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Sequential monolayer-suspension culture of human airway epithelial cells

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Abstract

In this article, we describe a technique for culturing human airway epithelial cells, developed in Leuven, as a new tool for a most reliable diagnosis for ciliary disorders. This technique that allows to keep both structural and functional primary abnormalities of inherited ciliary abnormalities, while avoiding the secondary ones, can also be useful to a number of other studies, namely in cystic fibrosis.

Keywords: Primary ciliary dyskinesia; Ciliogenesis; Cell culture; Secondary abnormalities

1. Introduction

Although the diagnosis of inherited ciliary abnormalities (PCD) is most frequently based on a dynein deficiency found in transmission electronic microscopy (TEM), there are many differential diagnostic problems both ultrastructurally and functionally and in many cases even no ultrastructural abnormality is found. Since after ciliogenesis in the sequential monolayer-suspension culture primary abnormalities (both structural and functional) are expressed while all secondary abnormalities are absent, the culture technique was further developed in Leuven as a new and most reliable diagnostic tool for ciliary disorders. Hereafter, you will find our technical protocol on culturing human nasal (bronchial) epithelial cells which is also available in detail elsewhere [1–4].

2. Materials

- *Pronase*: 0.1%, protease XIV in DMEM-Ham's F12 supplemented with AB;
- *Monolayer medium*: DMEM-Ham's F12 from Life Technologies supplemented with AB (50 U/ml penicillin and 50 μg/ml streptomycin), cholera toxin (10 ng/ml) and Ultroser G (Life Technologies, 2% end concentration);
- *Suspension medium*: DMEM-Ham's F12 from Life Technologies supplemented with AB (50 U/ml penicillin and 50 μg/ml streptomycin), and NUSerum (Becton Dickinson, 10% end concentration);
- *Collagen-coated tissue flasks*: 0.2% of collagen (rat tail extracted) is used as substrate for the cells in monolayer culture.

3. Procedure

The human respiratory mucosa or nasal polyps are placed in Pronase overnight at 4 °C on a slowly continuously rotating apparatus (16–24 h). On the next day, 1 ml of serum is added to the solution with the dissociated cells and the cells are washed by means of centrifugation (three times). The cells are placed for 1 h at 37 °C in an uncoated tissue flask of 25 cm^2 to remove eventually contaminating fibroblasts. Hereafter, the cells are plated into collagen coated tissue flasks (T25 or T75). Culture medium is changed after 1 day and then 3 weeks. After 3 weeks, cells have reached confluency and ciliated cells have disappeared. Collagenase is added to the culture flasks and placed in the incubator (37 °C) for at least 30 min. Releasing cell sheets may be "cut" into smaller ones with a cell scraper to obtain smaller spheroids (Fig. 1). The

Abbreviations: PCD, primary ciliary dyskinesia; TEM, transmission electronic microscopy.

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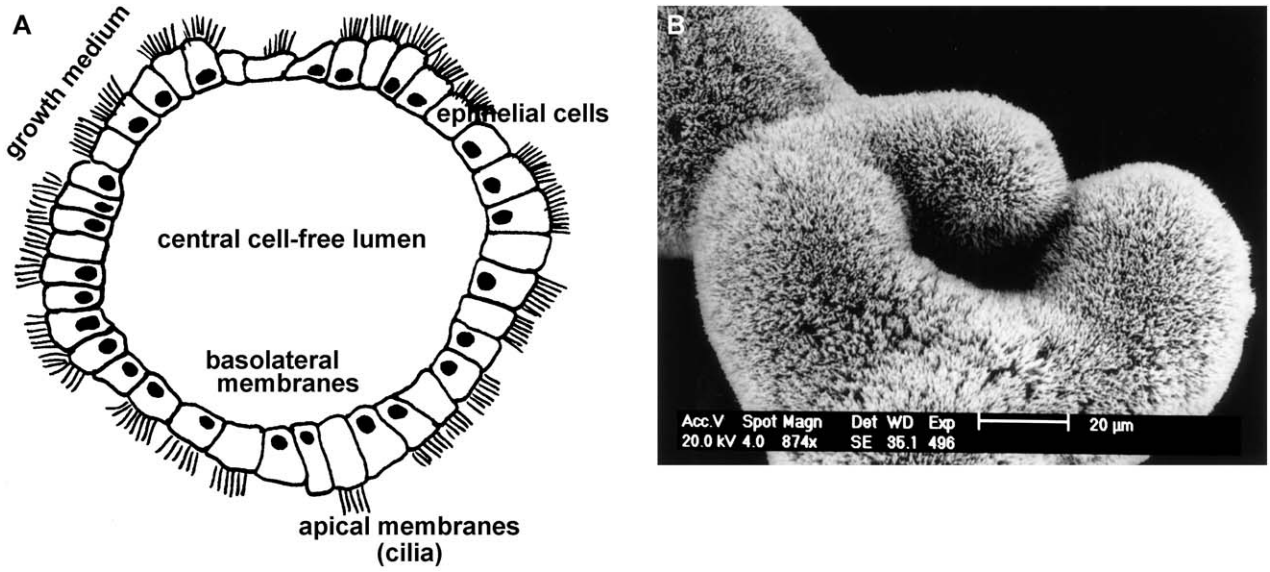

Fig. 1. (A) Schematic representation of a spheroid in suspension culture; (B) SEM picture of spheroid after 6 weeks in suspension culture.

cell sheets are washed by means of centrifugation (two times). The cells are finally placed into an uncoated tissue flask (T25) and placed on the rotary shaker (80 rpm, 37 °C) for 1 week. On the following 2 weeks, the flasks are placed stationary in the incubator at 37 °C. After 2 weeks of suspension culture functional cilia reappear on the spheroids and these ciliated aggregates can be kept in culture for more than several months.

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