Abstract

Limbal stem cell deficiency is a disease caused by the impairment of limbal epithelial stem cells (LESCs), leading to the replacement of the corneal surface with nontransparent conjunctiva, and its treatment is not standardly available worldwide. This work focuses on preparing cells for treating both uni- and bilateral forms of the disease using advanced cell-based therapy. It investigates LESCs in various scaffolds (fibrin, nanofibers) and explores non-limbal cell sources like oral mucosal epithelial cells (OMECs) under standard complex and xenobiotic-free culture conditions. The study uses immunofluorescence and gene expression to detect stem cell markers, proliferation, and differentiation capacity of cultured cells. Moreover, long-term OMECs storage with different media and cryoprotective agents and the healing properties of the amniotic membrane (AM) were also examined.

We found that adding interleukin-13 to the culture media enhanced LESCs' stemness. LESCs on fibrin gels showed higher expression of stemness markers, while those on polymers expressed more mesenchymal ones. Using standard and xeno-free media, we successfully prepared OMEC-containing cell sheets on fibrin gel substrates. The cultured cells exhibited high expression of stemness genes (*ΔNp63α*, *NGFR*, *KLF4*) and decreased levels of differentiation (lower *KRT13* expression). Keratins related to basal layer and progenitor cells (*KRT14*, *KRT15*, *KRT17*, *KRT19*) were highly expressed in both conditions. The cells in complex media had a higher proliferation rate, evidenced by the upregulation of *MKI67*, with an earlier onset of the growth and reaching confluence sooner than xeno-free cultures. OMECs formed a confluent cell sheet even after storage in liquid nitrogen. Better outcomes (confluence, viability) were observed for OMECs stored in complex media alone or with 5% glycerol, compared to complex media with 10% glycerol or 10% dimethyl sulfoxide, particularly when stored after the first passage instead of using primary cells. Lastly, we showed that cryopreserved AM is a safe and effective treatment for non-healing wounds, with consistent interplacental quality among AM grafts and a strong analgesic effect.

In conclusion, we have prepared and finalized protocols for cultivating limbal and oral mucosa cells. The cell culture can now be transferred to the cleanroom conditions of the tissue bank for verification, and the protocols can be forwarded to the State Institute for Drug Control for approval for clinical use.

Keywords: limbal stem cell deficiency, stem cells, ocular surface, oral mucosa, cell culture, amniotic membrane, transplantation