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Cell suspension cultures and adenoid epithelium: An assessment of the source of material for human ciliary function experiments *in vitro**

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SUMMARY

The aim of this study was to explore the usefulness of two different in vitro models for studying the function of human upper respiratory cilia, i.e. cell suspension cultures of human upper airway epithelium, and ciliated adenoid epithelium. Ciliary beat frequency (CBF) and signal consistency (SC), as parameters of ciliary function, were measured by a computerized photo-electrical method. Measurements after one week revealed that CBF of ciliated aggregates from cell suspension cultures had deteriorated to a mean of 5.8 Hz. In the subsequent period, it remained at this rather low and non-physiological level. SC decreased too, although not as dramatically. These results indicate that ciliated aggregates from cell suspension cultures cannot be used for human ciliary function experiments in vitro. On the other hand, in ciliated adenoid epithelium, CBF remained constant for a period of 5 h, although SC decreased after 30 min. Because of this result and the fact that ciliated adenoid epithelium is easily obtainable, we regard this material as suitable for studying human ciliary beat in vitro.

Key words: human cilia, cell suspension cultures, adenoid epithelium, ciliary beat frequency, ciliary beat harmony

INTRODUCTION

The mucociliary transport system is an important defense mechanism of the airways, and a major factor in sustaining mucociliary transport is ciliary beat. Several methods for testing ciliary function have been described. For this purpose, both animal and human ciliated epithelia have been used, taken from the upper and lower respiratory tracts. For pharmacological studies the use of human specimens has to be preferred, because of the reported interspecies variation in ciliary reaction (Van de Donk et al., 1982). Human specimens can be obtained from the lower airways and the nasal mucosa, the latter being the more accessible location. Both brushing (Han et al., 1990; Deitmer and Scheffler, 1993) and curette techniques (Ingels et al., 1991) yield ciliated epithelium without a proper lining, since many intercellular connections are mechanically disrupted during harvesting. Mucosal biopsies provide much better specimens, as they have an intact lining of ciliated cells (Ingels et al., 1991). However, the biopsy method has certain practical disadvantages. Firstly, local anaesthetics cannot be used in view of their ciliotoxic effects (Ingels et al., 1994). Secondly, there is the risk of nasal bleeding after biopsy. Alternatively, mucosa can be harvested from patients undergoing nasal or sinus surgery (Wolf et al., 1988), but an important drawback of this method is that the mucosa is usually in a pathological condition. To circumvent the above-mentioned disadvantages, ciliated epithelium obtained from patients undergoing adenoidectomy, can be used (Van de Donk et al., 1982; Khan et al., 1986; Staskowski and McCaffrey, 1992). Furthermore, it has been suggested that preserved or cultured respiratory epithelium can be used. Proetz and Pfingsten (1936, 1939) have processed explants of animal ciliated epithelium, whereas Rose et al. (1949) have used human nasal epithelium. In an explant, the specimens are preserved under optimal physiological conditions, although mitosis of ciliated cells does not take place (Drucker et al., 1976). On the other hand, mitosis is not affected in a monolayer culture of dissociated cells, but the specific properties of the respiratory epithelium, i.e. the cilia and the mucus-producing cells, are lost in these cultures (Wu et al., 1985; Rautiainen et al., 1993).

Jorissen et al. (1989) succeeded in preserving nasal epithelial cells with beating cilia in cell suspension cultures, which could be maintained for up to seven months. The dissociated cells formed aggregates of 50 μm to 2 mm in diameter, and with the cilia directed outwards. However, ciliary activity was not quantitatively determined.

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Sufficient amounts of viable ciliated cells, in a functional state comparable to the physiological condition, are a prerequisite for performing *in vitro* studies on human ciliary function. The present study was designed to explore the usefulness of two different sources of human upper respiratory epithelium for *in vitro* function experiments: (1) ciliated aggregates taken from cell suspension cultures; and (2) ciliated epithelium of freshly harvested adenoids. Cell suspension cultures have the advantage of being at the researcher's disposal at any moment. An argument in favour of using adenoid epithelium is its ample availability in ENT practice.

Ciliary beat frequency (CBF) and signal consistency (SC) have been studied as parameters of ciliary activity. CBF is assumed to be a decisive factor in mucociliary transport (Duchateau et al., 1985), whereas SC may give an indication of ciliary harmony (Ingels et al., 1992).

MATERIAL AND METHODS

Preparation of cell suspension cultures

Cell suspension cultures were prepared from the epithelium of nasal polyps (n=16) and the mucosa of inferior turbinates (n=7). Tissues were obtained from patients undergoing polypectomy or turbinate surgery under general anaesthesia. The specimens were processed according to the method described by Jorissen et al. (1989). First, they were rinsed three times in medium consisting of Ham's F12-DME 1/1 (Gibco, Paisley, UK), NU serum (10%), penicillin G (50 IU/ml) and streptomycin (50 μg/ml). The tissues were then digested in 0.1% pronase (Sigma, St. Louis, MO, USA) at 4°C under continuous rotation over a period of 24 h. Next, the suspension was washed three times with medium and preplated in plastic culture dishes for 1 h at 37°C to remove fibroblasts. Cell suspensions were then placed on a shaker at 80 rpm for seven days at 37°C, enabling the formation of ciliated aggregates. They were stored under 5% CO_2 in an incubator. The medium was changed after the first day and subsequently three times per week.

Adenoid specimens

Adenoids were obtained from children undergoing adenoidectomy (n=7). In order to remove blood and other debris, the specimens were rinsed in physiological saline shortly after adenoidectomy. They were then transferred to medium, which consisted of CMRL-1066 (Gibco, Paisley, UK) containing glutamine, 5% inactivated fetal calf serum, hydrocortisone hemisuccinate (0.1 μg/ml), crystalline porcine insulin (1 μg/ml), penicillin G (100 IU/ml) and streptomycin (100 μg/ml; cf. Yager et al., 1978; Ingels et al., 1991). Pieces of approximately 0.3 cm in diameter were cut from the ciliated epithelium and examined microscopically. Specimens were only used for measurement when a proper row of beating cilia was present.

Measurement of CBF and SC

CBF and SC were measured by the photoelectrical method, as described by Ingels et al. (1991). The specimens were inserted into a perfusion chamber, which consists of a standard glass slide and a cover slip with a silicone ring in between, mounted in an aluminium frame. The perfusion chamber was placed onto a microscope stage that was kept at a temperature of 34°C by means of an electronic heating device. A phase-contrast microscope (Leitz, Wetzlar, Germany) was adapted by attaching a triocular tube, using a ×100 oil-immersion objective and a ×10 ocular lens. A square-angled diaphragm was mounted in the same tube with a beam splitter and a visaflex house (Leitz) in order to view the measured area. A 12 V/250 W halogen lamp was used as light source. Variations in light intensity, caused by the beating action of the cilia, were registered by a photometer. Using a 12-bit A/D converter, the digitalized signal was recorded by a personal computer (IBM, Grenock, UK) with a sample frequency of 200 Hz. A fast Fourier transform analysis (FFT) of the recorded signal was performed over a period of 20 s. CBF was determined from the first harmonic of the power spectrum obtained by FFT. On the basis of the CBF signal, the SC was computed.

Experimental design

The cell suspension cultures were examined daily in order to study the survival of ciliary activity over time. Furthermore, ciliary activity was determined by measuring CBF and SC at the outset, and at weekly intervals up to seven weeks. Because the process of forming aggregates took one week, the first measurements could only be made after one week. To avoid microbial contamination, aggregates were not returned to their original cell suspension culture after measurement.

Adenoid specimens were investigated by measuring CBF and SC at the start and subsequently every 30 min for 5 h. Since CBF of different cells in the same specimen can vary considerably (Ingels et al., 1991), all measurements in each experiment were performed on one single ciliated cell. Only cells adjacent to others and beating freely were examined. Statistical analysis of the results was carried out by analysis of variance (ANOVA). A value of $p < 0.05$ was considered significant.

RESULTS

Cell suspension cultures

The number of aggregates with beating cilia was found to diminish with time. The mean functional survival times amounted to 19.7 days (range: 3-50 days) for cell suspension cultures derived from polyp epithelium, and 9.4 days (range: 3-22 days) for those from turbinate mucosa (Table 1).

In Figure 1, CBF and SC are presented graphically in relation to time. The mean initial value (±SEM) of CBF was 8.8±0.2 Hz. After one week a mean value (±SEM) of 5.8±0.2 Hz was found. In the subsequent period, CBF stayed at a relatively low and varying level, although some increase was observed after 5 weeks. SC, with a mean initial value(±SEM) of 2.3±0.1, also showed lower values with time, although less dramatically than CBF.

Table 1. Human polyp and turbinate epithelium in cell suspension cultures; survival of viable cells with beating cilia (mean time and range).

	number of cultures	time (days); mean ± SEM	range
polyps	16	19.7±3.9	3-50
turbinates	7	9.4±2.5	3-22

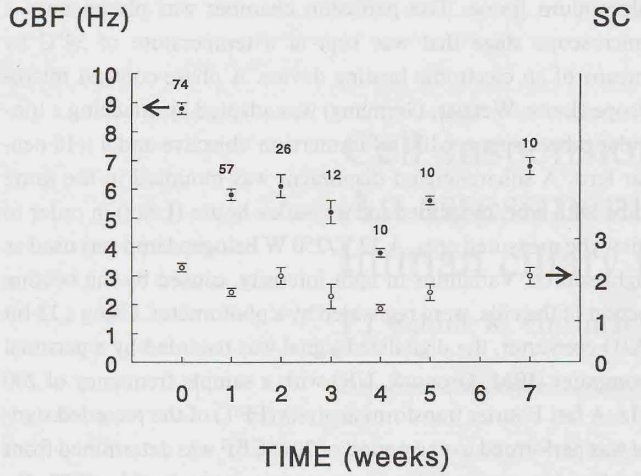

Figure 1. CBF (mean±SEM; closed circles) and SC (mean ± SEM; open circles) of aggregates of cell suspension cultures. The number of measured ciliated cells is indicated.

Adenoid specimens

The results obtained in adenoid specimens are presented in Figures 2 and 3. CBF amounted to 9.3 Hz initially, and it remained at a constant level during the complete test period of 5 h. Statistical analysis showed no significant CBF change over time ($p > 0.05$; ANOVA). For SC, a statistically significant time-dependent decrease was found ($p < 0.05$; ANOVA). After 90 min, SC was about 60% of its initial value, and no further decrease was observed.

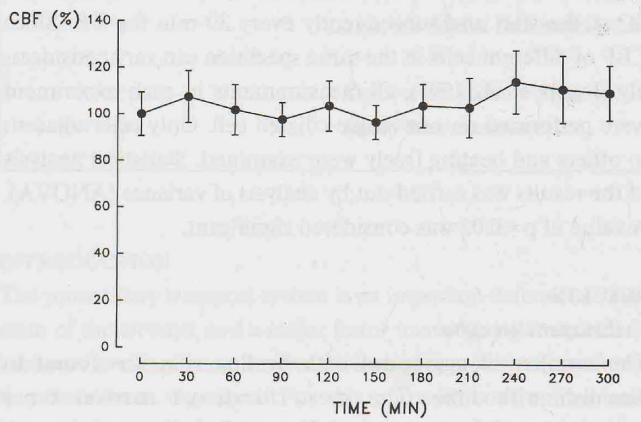

Figure 2. CBF (mean±SEM; n=7) of adenoid cilia in relation to time. Initial mean CBF (9.3 Hz) is set at 100%.

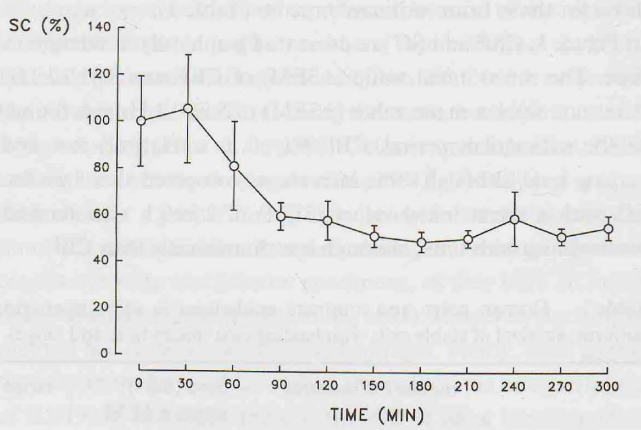

Figure 3. SC (mean±SEM; n=7) of adenoid cilia in relation to time. Initial mean SC (3.0) is set at 100%.

DISCUSSION

Ciliary beat in cell suspension cultures

We confirmed that in cell suspension cultures of ciliated epithelium, beating cilia remain present for a relatively long period. In the majority of the specimens ciliary beat was lost within 2-3 weeks, however. In one specimen ciliary beat lasted for more than seven weeks. Jorissen et al. (1989) were able to preserve aggregates with beating cilia even longer in some cases. With the monolayer culture technique, as used by Rautiainen et al. (1993), all ciliated cells were lost much sooner.

As the number of aggregates in a cell suspension culture decreased, the number of ciliated cells that could be measured diminished. In our experiments the initial mean CBF amounted to 8.8 Hz. After one week a mean CBF value of 5.8 Hz was found; even lower values were recorded after three and four weeks. After five weeks a small increase was seen, but the initial level was never reached again. A possible explanation for this finding would be that cells measured after five weeks and later are the most viable ones, retaining their ciliary beating capacity for a longer period.

Also, a minor decrease in SC was found in the course of time. The relevance of this parameter is not yet fully understood, but this decrease in SC could indicate a loss of co-ordination of the beating of the cilia. Apparently, ciliary beat becomes slower as well as less harmonic the longer the cultures are kept. Similar phenomena were observed in monolayer cell cultures (Rautiainen et al., 1993). Our results show that it is possible to maintain ciliary activity in a cell suspension culture over a longer period. However, as ciliary beat deteriorates rather rapidly, this model is less appropriate for function experiments.

Ciliary beat of ciliated adenoid epithelium

In adenoid specimens CBF remained unchanged for a period of 5 h. Ciliated adenoid epithelium can thus be used in experiments studying various factors affecting CBF *in vitro*. Yet it should be realized that adenoid tissue may be infected, and infection may decrease CBF (Wilson et al., 1985). In our experiments this apparently did not play a role, as initial CBF (9.3 Hz) was equal to that in biopsies of the human nasal mucosa (Lioté et al., 1989; Ingels et al., 1991).

SC was found to decrease significantly, although it remained at a constant level after 1.5 h. It is likely that the *in vitro* conditions for the epithelium to sustain ciliary beat are not as optimal as those *in vivo*. Thus, a decrease in SC could be the first sign of impaired ciliary function with CBF being unaffected. This finding supports the hypothesis that SC has a certain value as another parameter of ciliary activity. However, the significance of SC with regard to the results of function experiments is still unclear.

CONCLUSIONS

Processing human upper respiratory ciliated epithelium for cell suspension cultures is a method that can be used to preserve cells with beating cilia for several days to weeks. The first measurement of CBF can be performed one week after initiating the cultures. However, after one week, CBF is already at a rather

low and non-physiological level. Therefore, we regard this model as inappropriate for studying the effects of several substances on ciliary function. Besides, the method is quite complicated as well as time-consuming.

On the other hand, ciliated adenoid epithelium retains a constant CBF for many hours and it is amply available in ENT practice. Therefore, it is very suitable for studying human ciliary beat *in vitro*.

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