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Research Paper

Culture of Calu-3 Cells at the Air Interface Provides a Representative Model of the Airway Epithelial Barrier

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Purpose. The aim of this study was to compare the effect of liquid-covered culture (LCC) and air-interfaced culture (AIC) on Calu-3 cell layer morphology and permeability, thus assessing the fitness of these culture systems as models of airway epithelium barrier function.
Methods. Cell layers were grown on 0.33 cm^2 Transwell polyester cell culture supports. Cell layers grown using LCC and AIC were evaluated by using light and electron microscopy, transepithelial electrical resistance (TER), and permeability to the transepithelial flux of fluorescein sodium (flu-Na), and by varying molecular weight dextrans labeled with fluorescein isothiocyanate (FITC-dex). The tight junction protein, zona occludens protein-1 (ZO-1), was visualized by confocal microscopy and apical glycoprotein secretions were identified by using alcian blue.
Results. Cells grown via AIC produced a more columnar epithelium with a more rugged apical topography and greater glycoprotein secretion compared to cells grown via LCC. Apical protrusions appearing to be cilia-like structures were observed on occasional cells using AIC, but typical airway ciliated cell phenotypes were not produced under either condition. Secretory granules were observed in cells cultured under both conditions. Cells cultured using LCC exhibited higher levels of ZO-1 protein than the AIC counterpart. The maximal TER of cells using LCC, 1,086 ± 113 Ω cm^2 at 11–16 days, was significantly greater than the TER of cells cultured using AIC, 306 ± 53 Ω cm^2 at 11–13 days. Apparent permeability (P_{app}) values for the transport of flu-Na using LCC and AIC were $1.48 \pm 0.19 \times 10^{-7}$ and $3.36 \pm 0.47 \times 10^{-7}$ cm s^{-1}, respectively. Transport rates of flu-Na and FITC-dex were inversely proportional to molecular weight, and were significantly lower ($p < 0.05$) in cell layers grown using LCC than AIC. Renkin analysis fitted the data to single pore populations of radii 7.7 and 11.0 nm for LCC and AIC, respectively.
Conclusion. Distinct differences in morphology and permeability result when Calu-3 cells are grown using AIC or LCC. Cells cultured using AIC generate a model more morphologically representative of the airway epithelium than cells cultured using LCC.

KEY WORDS: drug delivery; permeability; respiratory cell culture; toxicology.

INTRODUCTION

The Calu-3 cell line is one of the few respiratory cell lines that form tight junctions *in vitro*, allowing it to be used for modeling the airway epithelial barrier in lung research (1–5). The cell line produces features of differentiated, functional human airway epithelial cells (4), and has become the principal cell line for *in vitro* research into tracheobronchial epithelial permeability. Calu-3 cells are finding increasing application in drug delivery and toxicological research (6–8), and characterization of the epithelial cell layers generated under different culture conditions is important before investing significant resource into these studies.

The culture conditions employed in primary respiratory epithelial airway cell culture are critical to the characteristics of the resultant cell layer (9,10). When grown by using air-interfaced culture (AIC), primary culture cell layers (11–13) resemble the native epithelium (14–16) to a greater extent than cells grown by using liquid-covered culture (LCC). This is also generally the case for the Calu-3 cell line, with some researchers reporting enhanced ciliogenesis, increased mucus secretion, and more physiological transepithelial electrical resistance (TER) values in cell layers grown using AIC than cells grown using LCC (see Table I). AIC conditions are also favored for Calu-3 cultures being used to study airway lining fluid regulation (17,18), following the finding that AIC Calu-3

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ABBREVIATIONS: A, surface area of filter insert (cm^2); AIC, air-interfaced culture; C, initial concentration (μg/mL); D, diffusion coefficient (cm^2/s); F, flux (rate of change in cumulative mass transported) (μg/s); FITC-dex, fluorescein isothiocyanate labeled dextran; flu-Na, fluorescein-sodium salt; LCC, liquid-covered culture; P_{app}, apparent permeability (cm/s); r_i, molecular radius of solute (nm); r_p, molecular radius of pore (nm); TER, transepithelial resistance (Ω cm^2); ZO-1, zona occludens protein-1; ε/L, ratio of pore area to length.

Table I. Calu-3 Epithelial Cell Morphology and Permeability Reported in Studies using Air-Interfaced Culture (AIC) and Liquid-Covered Culture (LCC)

	Liquid-covered culture			Air-interfaced culture			
Study	Characterization	P_{app} ($\times 10^{-7}$ cm/s)	TER max (Ω cm^2)	Characterization	P_{app} ($\times 10^{-7}$ cm/s)	TER max (Ω cm^2)	Cell layer morphology
(2)	Immunochemistry	Flu 1[a]	1,185	Immunochemistry	Flu 1[a]	500	ZO-1 and E-cadherin staining appear more prominent using LCC than AIC
(23)	Immunochemistry Mucus staining	Flu 2.2	>2,000	Immunochemistry, Mucus staining, SEM	Flu 2.2	~700	Similar expression of junctional proteins (ZO-1, E-cadherin) using AIC and LCC was shown. Strong staining for acidic glycoproteins was shown using AIC but not LCC
(3)	TEM/SEM	Man 0.44	697	TEM/SEM	Man 0.88	816	Junctional structures and mucus vesicles reported using LCC and AIC, passage 19. (Ref 3, reporting ref 37). Cells grown using LCC reported to exhibit short thick cilia, whereas cells grown using AIC exhibited longer thinner cilia (micrograpis not shown)
(22)	–	Flu 1[a]	1,000	–	Flu 9[a]	550	Morphology not reported
(29)	–	–	–	Pore size analysis, TEM	Man 1.3	>1,000	Junctions reported to be 5.7 nm. Some multilayering and secretory granules (cell passage 20–40)
(5)	–	–	–	Light microscopy, TEM	–	~600	Apical microvilli and tight junctions reported. No report of cilia present. Light microscopy of cells in culture shown
(4)	–	–	–	TEM	–	116	Single-cell layer with 20% of cells containing secretory granules. Apical microvilli, tight junctions and desmosomes shown. No report of cilia present
(34)	–	–	–	Immunochemistry	Man 3.1	359	Expression of junctional proteins
(20)	TEM/SEM/Light	Man 4.7	1,000	–	–	–	Tight junctions and cilia present + mucus secretion (cell passage 20–40)
(36)	TEM	–	700–2,500	–	–	–	Single layer with some areas which were 2 cells deep
(21)	SEM/TEM	Man 3.7	900	–	–	–	Single layer of cells with short and thick cilia with mucus secretion (cell passage 20–40)
Present study	TEM/SEM, Immunochemistry, Mucus stain, Pores size analysis	Flu 1.48	1,080	TEM/SEM, Immunochemistry, Mucus stain, Pore size analysis	Flu 3.36	303	Apical microvilli and tight junction structures shown using AIC and LCC (cell passage 38–50). Pseudostratified cell layer with mucus cover using AIC with apical structures resembling immature cilia/extended microvilli. Single-cell layer using LCC with no mucus covering or other apical structures

– = Not performed, Flu = fluorescein sodium, Man = mannitol.
[a] Taken from figure.

cells provided a much greater basal short circuit current than LCC cells (19).

However, there are inconsistencies in the literature regarding Calu-3 morphology and permeability. For example, some Calu-3 cells grown using LCC have been reported to exhibit characteristics more usual in cultures grown by using AIC, including the appearance of abundant cilia and visible mucus production (20,21). Permeability to fluorescein sodium (flu-Na) has been found to be markedly different between certain cell layers grown using LCC and AIC (22), whereas permeability proved similar in other studies (2,23).

To our knowledge, there are limited reports in which the effect of culturing Calu-3 cell layers using LCC and AIC on cell morphology (23) or the cell permeability barrier (22,23) have been compared directly. Of these reports, one is a conference proceedings (37), and the other two were primarily focused on the deposition of particles to the cell layer (22,23). The aim of this work was to evaluate the Calu-3 cell line with respect to its morphology and permeability by using identical culture conditions except for the air-interfaced and liquid-covered distinction. This was to provide a direct and more complete evaluation of the fitness of these culture systems as models in which to study the permeability of airway epithelium.

MATERIALS AND METHODS

Materials

All materials were obtained from Sigma-Aldrich (Dorset, UK) unless otherwise stated. Cell culture flasks (75 cm^2 with ventilated caps) and Transwell cell culture systems (0.33 cm^2 polyester, 0.4 μm pore size) were from Costar (through Fisher Scientific, Leicestershire, UK). Cell culture reagents included trypsin–EDTA solution (2.5 g/L trypsin, 0.5 g/L EDTA), trypan blue solution (0.4% w/v), Hanks balanced salt solution [HBSS, no phenol red, including $NaHCO_3$ at 0.33 g/L with HEPES buffer (0.01 M)], and phosphate-buffered saline (PBS; Oxoid, Hampshire, UK). Black 96-well plates were from Nunc (through Fisher Scientific, Leicestershire, UK).

Calu-3 Cell Culture

The Calu-3 human bronchial epithelial cell line was purchased from the ATCC (Rockville, MD, USA) and used between passages 38 and 50. Cells were cultured in 75 cm^2 flasks using 20 mL medium and maintained in a humidified, 5% CO_2–95% atmospheric air incubator at 37°C. Cell culture medium was prewarmed to 37°C and comprised 500 mL Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Hams (1:1), 50 mL fetal calf serum (Lot no 34K3399; FCS), 5 mL nonessential amino acid solution (×100), 5 mL L-glutamine solution (200 mM), and 0.5 mL gentamycin (50 mg/mL). Medium was exchanged twice per week and cells were passaged weekly at a 1:3 split ratio by using a 3 mL trypsin–EDTA solution.

Cells cultured on Transwell cell culture supports using LCC and AIC were seeded at a density of 5×10^5 cells/cm^2 and were introduced into the apical surface of the Transwell cell culture support in 0.1 mL medium with 0.5 mL medium added to the basolateral chamber. The cells were incubated at 37°C, 5% CO_2 for 2 days. After this time, medium was aspirated from the apical and basolateral cambers. For LCC, medium was replaced in both apical and basolateral chambers, whereas medium was only replaced in the basolateral chamber for AIC. Subsequently, medium in the respective chambers was replaced every 2 days.

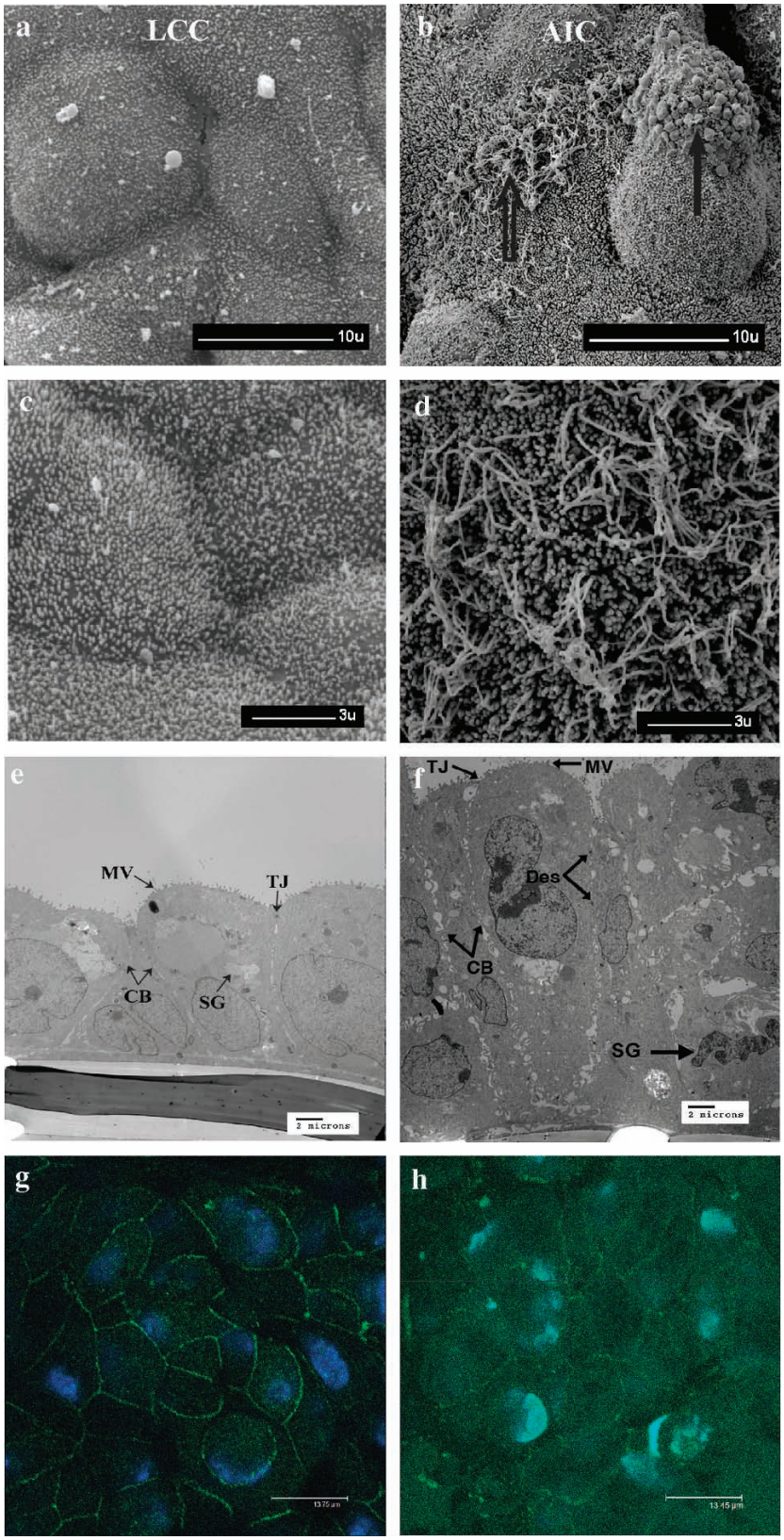
Cell Surface Staining

Cell layers were washed twice with 100 μL HBSS and fixed for 10 min by using 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer. The cells were washed again with HBSS and 100 μL alcian blue stain [1% (w/v) alcian blue in 3% (v/v) acetic acid/water at pH 2.5] added for 1 h. The dye was then removed and cell layer washed with HBSS until the rinsate ran clear.

Microscopy

For electron microscopy, a 1:1 mixture of medium and fixing solution [2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2] was added to both chambers and the cell layer was rotated gently. After 5 min, the solution was removed and replaced with 100% fixing solution and stored at 4°C for up to 2 weeks. Cells for scanning electron microscopy (SEM) were bathed in 1% (w/v) osmium tetroxide in water for 90 min. The cells were then dehydrated in progressively increasing concentrations of ethanol, before being transferred to a critical point dryer. Samples were then mounted on aluminum stubs (Quorum Technologies, Sussex, UK) with adhesive carbon tape and gold coated using a Polaron E5100 sputter coater (Quorum Technologies, Sussex, UK). Cells were analyzed at 15 kV (Philips SEM501B scanning electron microscope (Philips Electron Optics, Cambridge, UK), fitted with a Deben Pixie 3000 digital scan generator (Deben UK Ltd, Suffolk, UK). For transmission electron microscopy (TEM), samples were exposed to 1% (w/v) osmium tetroxide in water for 90 min and then dehydrated in acetone, followed by sequential immersion in 3:1, 1:1, and 1:3 acetone/Spurr resin each for 2 h and finally for 48 h in 100% Spurr resin. Ultrathin sections were cut by using a Reichart OMU4 Ultracut ultramicrotome (Leica Microsystems, Milton Keynes, UK), stained by using uranyl acetate and lead citrate, and viewed via a JEOL 100CX transmission electron microscope (JEOL UK Ltd, Welwyn Garden City, UK). For both TEM and SEM analyses, at least three cell preparations were examined and representative micrographs were selected from several sections.

For confocal microscopy, cells were washed in 10 mL PBS and fixed for 10 min using 3.7% (v/v) paraformaldehyde in PBS at room temperature. The cells were then washed with 10 mL PBS and exposed to 0.1% (v/v) Triton X 100 with 0.5% (v/v) FCS in PBS for 60 min. The cells were washed again with 10 mL PBS and then 50 mM ammonium acetate in PBS was added for 10 min. After washing with a further 10 mL PBS, cells were incubated at 37°C with rabbit anti-ZO-1 (1 μg/mL in PBS) (Zymed, Cambridge BioScience, Cambridge, UK) for 60 min. A further washing with 10 mL PBS was carried out and AlexaFlour 488 chicken antirabbit IgG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(10 μg/mL in PBS) (Molecular Probes, Cambridge BioScience, UK) added. The cell layer was again washed with 10 mL PBS, counterstained with 1 μg/mL 4′-6-diamidino-2-phenylindole (DAPI) in water for 10 min, and then washed with 10 mL PBS. The Transwell cell culture support membrane was cut from the plastic support and mounted on a microscope slide in 10% (w/v) glycerol and sealed. Slides were stored at 4°C and viewed the following day. The cell layer was viewed via a Leica DMIR E2 confocal microscope (Leica Microsystems).

Electrical Measurements

TER measurements were made by using chopstick electrodes and an EVOM voltohmmeter (STX-2 and Evom G, World Precision Instruments, Stevenage, UK) on cells cultured using LCC immediately upon removal from the incubator. For cells cultured using AIC, warmed medium (0.1 mL, 37°C) was added to the apical chamber before returning them to the incubator to equilibrate for a further 30 min, and then measuring the electrical resistance. TER was calculated by subtracting the resistance of a cell-free culture insert and correcting for the surface area of the Transwell cell culture support.

Permeability of Calu-3 Cell Layers

To prepare the cell layers on the day of transport studies, TER was taken and the medium on the apical and basolateral chambers was aspirated and the cell layers washed twice with warm HBSS (37°C). HBSS (0.1 mL) was introduced to the apical chamber and (0.6 mL) to the basolateral chamber (using AIC, HBSS was not placed in the apical well after the final washing). The cells were returned to the incubator at 37°C for 1–2 h to equilibrate. The TER was measured immediately prior to experimentation.

Fluorescein isothiocyanate labeled dextrans (FITC-dex) with average molecular weights of 4, 10, 20, 40, and 70 kDa and flu-Na (0.367 kDa) were dissolved in warm HBSS to produce 2.5 mg/mL test solutions. HBSS in the apical chamber of each well was aspirated, and the experiment was initiated by introducing the test solution (215 μL). A sample of this solution (100 μL) was then immediately removed for determination of the initial starting concentration. The cells were incubated at 37°C and rotated at 100 rpm. Basolateral sampling (100 μL) was carried out for 4 h, with samples being replaced with fresh warmed HBSS (37°C, 100 μL). At the final time point, a 100 μL sample was also removed from the apical chamber. TER was measured after the final sample was taken.

Sample Assay

All samples were transferred to a black 96-well plate, and 100 μL of 40 mg/mL NaOH aqueous solution was added to each sample. Fluorescence was measured via excitation and emission wavelengths of 485 and 530 nm, respectively, by using a fluorometer (Cytoflour, Series 4000, Foster City, CA, USA).

Data Analysis

Apparent permeability coefficients (P_{app}) were calculated by Eq. (1), where F is flux (rate of change in cumulative mass transported), A is surface area of cell culture support, and C_0 is the initial concentration in donor chamber.

$$P_{app}(\mathrm{cm/s}) = F\left(\frac{1}{AC_0}\right) \tag{1}$$

Statistical testing was performed by using SPSS version 11.0 software and data were analyzed by Student's t test, with significance reported if $p < 0.05$.

Theoretical Pore Size Calculation

The theoretical pore size of the cell layers was estimated by calculating the Renkin function (24) [Eq. (2)] for the range of solutes, where r_i is the molecular radius of the solute (nm) and r_p is the theoretical pore radius (nm).

$$\textit{Renkin function}\left(\frac{r_i}{r_p}\right) = \left[1-\frac{r_i}{r_p}\right]^2\left[1-2.109\left(\frac{r_i}{r_p}\right)+2.09\left(\frac{r_i}{r_p}\right)^3-0.95\left(\frac{r_i}{r_p}\right)^5\right] \tag{2}$$

First, diffusion coefficients (D) for each solute were calculated according to the method of Seki and coworkers (25), which has been applied to the diffusion of dextrans (26).

$$\log D = -0.434 \log \mathrm{MW} - 0.4059 \tag{3}$$

Using these values of D, solute molecular radius was derived by using the Stokes-Einstein equation. The Renkin function was then used to calculate ε/L (pore area and length, respectively) using experimental P_{app} data [Eq. (4)].

$$P_{app} = \left(\frac{\varepsilon}{L}\right)\left(\frac{r_i}{r_p}\right)D \tag{4}$$

Fig. 1. SEM, TEM, and confocal images of Calu-3 cells using LCC (a, c, e, and g) and AIC (b, d, f, and h). (a, c) Cell layers grown using LLC at day 16 with short and thick microvilli and clear cell–cell boundaries. These are compared to cell layers grown using AIC at day 11 (b, d), showing a heterogenous population of apical extensions, some short microvilli (similar to LCC), and others much more extended and thinner or immature cilia (open arrow). Exuding mucus can also be seen (filled arrow). (e, f) TEM of Cell layers cultured using LCC at day 13 and AIC at day 10, respectively. It can be seen that AIC produces a thicker cell layer with pseudostratification being more apparent than cells grown using LCC. Some secretory granules (SG), tight junctional complexes (TJ), microvilli (MV), desmosomes (DES), and cell boundaries (CB) are clearly apparent. The Transwell insert membrane can be seen at the bottom of the micrographs. (g, h) Confocal morphology of the tight junction complex (ZO-1, green) and nuclei (DAPI, blue) in Calu-3 cells grown using LCC (g) and AIC (h) after 11 days of culture. Images are representative single XY 2D planar sections, taken using a 640× oil immersion lens (scale bar = 13.45 and 13.75 μm for AIC and LCC, respectively).

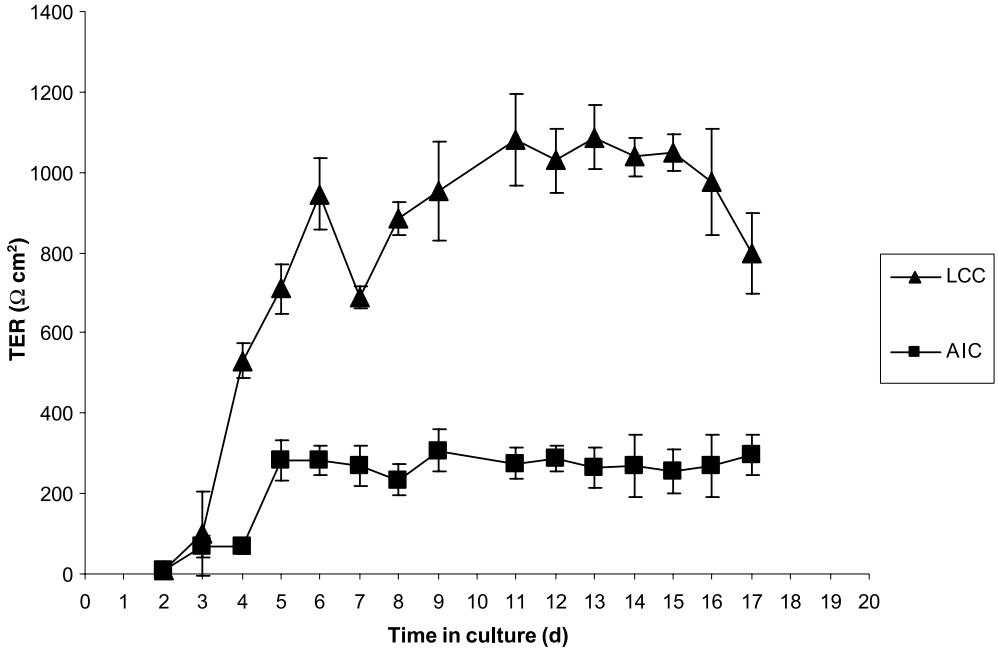

Fig. 2. Transepithelial electrical resistance of Calu-3 cells cultured using LCC and AIC on 0.33-cm^2 cell culture supports plotted as a function of time from three separate experiments. Data mean ± standard error (n = 36).

Predicted P_{app} was plotted against experimental values, and r_i and ε/L were varied to find the minimum sum of the squared differences between the predicted and experimental values.

RESULTS

Calu-3 Cell Culture

By day 4, cell layers grown under both set of conditions in Transwell inserts were confluent, producing an effective barrier to hydrostatically driven medium seepage from the basolateral to apical well. In cell layers that were stained with alcian blue, there was much more prominent staining for the cell layer grown using AIC compared to that of the cell layer grown using LCC (data not shown), suggesting that a greater quantity of glycoprotein (mucus) was present covering the cell surface.

Epithelial Cell Layer Morphology

SEM revealed microvilli to be present from day 2 (data not shown) and to increase only marginally in length, width, and density to day 16 using LCC (Fig. 1a, c). These features

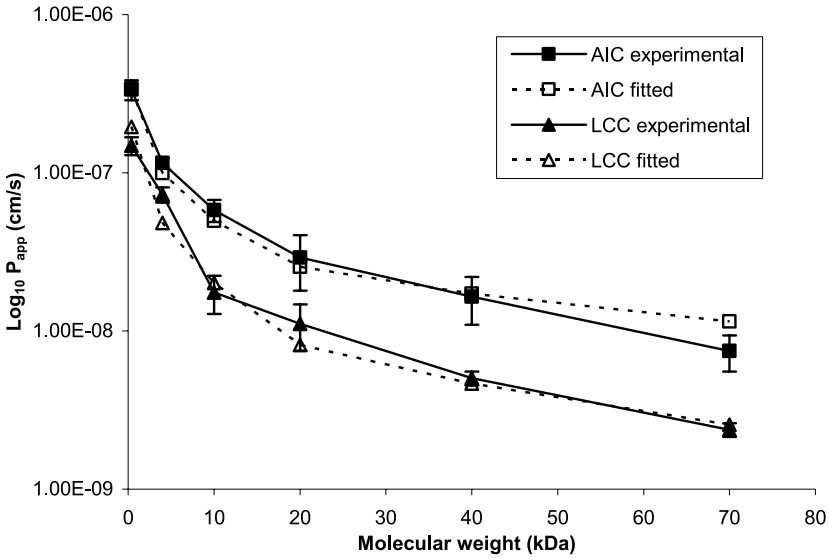

Fig. 3. Permeability of FITC-labeled dextrans and sodium fluorescein across Calu-3 cell layers cultured using AIC and LCC for 11 days. Fitted lines show predicted P_{app} based on cell layers with a single pore population of radii 7.7 and 11.0 nm for LCC and AIC conditions, respectively. Data represent the mean of three experiments, for each data point ± standard error (n = 6–16).

were more pronounced using AIC (Fig. 1b, d). Apical projections extending beyond the microvilli were observed on the surface of some cells using AIC (but not LCC), possibly being immature cilia. These extended protrusions were localized to individual cells, occurring at an approximate rate of 1/100 cells over the preparations examined. In all instances, the microvilli were more pronounced in preparations grown using AIC than those grown using LCC. Individual cells and cell–cell boundaries were seen clearly under both conditions.

Cell layers grown using LCC and viewed by TEM were shown to exist as a monolayer (Fig. 1e), whereas AIC produced a pseudostratified layer of more columnar cells (Fig. 1f). The thickness of the layer was generally 15–20 μm for LCC but varied more using AIC, ranging between 20 and 45 μm. Apical microvilli, tight junctions, and desmosomes were observed using both LCC and AIC, but no ciliated structures were observed by TEM. Secretory vesicles were observed in cell layers grown under both types of culture condition.

The zona occludens protein-1 (ZO-1; a major component of the tight junction) in cells cultured using both AIC and LCC was detected by using an anti-ZO-1 label; a nuclear stain was also used (Fig. 1g, h). It was apparent that the tight junctions were more heavily stained when the cells were grown using LCC rather than AIC, suggesting that the ZO-1 antibody was binding with higher amounts of protein expressed under the former conditions. This can be indicative of tighter junctions and a less permeable cell layer, as was borne out by solute permeability measurements.

Epithelial Cell Layer Permeability

The TER of cell layers grown using AIC and LCC increased above baseline values after 2–3 days in culture. Using AIC, cell layer TER reached a plateau (approximately 300 Ω cm^2) around day 5 post-seeding, whereas cell layers cultured using LCC expressed a significantly higher TER, reached a maximum peak around day 11, and maintained a resistance of approximately 1,000 Ω cm^2 (Fig. 2). As a consequence of the maximum TER values and stable morphology being attained between days 11 and 13 under both culture conditions, this time frame was selected as being suitable for performing solute permeability studies.

FITC-dex permeability across the cell layers was inversely proportional to molecular weight (Fig. 3). In this experiment, cells cultured using LCC were less permeable to solute flux than cells cultured using AIC ($p < 0.05$), suggesting that the former exhibit a lower molecular weight permeability exclusion threshold than cell layers grown using AIC. The P_{app} values and molecular weights of the compounds can be employed to determine the theoretical equivalent pore size of the cell layer in question. Fitted lines show predicted P_{app} based on cell layers with a single pore population of radii 7.7 and 11 nm for LCC and AIC conditions, respectively (Fig. 3).

DISCUSSION

This study has demonstrated, by direct comparison, that Calu-3 cells cultured using AIC produce a cell layer with greater similarity to the airway epithelial morphology and electrical resistance *in vivo* (14–16,27) than cells cultured using LCC. Permeability studies showed that an effective barrier to paracellularly transported compounds was also achieved and thus air-interface cultured cell layers can be used to assess solute permeability in the airway epithelium.

To date, there is no consensus in the literature regarding how Calu-3 cells should be cultured for this purpose, and differences in morphology and functionality of the cell layer have been reported, most probably due to interlaboratory variations in growth conditions and cell passage number. To examine this, results from the present study on the structure and barrier properties of Calu-3 cells grown using both AIC and LCC were compared with those that have been previously reported (Table I). This allows the impact of these conditions on cell characteristics and model permeability to be compared.

In this study, secretory vesicles were observed in cells grown under both conditions, but only when AIC was used was glycoprotein detected at the cell surface. Little glycoprotein staining occurred in the cell layer grown using LCC, whereas heavy staining was observed using AIC. This observation concurs with that of Fiegel and co-workers (23), who stained cell layers cultured under both conditions with eosin Y, aniline blue, and orange G to show acidic glycoproteins typically found in mucus. It is possible that using LCC, any mucus secreted is dispersed into the culture medium and removed by medium exchange. Other workers have shown that a number of mucin mRNA transcripts were present in Calu-3 cells (cultured in flasks), and that MUC5/5AC mucin was secreted into the surrounding medium (28).

The presence of cilia on Calu-3 cells has been reported by some investigators (20,21,37), whereas others do not report the finding (4,5,23,29). In this study, thin apical protrusions were observed to extend beyond the microvilli using AIC (Fig. 1d). It is not clear whether these structures are cilia and no characteristic cilia procentriole formation was observed via EM analysis. No micrographs of Calu-3 cells have been published that show the typical airway ciliated cell phenotype seen in primary airway cell culture (30), and the capacity of Calu-3 cells to express this morphology remains to be demonstrated.

The Calu-3 line is derived from submucosal gland acini and produces secretory IgA component, lactoferrin, and lysozyme mRNA, typical of serous submucosal gland secretions, but not 15-lipoxygenase (an epithelial surface marker) (31). When primary submucosal acini cells were cultured, tight junctions, microvilli, and a TER of 578 ± 89 Ω cm^2 were produced, but the presence of cilia was not reported (32). *In vivo*, only the upper regions of the gland collecting duct are populated with cilia, this being an extension of the epithelial cell layer (33). It is interesting that although the Calu-3 cell line is of submucosal gland origin, a location that would suggest liquid covered conditions *in vivo*, these cells can be influenced to display more airway surface-like features through exposure to the atmosphere.

The presence of only a single cell type is one of the limitations of using the Calu-3 monoculture cell model, and using cocultures or primary cells would clearly produce a more faithful representation of the native phenotype. Although they do not offer the convenience of cell line-based models, normal human bronchial epithelial cell layers composed of ciliated and goblet cells have been cultured to

provide a permeability barrier, which is also suitable for drug permeability studies (13).

TER values of the Calu-3 cell layers grown using AIC were significantly lower than LCC, indicating less restrictive tight junctions, with values falling within the range found in other studies (2,4,22,34). This finding is supported by the qualitative data obtained relating to the intensity of expression of ZO-1 fluorescent label, which could indicate greater levels of ZO-1 in the LCC monolayer. The transport rates of the test compounds in Calu-3 cells cultured using LCC were lower than in cells cultured using AIC, a finding that has been previously reported (22). However, other groups have compared the rate of fluorescein movement across the Calu-3 cell layers grown using LCC and AIC and found no difference (2,23). The latter results were attributed to the existence of a threshold TER barrier above which similar transport rates result, although this phenomenon was not seen by Cooney and co-workers (22). LCC conditions have also been reported to promote the cell layer barrier properties of another airway cell line, 16HBE14o- (35). In the latter instance, the volume of apical fluid was found to influence barrier properties, with LCC being required to generate an adequate cell barrier, although other researchers have cultured suitable drug absorption models using AIC with 16HBE14o- cells (6).

In this study, TER values of approximately 1,000 Ω cm^2 were obtained by using LCC and P_{app} values for flu-Na were in-7 cm s^{-1}. For AIC, a TER of approximately 300 Ω cm^2 resulted in P_{app} values being in the range of $2–4\times10^{-7}$ cm s^{-1}. Using the transport data obtained from the molecular weight markers and the cells cultured using AIC, the theoretical pore diameter proved to be almost twice as wide as that reported by Mathias and coworkers (29). This corresponds with the greater permeability of the solutes in the cell layers reported here.

The mechanism(s) by which different culturing regimes determine morphology and electrical properties requires clarification. Oxygen is thought to play a large part in this process, having been shown previously to increase the amount of sodium transported across the epithelium, with cells shifting to a more oxidative pattern of metabolism through placement at an air interface (12). Relatively little research has been undertaken to assess what molecular and biochemical modulation signals are being produced in response to different culture conditions, which in turn influence cell physiology within the cell layer. This is an area worthy of further research to expand our understanding of the mechanisms of cellular differentiation under these circumstances.

CONCLUSION

As a consequence of interlaboratory variation in culture conditions, it is important to characterize cell layers before using Calu-3 cells as a model of the airways. AIC and LCC for human Calu-3 cell line culture were directly compared in the present study. The growth of the cells using AIC produced a cell layer with an ultrastructure, secretory component and electrical resistance that is more suitable as a model of the tracheobronchial epithelium than was produced using LCC. Air-interface cultured cell layers will provide a platform for the evaluation of solute permeability, transport mechanisms, and particle-cell interactions.

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