Fabrication of Transplantable Human Oral Mucosal Epithelial Cell Sheets Using Temperature-responsive Culture Inserts without The Use of Xneogeneic Products

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Background: The oral mucosal epithelium is an attractive cell source for regenerative therapies due to its strong proliferative potential, and its availability from relatively non-invasive biopsies. Recently, for various applications, we have used the ectopic transplantation of oral mucosal epithelial cell sheets to regenerate tissues such as the cornea[1)] and esophagus[2)]. However, current conditions to create stratified epithelial cell sheets use fetal bovine serum (FBS) and murine 3T3 feeder cells to promote cell attachment and proliferation, allowing for serial propagation.

Aims: Currently, significant concerns for the clinical application of tissue engineered epithelial cell sheets are due to the potential contamination by feeder cells, as well as a risk of pathogen transmission such as mycoplasma, viruses and prions (i.e. bovine spongiform encephalopathy, BSE). We therefore sought to develop culture conditions without the use of xenogeneic products, and robust epithelial cell sheets could be fabricated. Then we investigate these epithelial cell sheets, compared with native oral tissue and cell sheets with 3T3 feeder cells and FBS.

Materials and Methods: Canine oral mucosal epithelial cells were cultured on temperature-responsive culture dishes under various conditions without feeder cells, and in either autologous serum or serum-free environments. Additionally, the initial cell density and the effect of temperature-responsive culture inserts, the surface of which have micropores, were also examined.
On the basis of the canine data, human oral mucosal epithelial cells isolated from small, non-invasive biopsies were cultured on temperature-responsive culture inserts in the absence of xenogeneic products, such as murine 3T3 cell feeder layers and FBS. Upon non-invasive harvest, the fabricated cell sheets were investigated by histology, immunohistochemistry, and electron microscopy.

Results: When canine and human oral mucosal epithelial cells were seeded at subconfluent densities on temperature-responsive culture inserts, cells proliferated to form stratified sheets without the use of feeder cells or serum[3.4)]. Upon reducing temperature, all the cultured cells were detached from the dish surfaces as a transplantable single contiguous cell sheet under xneogeneic product-free culture condition(Fig.1). Immunohistochemistry showed that fabricated cell sheets were composed of epithelial cells having a similar expression profile of cytokeratins, which are markers of differentiated epithelial cells, to that of native oral mucosal epithelium. The maintenance of epithelial stem/progenitor cells in fabricated cell sheets was indicated by p63 expression, which was observed throughout the basal layer of the native oral epithelial tissue. Scanning electron microscopy revealed human oral mucosal epithelial cell sheets had flat cells in the apical side, and smooth and flat basal cell surfaces, which numerous processes were observed with higher magnifications. Transmission electron microscopy show small microvilli in the apical cell menbrances of superficial cells. Prominent desmosomes were also observed along cell-cell junctions of middle cells.

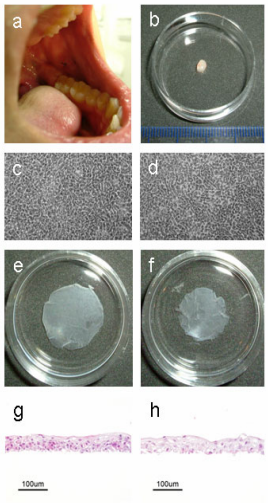

Fig.1 Fabrication of human oral mucosal epithelial cell sheets. A 5-mm punch biopsy of human oral mucosal tissue was surgically excised(a.b). Isolated epithelial cells were cultured on temperature-responsive culture inserts(c.d) supplemented with FBS(c.e.d) or autologous serum(d.f.h) in the absence of feeder layers. Macroscopic views(e.f) and HE-stained paraffin-sections(g.h) of the harvested cell sheets.

Conclusions: Using temperature-responsive culture inserts, stratified and transplantable human oral mucosal epithelial cell sheets were fabricated without xenogeneic products.

Reference:

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