World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:9, No:09, 2015

Pluripotent Stem Cells as Therapeutic Tools for Limbal Stem Cell Deficiencies and Drug Testing

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Abstract : Background and Rationale: Transparent avascularised cornea is essential for normal vision and depends on limbal stem cells (LSC) that reside between the cornea and the conjunctiva. Ocular burns or injuries may destroy the limbus, causing limbal stem cell deficiency (LSCD). The cornea becomes vascularised by invaded conjunctival cells, the stroma is scarring, resulting in corneal opacity and loss of vision. Grafted autologous limbus or cultivated autologous LCS can restore the vision, unless the two eyes are affected. Alternative cellular sources have been tested in the last decades, including oral mucosa or hair follicle epithelial cells. However, only partial success has been achieved by the use of these cells since they were not able to uniformly commit into corneal epithelial cells. Human pluripotent stem cells (iPSC) display both unlimited growth capacity and ability to differentiate into any cell type. Our goal was to design a standardized and reproducible protocol to produce transplantable autologous LSC from patients through cell reprogramming technology. Methodology: First, keratinocyte primary culture was established from a small number of plucked hair follicles of healthy donors. The resulting epithelial cells were reprogrammed into induced pluripotent stem cells (iPSCs) and further differentiate into corneal epithelial cells (CEC), according to a robust protocol that recapitulates the main step of corneal embryonic development. qRT-PCR analysis and immunofluorescent staining during the course of differentiation confirm the expression of stage specific markers of corneal embryonic lineage. First appear ectodermal progenitor-specific cytokeratins K8/K18, followed at day 7 by limbal-specific PAX6, TP63 and cytokeratins K5/K14. At day 15, K3/K12+-corneal cells are present. To amplify the iPSC-derived LSC (named COiPSC), intact small epithelial colonies were detached and cultivated in limbal cell-specific medium. In that culture conditions, the COiPSC can be frozen and thaw at any passage, while retaining their corneal characteristics for at least eight passages. To evaluate the potential of COiPSC as an alternative ocular toxicity model, COiPSC were treated at passage P0 to P4 with increasing amounts of SDS and Benzalkonium. Cell proliferation and apoptosis of treated cells was compared to LSC and the SV40-immortalized human corneal epithelial cell line (HCE) routinely used by cosmetological industrials. Of note, HCE are more resistant to toxicity than LSC. At P0, COiPSC were systematically more resistant to chemical toxicity than LSC and even to HCE. Remarkably, this behavior changed with passage since COiPSC at P2 became identical to LSC and thus closer to physiology than HCE. Comparative transcriptome analysis confirmed that COiPSC from P2 are similar to a mixture of LSC and CEC. Finally, by organotypic reconstitution assay, we demonstrated the ability of COiPSC to produce a 3D corneal epithelium on a stromal equivalent made of keratocytes. Conclusion: COiPSC could become valuable for two main applications: (1) an alternative robust tool to perform, in a reproducible and physiological manner, toxicity assays for cosmetic products and pharmacological tests of drugs. (2). COiPSC could become an alternative autologous source for cornea transplantation for LSCD.

Keywords : Limbal stem cell deficiency, iPSC, cornea, limbal stem cells
Conference Title : ICCSCE 2015 : International Conference on Cell and Stem Cell Engineering
Conference Location : Rome, Italy
Conference Dates : September 17-18, 2015