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Mycophenolate Mofetil Increases Mucin Expression in Primary Cultures of Oral Mucosal Epithelial Cells for Application in Limbal Stem Cell Deficiency

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Abstract : Autologous cultured explants of human oral mucosal epithelial cells (OMEC) are a potential therapeutic modality for limbal stem cell deficiency (LSCD). Injury or inflammation of the ocular surface in the form of burns, chemicals, Stevens Johnson syndrome, ocular cicatricial pemphigoid etc. can lead to destruction and deficiency of limbal stem cells. LSCD manifests in the form of severe ocular surface diseases (OSD) characterized by persistent and recurrent epithelial defects, conjuntivalisation and neovascularisation of the corneal surface, scarring and ultimately opacity and blindness. Most of the cases of OSD are associated with severe dry eye pertaining to diminished mucin and aqueous secretion. Mycophenolate mofetil (MMF) has been shown to upregulate the mucin expression in conjunctival goblet cells in vitro. The aim of this study was to evaluate the effects of MMF on mucin expression in primary cultures of oral mucosal epithelial cells. With institutional ethics committee approval and written informed consent, thirty oral mucosal epithelial tissue samples were obtained from patients undergoing oral surgery for non-malignant conditions. OMEC were grown on human amniotic membrane (HAM, obtained from expecting mothers undergoing elective caesarean section) scaffold for 2 weeks in growth media containing DMEM & Ham's F12 (1:1) with 10% FBS and growth factors. In vitro dosage of MMF was standardised by MTT assay. Analysis of stem cell markers was done using RT-PCR while mucin mRNA expression was quantified using RT-PCR and q-PCR before and after treating cultured OMEC with graded concentrations of MMF for 24 hours. Protein expression was validated using immunocytochemistry. Morphological studies revealed a confluent sheet of proliferating, stratified oral mucosal epithelial cells growing over the surface of HAM scaffold. The presence of progenitor stem cell markers (p63, p75, β1-Integrin and ABCG2) and cell surface associated mucins (MUC1, MUC15 and MUC16) were elucidated by RT-PCR. The mucin mRNA expression was found to be upregulated in MMF treated primary cultures of OMEC, compared to untreated controls as quantified by q-PCR with β-actin as internal reference gene. Increased MUC1 protein expression was validated by immunocytochemistry on representative samples. Our findings conclude that OMEC have the ability to form a multi-layered confluent sheet on the surface of HAM similar to a cornea, which is important for the reconstruction of the damaged ocular surface. Cultured OMEC has stem cell properties as demonstrated by stem cell markers. MMF can be a novel enhancer of mucin production in OMEC. It has the potential to improve dry eye in patients undergoing OMEC transplantation for bilateral OSD. Further clinical trials are required to establish the role of MMF in patients undergoing OMEC transplantation.

Keywords : limbal stem cell deficiency, mycophenolate mofetil, mucin, ocular surface disease
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