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CULTURED HUMAN BUCCAL MUCOSA EPITHELIAL CELLS CAN BE DIFFERENTIATED TO CORNEAL LINEAGES PROVEN BY PRESENCE OF β-INTegrIN AND C/EBPβ

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Background:
Limbal stem cell deficiency may lead to severe corneal opacification and consequently, severe losses of vision as a result of complete loss of corneal epithelial stem cells. The aim of this study was to differentiate human buccal mucosa epithelial cells to corneal epithelial lineages.

Materials and methods:
Buccal mucosa was obtained from excess mucosa in post-tonsillectomy. The tissue was minced into several pieces of about 1mmx1mm in size and digested with 0.3% Collagenase Type I. Cells were re-suspended in mixed medium of Define Keratinocytes Serum Free Medium (DKSFM) and Ham’s F12 medium added with Dulbecco’s Modified Eagle Medium supplemented with 10% foetal bovine serum (F12: DMEM+10%FBS). Fibroblast cells were removed after confluence was reached by differential trypsinization technique and epithelial cells were divided into two plates containing DKSFM and limbal medium for up to ten days respectively. Total RNA for both types of cells were extracted and subjected to quantitative Real Time Polymerase Chain Reaction (RT-PCR) to detect β-integrin and C/EBPβ expression. β-integrin acts as a cell adhesion regulator which is very important in maintaining epithelial cellular attachment and plays a vital role in normal corneal phenotype maintenance and wound healing. Meanwhile C/EBPβ functions as a regulator of cell cycle and self-renewal in human limbal stem cells.

Results:
Induced buccal epithelial cells showed up-regulation (β-integrin: 3.24×10⁻¹±1.01×10⁻¹; C/EBPβ: 1.62×10⁻²±6.29×10⁻³) of both corneal markers as compared to uninduced buccal epithelial cells (β-integrin: 4.55×10⁻⁵±3.46×10⁻³; C/EBPβ: 2.34×10⁻⁶±4.59×10⁻⁷) (p=0.01).

Conclusion:
We conclude that buccal mucosa epithelial cells have the potential to differentiate to corneal lineages.

Keywords:
corneal lineages, buccal mucosa, limbal medium, RT-PCR, tissue engineering.