Subcutaneous transplantation of autologous oral mucosal epithelial cell sheets fabricated with temperature-responsive culture dishes

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Introduction: Transplantable oral mucosal epithelium cell sheets were fabricated on temperature-responsive culture dishes. We have succeeded clinical application of the cell sheets for the corneal epithelial regeneration (*N Engl J Med.* 2004;351:1187-96). Recently, we also reported endoscopic transplantation of the cell sheets for the prevention of inflammation and constriction of esophagus after endoscopic submucosal dissection (*Gut, in press*). However, the fate of autologous oral mucosal epithelial cell sheets after ectopic transplantation is not well elucidated. Here, we examined the fate of autologous oral mucosal epithelial cell sheets after subcutaneous transplantation in a rat model

Methods: Oral mucosal biopsy specimens were taken from Lewis rats under deep anesthesia. Oral mucosal epithelial cells were isolated by dispase- and trypsin- treatment, then seeded on temperature-responsive culture dishes with mitomycin C-treated 3T3 feeder layer cells. After 7-10 day culture, epithelial cells were harvested as a single contiguous cell sheet. Subcutaneous transplantation was performed as described previously (*J Invest Dermatol*. 1988;91:315-8). The dorsal skin of the anesthetized male Lewis rats were incised by scissors and lifted as a rectangular flap. Harvested autologous epithelial cell sheets were washed with Dulbecco's phosphate buffered saline, and transplanted on the flap with its basal surface contacted to it. The apical sides of transplanted cell sheets were covered with thin silicone sheets to avoid adhesion with thoracic wall to mimic the environment of original epithelial layer. After transplantation, the incisions were closed with suturing. To observe time course of transplanted oral mucosal cell sheets, skin flaps was taken up at 1 day, 3 day, 5 day, 7 day, 10 day and 14 day after transplantation and examined with hematoxylin-eosin staining. Regenerated tissues were also examined by immunohistology for keratin expression profiles, expressions of p63 and PCNA, and compared with fabricated cell sheets, native oral mucosa and skin.

Results and Discussion: Subcutaneously transplanted autologous oral mucosal epithelial cell sheets became thicker and thicker reaching approximately 15 cell layers within 7 days (Fig. 1a-e). Basement membrane-like structure was also regenerated within 5 days. However, these transplanted cells survived only for 2-3 weeks implying the lack of niche for the maintenance of epithelial stem cells. Expression of p63, a putative epithelial stem/progenitor marker, and that of PCNA, a cell cycle progression marker, were detected 3 day after transplantation, but faded out within 10 days. Both of cytokeratin 4 and 13 were hardly expressed in fabricated epithelial cell sheets, while they were expressed in all the cell layers except basal cells in regenerated epithelium. Cytokeratin 14 was strongly expressed in cell sheets, but the expression was limited in basal and lower layer cells in regenerated epithelium.

These results indicate some factors secreted from host tissue induced proliferation and differentiation of transplanted epithelial cells, but could not support stem cell maintenance.

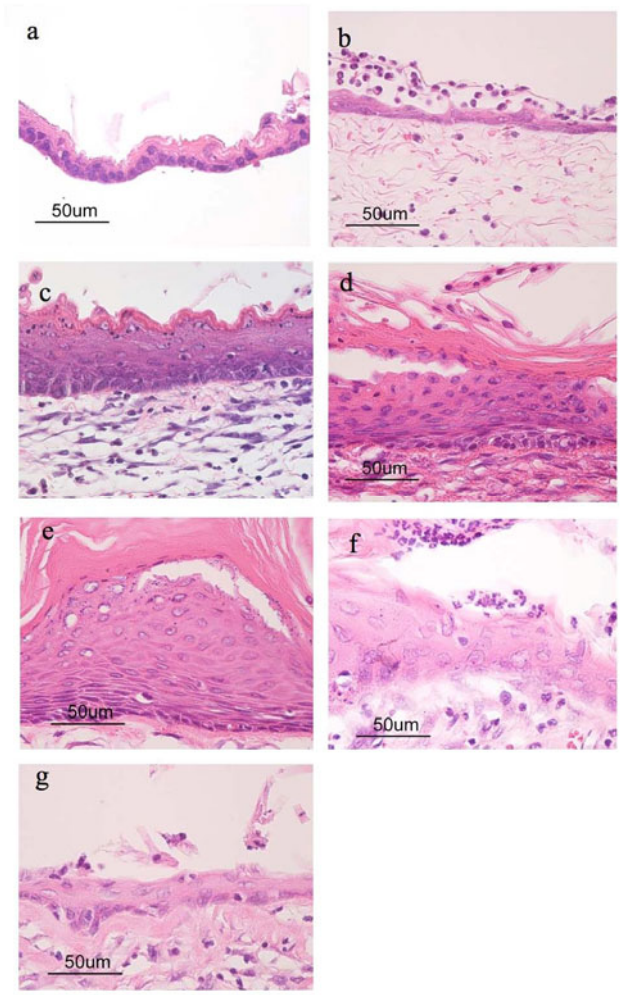

Figure 1. Histological examination of subcutaneously transplanted autologous oral mucosal epithelial cell sheets. Fabricated oral mucosal epithelial cell sheet (*a*), 1 (*b*), 3 (*c*), 5 (*d*), 7 (*e*), 10 (*f*), and 14 days (*g*) after transplantation.

Conclusions: This model can provide a unique tool for the investigation of epithelial proliferation and stem cell niche.