DOI 10.18699/SBB-2022-464

ReLEx SMILE lenticules as a source of obtaining stromal corneal cells

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Key words: cornea, stromal cells, keratocyte, fibroblast, lenticules

Motivation and Aim: It is well-known that the corneal blindness takes the third leading place in the structure of the causes of eye diseases [1]. The impaired function of keratocytes in degenerative diseases of the cornea is accompanied by its clouding [2]. The transparency is a key property of the cornea, providing its optical properties. Corneal transplantation remains the treatment for patients with severe corneal lesions, but has some limitations, including the deficiency of donor corneal tissue and post-transplant complications. Cellular therapy using human corneal stromal cells (hCSC) is an alternative approach for treating the cornea. Aim of this study to isolate hCSCs from a new source – ReLEx SMILE lenticules and conduct a comparative of the phenotype and functional properties for fibroblasts and keratocytes, with the prospect in the treatment of diseases associated with corneal opacity.
Methods and Algorithms: hCSCs were isolated from lenticules and cultivated in DMEM/F12 media with 10 % FCS, L-glutamin, antibiotics. The differention of corneal fibroblasts into keratocytes was performed in a selective KGM (keratocyte growth medium). The cell culture phenotypes were determined by flow cytometry. The proliferation and migration of cells were assessed using a real time cell analyzer RTCA. The cell production of EPO, BDNF, VEGF, TNF-alpha, IGF-1, SDF-1a, sICAM-1, vimentin, fibronectin, and total collagen was determined immunosorbent assay (ELISA).
Results: The hCSCs had the morphology and phenotype of fibroblasts in the growth medium with the addition of 10 % FCS and the expressed markers of mesenchymal cells CD73 (99.2 ± 1.62 %), CD105 (22.8 ± 2.91 %) and were negative for CD90, CD34, CD45, HLA-DR, as well as keratocan and lumican. Keratocytes cultured in KGM expressed high levels of keratocyte markers: keratocan (98.8 ± 1.93 %) and lumican (81.4 ± 26.64 %) but not fibroblast markers. Corneal fibroblasts had more pronounced proliferative and migratory activity than keratocytes according to real time cell analyzer RTCA. The fibroblast conditioned medium contained the higher levels of VEGF and vimentin. The keratocytes produced higher levels of EPO and BDNF. The level of fibronectin, collagen, SDF-1a, IGF-I, TNF-alpha, and sICAM-1 was similar in the conditioned mediums of the fibroblast and keratocytes.
Conclusion: Our data indicate that lenticules obtained with the ReLEx SMILE can be a source of hCSCs and keratocytes for various tasks of regenerative medicine.

References

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