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Culture of cells harvested with nasal brushing: a method for evaluating ciliary function

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SUMMARY

Objectives: *Usefulness and reliability of nasal brush samples in a monolayer cell culture was studied for evaluation of ciliary movement.*

Methods: *Cells for cultures were harvested under the middle turbinate from patients with chronic sinusitis and from controls. Ciliary function was analysed using a microscope equipped with a high-speed video camera. Ciliary beat frequency (CBF), ciliary amplitude, waveform and coordination were analysed from the cultures 4-6 h after the harvest of brush samples and 3 days after the culture.*

Results: *The average success rate of cell cultures was 82, 5%. There were not statistically significant differences in CBF between patients and controls but there was significant difference between brush biopsy and cultured brush biopsy in controls. The ciliary beat amplitude and the waveform were normal in all samples in both groups.*

Conclusions: *Miniculture method for culturing nasal cells from brush biopsies proved reliable and non-invasive for detailed analysing of ciliary function and for excluding possible secondary ciliary dyskinesia. Compared to conventional mucosal cell cultures where thick and invasive specimens are needed for successful cultures, it is easier and quicker to perform and well tolerated by patients. Thin monolayer cultures enable the evaluation of frequency, amplitude and the waveform of the cilia beat under the microscope whereas floating cells and cell clusters do not give this opportunity.*

Keywords: ciliary beat frequency, cell culture, primary ciliary dyskinesia, secondary ciliary dyskinesia

INTRODUCTION

Mucociliary clearance is an important defence mechanism in the respiratory tract and ciliary function is one of its major properties. Ciliary function includes coordinated ciliary activity, ciliary beat frequency (CBF), ciliary beat amplitude (CBA) and waveform and can be estimated in cell culture with high-speed video recordings and a phase-contrast microscope [1,2]. All phases of ciliary motility can be analysed with a microscope equipped with a high-speed video camera recorder. Ciliary function can be examined in monolayer culture without being disturbed by effects from the autonomic nervous system or mucosally secreted factors [2,3].

Primary ciliary dyskinesia (PCD) is one of the possible causes of chronic otitis media, sinusitis, bronchiectasis and male infertility [4-6]. The diagnosis of PCD is complicated. Electron microscopy (EM) has played a leading role in the exploration of PCD, although the ultrastructural findings in respiratory cilia can be complex and the use of EM as a criterion for the diagnosis has some limitations. The major problem in EM is differentiation of primary and secondary dyskinesia [7]. The presence of coordinated, effective ciliary beating has been regarded as an exclusion criterion for PCD [8-10]. In cell culture the secondary ciliary dyskinesia can be excluded and it is possible to examine primary disturbances of ciliary function. Brush biopsy is less invasive and all phases of ciliary motility can analyse better in cell culture than in conventional mucosal biopsies. Rutland and Cole [11] reported a brush biopsy method for measurement of CBF already 1980 and also nasal biopsies have been widely used for studies of both ciliary ultrastructure and the motility of cilia.

The purpose of this study was to examine the usefulness of brush biopsy in cell culture and to determine the reliability of ciliary movement examination with an inverted phase-contrast microscope equipped with a high-speed video camera. Cell culture of brush biopsy has been used to examine abnormal apoptotic mechanism in asthmatic bronchial mechanism [12]. We used this method in analysis of ciliary function for the first time to our knowledge.

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MATERIALS AND METHODS

We studied 10 consecutive patients (7 females, 3 males, mean age 51.9 yrs) suffering from chronic sinusitis who underwent sinus operations. The control group (CG) included 10 patients (7 females, 3 males, mean age 37.22 yrs), who were operated on for other diseases (e.g. parotid tumour) and suffered from no upper respiratory tract infections. None of our patients or controls had diagnosed with PCD. In the chronic sinusitis group (CSG) nasal mucosa specimens were taken during sinus operations for use in 10 cell cultures. In addition, ciliated cells were taken from the middle nasal turbinate on both sides of the nose using brush biopsy. Brush biopsies were taken from the CG similar to those from the CSG under local anaesthesia induced by cocaine and adrenalin. Both the specimens and brushed cells were immediately immersed in cell culture medium (1:1 mixture of Dulbecco´s modified Eagle´s medium (DME) and Ham´s nutrient F12 (DME/F12) supplemented with penicillin (50 IU/ml) and streptomycin (50 mg/ml)).

Ciliary activity was analysed from part of the brush biopsy cells of both groups 4-6 h after the harvest. The remaining harvested cells were then cultured in 2 cultures for each patient using a thin collagen gel layer on a cover glass. The medium with brushed cells was centrifuged at 1000 rpm for 5 min. The supernatant was discarded and 2 ml fresh medium added to the centrifuged brushed cells. The medium used for culture consisted of a 1:1 mixture of DME/F12 supplemented with 10% NU-serum, choleratoxin (10 ng/ml), retinoic acid [10^{-7}], penicillin (50 IU/ml) and streptomycin (50 mg/ml).

The mucosal specimens from the CSG group were conventionally cultured. For ethical reasons, large mucosal specimens were not taken from controls. Instead this biopsy method was used as control method for brush biopsies. After washing, the mucosal specimens were digested with 0.75 % pronase (Type 14 protease, Sigma) in DME/F12 at +4 °C overnight under continuous rotation. After removing the specimens, the cell suspension was preplated for 2 h on a plastic dish to reduce the number of contaminating fibroblasts. The medium was then transferred from the dish and centrifuged, after removing the supernatant and adding fresh medium. Cover glasses with a diameter of 30 mm were placed in the cell culture dishes and coated with collagen. The cell suspensions from the harvested cells and nonadherent cells from the mucosal specimens were plated on the collagen layer and incubated at + 37 °C in a 5% CO^2 atmosphere.

On the third day the cover glasses were removed from the cell culture dishes and fixed to the bottom of a Rose´s chamber, which was immediately filled with medium.

Ciliary motility was analysed both from the brush biopsy cells and cultured nasal cells using an inverted phase-contrast microscope (Nikon) equipped with a high-speed CCD video camera capable of taking 240 frames per second at 100x magnification.

The CBF, CBA, coordination and the waveform of cilia were analysed from slow speed recordings. The CBA was evaluated by semi-quantitative analysis of ciliary movement in 3 classes as follows: wide amplitude, intermediate, and no or tremor like movement.

Variables had normal distribution and the t-test was used for statistical analysis. Written informed consent was obtained from all volunteers before participation, and ethical approval was obtained from the Joint Ethics Committee of Tampere University Hospital.

RESULTS

The average success rate of cell cultures in cultured harvested cells was 82.5% (33/40). It was 85% (17/20) in patients (CSG) and 80% (16/20) in controls (CG). Cells for brush biopsies were taken from both nostrils in each patient and control and only in one control patient (1/20) both cultures failed and no analyses of ciliary function were able to perform. For every patient and control subject at least one sample succeeded and therefore 19/20 subjects had their ciliary function examined with brush method. All mucosal specimen cultures succeeded (20/20) taken from patients (Table 1).

Table 1. Success rate of cell cultures.

	Controls	Patients
Cultured brush biopsy	80% (17/20)	85% (16/20)
Mucosal specimens	no samples	100% (20/20)

The mean CBF in brush biopsy cells was 18.8 ± 4.3 Hz (mean ± SD) in the CSG and 16.9 ± 3.2 Hz in the CG. The mean CBF in cultured brush biopsy cells was 20.7 ± 2.9 Hz in the CSG and 20.9 ± 2.7 Hz in the CG. The mean CBF was 23.4 ± 2.7 Hz in cultured mucosal specimens (Table 2).

The CBA was normal (wide amplitude) in all samples in both groups and there was no difference between the groups. There was coordinated ciliary activity in both cell cultures between each cilium in every cell, in harvested cell clusters ciliary activity was coordinated in all samples. The waveform was normal in both cell culture groups (Table 2).

There was no statistical difference in CBF in brush biopsy ($P > 0.05$) or cultured brush biopsy cells ($P > 0.05$) between patients in the CSG and CG. However there was significant difference ($P < 0.01$) in CBF between brush biopsy and cultured brush biopsy cells in controls (Table 2).

DISCUSSION

Ciliary function can be studied in monolayer culture without

Table 2. Ciliary beat frequency (CBF) and ciliary beat amplitude (CBA) in brush cell biopsies, in cultured brush cell biopsies and in mucosal specimens from controls and patients with chronic sinusitis. * $p < 0.01$

	CBA and CBF in controls	CBA and CBF in patients	
Brush biopsy cells	wide amplitude, 16.9 ± 3.2 Hz	wide amplitude, 18.8 ± 4.3 Hz	$p > 0.05$
Cultured brush biopsy	wide amplitude, normal waveform *20.9 ± 2.7 Hz	wide amplitude, normal waveform 20.7 ± 2.9 Hz	$p > 0.05$
Mucosal specimens	no samples	wide amplitude, 23.4 ± 2.7 Hz	

being disturbed by effects from the autonomic nervous system, bacterial toxins or factors secreted from the mucosa. A definitive advantage of using monolayer cell cultures on collagen-coated cover glasses is the use of higher microscope magnifications for studying ciliary beat, so that even the movements of individual cilia can be recognized and all parts of ciliary beating can be evaluated at the same time. This enables the evaluation of amplitude, the waveform and the frequency of cilia beat. Conventional cell cultures instead need a large number of cells and invasive biopsies. Fresh samples are also problematic because the cells are not fixed and they move and turn under the microscope so that the ciliary movement is impossible to evaluate exactly. Jorissen et al. [12] has shown that secondary ciliary dyskinesia is virtually absent after ciliogenesis in sequential monolayer-suspension culture, and the results are 100% sensitive and 100% specific for PCD.

We used brush biopsy in cell culture to test the reliability of ciliary movement examination in monolayer cell cultures. The success rate of cell cultures was good (82.5% from harvested cells and 100% from mucosal specimens). The reasons for cell culture failures included the use of insufficient material or loosening of the thin collagen gel layer from the bottom of the cover glass. More ciliated cells occur in mucosal specimens than in brush biopsy, which may explain the difference, but still the success rate of cell cultures from harvested cells was very high. The CBF resembled that found in monolayer culture [2] but was clearly higher than the CBF measured from biopsy specimens from the respiratory mucosa [13,14] or suspension culture [15]. The difference may be due to +36.5 °C temperature used in our study. There was no statistical difference in CBF between patients in the CSG and CG; however, the difference in CBF between methods may demonstrate the effect of disturbing factors in brush biopsy cells, which could be avoided when cells are cultured. The mean CBF in cell culture of mucosal specimens was higher than in the culture of brushed biopsy cells, which may be explained by the higher density of cells in mucosal culture. It has been reported that cell density influences the frequency of CBF in cultured cells [3]. CBF was surprisingly good also in patients´ samples, which might be explained at least partly by the fact that cells have ideal environment in cultures. This also indicates that there are normal ciliated cells left at least in the nasal cavity in patients with chronic sinusitis and that these cells have normal ciliary function.

We examined the cell cultures on the third day when the cells had already flattened but the number of cilia or the CBF had not decreased. The movements of individual cilia can be recognized in recordings performed at lower speeds, even in slightly flattened cells. Coordination of each cilium could be estimated in every individual cell and was present in all successful cell cultures from both groups. When the cells were separated from each other in the cell cultures, coordination between the cells could not be estimated because the cells did not influence each other and the direction of ciliary movement was thus individual. Coordinated ciliary activity between cells could be estimated from harvested cell clusters, this activity was coordinated if there was transport of material along the ciliary lining, single cells or cell clusters rotating in the medium.

The brush biopsy method is less traumatic, easily performed in outpatients and better tolerated than normal biopsy. Therefore it can be used under local anaesthesia repetitively when it is clinically reasonable. Cultured harvested cells are a useful functional method for examining all aspects of ciliary function. It is also possible that after functional evaluation the same cells can be prepared for TEM for evaluation of ciliary ultrastructural. The method is also a simple way to exclude the effects of chronic infection on ciliary function and ciliary ultrastructural. We found that the cell culture of respiratory cells harvested with brush biopsy is a promising method for analysis of ciliary function and could be used also to analyse patients with suspected PCD. However, the method needs further evaluation of both functional and ultrastructural findings from PCD and non-PCD patients before genetic ciliary disorders can be ruled out by the use of this method.

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