INTRODUCTION

Vocal fold cover layer with a tissue-engineered structure containing epithelium and fibroblast of oral mucosa

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Evaluation of fabricated tissue

The morphological characteristics of the fabricated tissues were observed with scanning electron microscopy and with immunohistochemical staining of anti-cytokeratin antibodies, anti-b1 integrin antibodies and anti-vimentin antibodies. Native vocal fold and buccal mucosa were used as positive controls.

RESULTS

We successfully fabricated a vocal fold cover layer with a tissue-engineered structure containing epithelium and fibroblast of oral mucosa in vitro (Figure 2). Scanning electron microscopy revealed developed microvilli on the apical surface of the fabricated tissue (Figure 3).

The upper layer of the fabricated tissue resembled the native vocal fold epithelium (Figure 4 and 5), with two to three cell layers, small basal cells, and did not resemble the native oral epithelium (Figure 6) which was much thicker than the native vocal fold epithelium.

Native epithelial cells of vocal fold and oral mucosa expressed cytokeratin as proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelium, and the cultured cells of the upper layer also expressed cytokeratin (Figure 7A). b1 integrin, reported to be an epithelial stem-cell and progenitor-cell marker, immunostained in the cultured cells of the upper layer (Figure 7B) as well as the native basal cells.

In both native lamina propria of vocal fold and oral mucosa, smaller and spindle shaped cells expressed vimentin as an abundant intermediate filament protein, and the cultured cells of the lower layer also expressed vimentin (Figure 7C).

DISCUSSION

The vocal fold scarring leads to the replacement of healthy tissue by fibrous tissue in the lamina propria. A number of promising treatment approaches12 for subepithelial lesion in vocal fold have been reported, involving cell, growth factor, and pharmacological agents. However, in wound healing that results in a scar, the normal stratification of epithelium is also disrupted13. Epithelial cells have the ability to phagocytize debris such as dead tissue that would obstruct their path and to prevent bacterial infiltration4. Therefore, the promotion of the vocal fold re-epithelialization as well as the subepithelial treatment have the potential for the prevention of the vocal fold scarring. In this study, the morphological and immunohistochemical characteristics of the tissue-engineered mucosa with two-layer structure were similar to those of native vocal fold epithelium and lamina propria. Our study shows that this artificial vocal fold fabricated from autologous oral mucosal tissue may serve as effective substitutes for allografts in the reconstruction of the optimal vocal fold layers.

CONCLUSIONS

1. We successfully fabricated a vocal fold cover layer with a tissue-engineered structure containing epithelium and fibroblast of autologous oral mucosa.
2. The tissue-engineered vocal fold cover layer has the possibility to reconstruct the vocal fold surface, and to restore voice in patients with severe vocal fold scarring.

REFERENCES