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The wound healing capacity of undifferentiated and differentiated airway epithelial cells in vitro

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ABSTRACT

Introduction: Congenital or acquired tracheal lesions alter airway epithelial structure and can lead to long-segment tracheal defects. Tissue engineered tracheal grafts (TETG) have the potential to cure such defects; however, clinical applications have been plagued with numerous complications including delayed graft epithelialization. The knowledge that epithelial cells migrate from native tissue to the TETG raises the possibility that TETG performance can be improved by increasing the rate of epithelialization.
Objectives: We developed a model that can be used quantify epithelial migration in clinically-relevant conditions.
Methods: Existing histological analyses determined the differentiation status of the normal and injured human tracheal epithelium and were used to identify in vitro culture conditions that mimic these parameters. The classical scratch assay was adapted to permit analysis of migratory velocity as a function of differentiation status. Migration of undifferentiated (UD), partially-differentiated (PD), and well-differentiated (WD) epithelia was quantified.
Results: The normal and injured epithelium can be modeled using human cells that are cultured using a modified air-liquid-interface culture system. PD cell cultures are similar to the remodeled epithelium; whereas; WD cultures are similar to the normal epithelium. Preliminary results indicate that PD cells migrate more rapidly than WD cells and that PD and WD cells migrate more rapidly than UD cells.
Conclusion: Pending verification of these results, we suggest that epithelial migration is significantly altered by differentiation status. Thus, efforts to improve TETG epithelialization should use model systems that faithfully-represent the differentiation state of the native tissue.

1. Introduction

Reconstruction of long segment tracheal defects remains a surgical challenge [1]. While tissue engineered tracheal grafts (TETG) represent a potential solution, early clinical application of TETG has been fraught with complication, including a months to years-long delay in epithelialization [2]. Given the known functions of the airway epithelium, graft epithelialization may assist with airway protection and secretion clearance, as well as attenuate other complications such as graft stenosis [3,4].

In cases where an allograft was partially epithelialized, there is strong evidence that host-derived cells migrate from the native tissue to the graft [5]. Thus, in vitro models that quantify the rate of epithelial migration would permit extensive preclinical assessment of biologic and biosynthetic scaffolds for tracheal replacement. The purpose of this study was to develop such a model.

Clinical scenarios in which a long segment defect is reconstructed with a TETG frequently involve acquired defects, such as stenosis resulting from long-term intubation or infection [3]. After the stenotic segment is removed, reconstruction of a long segment tracheal defect may involve anastomosis of a segment of normal airway to the TETG. In this case, the native airway would be lined by a well-differentiated, pseudostratified, mucociliary epithelium. However, replacement of an airway segment could involve anastomosis of an abnormal airway region with the TETG, as may be observed in stenosis and chronic inflammation [6]. Depending on the type and extent of injury, the abnormal native epithelium would exhibit moderate to poor differentiation [7,8]. In the context of model development, these

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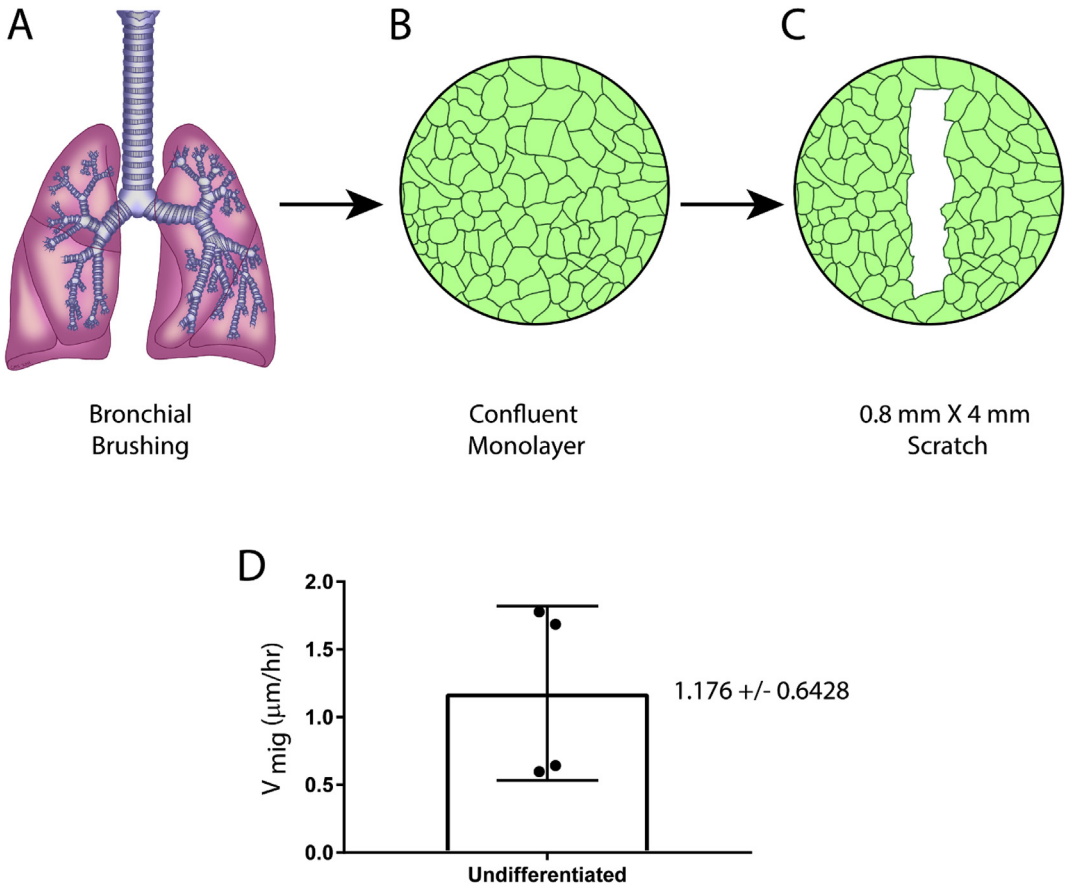

Fig. 1. Classical scratch assay. Human airway epithelial cells were recovered by brushing the bronchial airway (A) and propagated using the mCRC method (B). At confluence, a stylus was used to scratch the monolayer, resulting in a cell-free zone (C). The velocity of epithelial migration (v_{mig}) was determined for 4 donors (D). Data are presented as the mean and standard deviation.

observations raised the possibility that two systems would be needed to model epithelial migration in both normal and abnormal native airway contexts.

Epithelial cell migration is typically quantified using the in vitro scratch assay [9], which is also termed the wound healing assay [10]. Typically, undifferentiated cells are grown to confluence in a polystyrene cell culture dish and a stylus is used to create a small (~0.8 mm width, ~4 mm length) cell-free region, termed a "scratch". Phase contrast photomicrographs are collected at 6 h intervals over a 3–4 day period. Image analysis software is used to determine the width of the scratch. Changes in width over time are used to calculate the velocity of cell migration (v_{mig}). As a monolayer of undifferentiated cells is rarely if ever observed in the airway epithelium, the traditional migration model is unlikely to represent clinical situations requiring reconstruction.

We adapted the in vitro scratch assay to permit analysis of well- and poorly-differentiated airway epithelia. Bronchial or nasal epithelial progenitor cells were expanded using the modified conditional reprograming method [11] (mCRC) and differentiated on transwell inserts using a modification of the air-liquid-interface approach [12]. The impact of differentiation on migration velocity was evaluated by scratching partially-differentiated (Day 1) and well-differentiated (Day 25) cultures.

2. Materials and methods

2.1. Human subjects

The Institutional Review Board at Nationwide Children's Hospital approved this study. Donors received written, informed consent. This study included cells from 4 healthy subjects (mean age 17.75 ± 17.783, 3 male/1 female).

2.2. Airway brushing procedure

Human airway epithelial cells were collected from the nasal respiratory epithelium and bronchial epithelium as previously described [11].

2.3. Culture techniques

The mCRC technique was used to expand the initial cell inoculum [11]. At passage 3, cells were plated at $6.7 \times 10^3/cm^2$ on collagen-coated polystyrene cell culture plates and grown to confluence in FMed +10 µM Y27632. These cultures contained undifferentiated airway epithelial cells [11]. Passage 3 cells were also used to generate air-liquid interface (ALI) cultures. Cells were plated at 6.1×10^4 cells/cm^2 on collagen-coated 0.33 cm^2 transwell membranes and cultured in proliferation medium [12] plus 10 µM Y27632 for 24 h. The cells were then cultured in proliferation medium for an additional 4–5 days. At confluence, the medium was changed to Half & Half (50% Wu differentiation medium [12] and 50% Pneumacult ALI™ without the 100X maintenance supplement). Medium was present only in the basal compartment, which established an apical air-liquid-interface.

2.4. In vitro scratch assay

Undifferentiated, partially-differentiated, and well-differentiated airway epithelial cell cultures were scratched according to established