Mebiol Gel at 37°C. Cells were harvested from the dishes after 3 weeks by reducing temperature to 4°C. Autologous transplantation was undertaken to reconstruct the experimentally induced limbal stem cell deficiency in the rabbit eyes. The corneas of both eyes of all rabbits were harvested later for histological and RT-PCR studies. Reparative surgery was a total success in 7 (58.3%; score, 8-10), partial success in 2 (16.7%; score, 6-7) and failure in 3 (25%; score, <5). Histological and RT-PCR study documented successful growth of corneal epithelium onto the recipient surface. Our results suggest that transplantation of autologous limbal epithelial cells grown in thermo-reversible gel polymer may restore a nearly normal ocular epithelial surface in eyes with unilateral LSCD. ¹⁵

We believe that use biodegradable and thermo-reversible Mebiol Gel as the cultivation carrier will be most useful with no attending complication for corneal limbal stem cell transplantation in patients who need such a procedure.

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