Heliyon 10 (2024) e37977

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Development of a programmable automated cell culture system to study the lung pathophysiology of Cystic Fibrosis-related diabetes

Analia J. Vazquez Cegla [a,*], Cameron Hedden [b], Barry R. Imhoff [a], Guiying Cui [a], Nael A. McCarty [a]

[a] Division of Pulmonology, Asthma, Cystic Fibrosis, and Sleep, Department of Pediatrics, Emory + Children's Center for Cystic Fibrosis and Airways Disease Research, Emory University School of Medicine and Children's Healthcare of Atlanta, Atlanta, GA, USA
[b] Neuroscience and Behavioral Biology Program, Emory College of Arts and Sciences, Emory University, Atlanta, GA, USA

A B S T R A C T

The study of many diseases is limited by the *in vitro* systems available. Cystic Fibrosis-Related Diabetes (CFRD), the main co-morbidity of Cystic Fibrosis (CF), is a perfect example. Cells *in vivo* experience glucose fluctuations after meals. In contrast, cells cultured *in vitro* are initially exposed to high glucose media. Glucose gets progressively depleted until the next media change days later, which is not physiologically relevant and could negatively impact the results of research studies. To better study the mechanisms driving CFRD pathophysiology, we developed a programmable and automated cell culture system (PACCS) capable of mimicking acute hyperglycemic episodes experienced by CFRD patients after meals. We adapted a commercially available perfusion system and performed 3D modeling to develop this system. Results show that PACCS can be successfully used to culture airway epithelial cells, both immortalized and primary cells. Further, CF cells responded differently to meal-like conditioning when compared to controls, suggesting impaired adaptative responses in CF cells. Overall, PACCS will allow us to better study CFRD pathophysiology, and it could be used for a wide range of other applications.

1. Introduction

Cystic Fibrosis-Related Diabetes (CFRD) is the most common Cystic Fibrosis (CF) co-morbidity, and its pathophysiological consequences are severe [1]. CF is an autosomal recessive disease caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) [2,3]. CFTR is expressed at the surface of epithelial cells throughout the body, and is essential to the function of key systems, such as the respiratory and digestive tracts [4]. Recently, pharmacological advances in the CF field have greatly increased the life expectancy of people with CF (pwCF) [5]. As a result, CFRD has become more prominent. CFRD starts to develop during early childhood, and about half of pwCF develop CFRD by adulthood [6,7]. This is concerning since CFRD patients experience more rapid lung function decline, approximately six-fold greater than pwCF without diabetes, due to more frequent pulmonary exacerbations [8–10]. The link between CFRD and accelerated lung function decline is unknown, and there are no good *in vitro* models to study this devastating disease.

Immortalized and primary airway epithelial cells expressing either WT or mutant CFTR, the most common mutation being ΔF508, are available. However, common protocols used in the field involve culturing airway epithelial cells using media containing extremely high levels of glucose. DMEM for example, a common base media component, contains 450 mg/dL of glucose [11]. This is > 3.5 times higher than what is considered the upper limit for normal blood glucose at fasting (125 mg/dL) [12]. Further, cells *in vivo* experience

* Corresponding author. PACS Division, Dept. of Pediatrics, Emory University School of Medicine, Emory+Children's Cystic Fibrosis Center of Excellence (www.cfatl.org), 2015 Uppergate Drive, Atlanta, GA, 30322, USA.
E-mail addresses: ajvazqu@emory.edu, namccar@emory.edu (A.J. Vazquez Cegla).

https://doi.org/10.1016/j.heliyon.2024.e37977
Received 30 May 2024; Received in revised form 11 September 2024; Accepted 14 September 2024
Available online 19 September 2024

glucose fluctuations after meals and snacks, as shown by continuous glucose monitoring (CGM) data [13]. In contrast, cells cultured *in vitro* are initially exposed to media with high glucose concentration, which gets progressively depleted until the next media change, typically days later [14]. It is also possible that cells experience hypoglycemic periods, especially when cultured in small volumes, which might introduce harmful changes in cell behavior (Supplemental Fig. 1). Overall, current patterns of glucose exposure used to study CF *in vitro* do not resemble human physiology. Automation of cell culture, a rapidly growing field, could be used to address this issue.

Automation has been successfully used to culture adherent cells to tightly control their chemical and biological environment [15]. Custom chambers created using 3D printing and pumps are commonly used to control media flow in and out microfluidic chambers [16]. However, the field of cell culture automation has mostly focused on the development of lab-on-a-chip devices for high-throughput screening [17]. Limitations in the field include cost and ease of use of such devices. Further, there are currently no effective ways to automate cell culture of airway epithelial cells plated on permeable Transwell supports, necessary to better study the effects of CFRD on airway physiology.

To allow for better glycemic control while doing *in vitro* cell culture, we developed a Programmable Automated Cell Culture System (PACCS) capable of simulating meal-like glucose fluctuations (Fig. 1A–C). The system uses a combination of peristaltic pumps, pinch valves, and a programmable controller to change the media in custom-designed 3D-printed cell culture plates multiple times a day. The system requires minimal user intervention and can run meal-like glucose fluctuation programs for several days on multiple plates. This novel tool was used to evaluate the effects of episodes of acute hyperglycemia in WT and CF immortalized and primary airway epithelial cells, but it could be used for a wide range of applications. In this paper, we tested changes in the transepithelial electrical resistance (TEER) of airway epithelial monolayers in response to PACCS culture, as a measure of health of the epithelium. Overall, PACCS is a cost-effective and compact novel cell culture tool that addresses major limitations in the cell culture field and allows for better simulation of CFRD conditions *in vitro*.

2. Materials and methods

2.1. Cell culture of CFBE immortalized cell line

Immortalized CFBE41o- Human Bronchial Epithelial cells (CFBE) expressing WT-CFTR (CFBE-WT) were kindly provided by Dr. K. Oliver (Emory). Cells were cultured in MEM complete media containing 90 % MEM (Gibco, 11095-072), 10 % FBS (ThermoFisher, 26140), and 1 % Penicillin/Streptomycin (Gibco 15070-063). Cells were expanded in collagen-coated plates (PureCol, Advanced Biomatrix #5005-B) and 2 μg/mL puromycin (Sigma, P8833) was used as the selection agent. For PACCS experiments, cells were plated in collagen-coated Transwells (Corning, #3470) at a density of 100,000 cells per well at liquid-liquid interface. The next day after seeding, cells were put at air-liquid interface (ALI). Experimental Transwells were transferred to PACCS, while control Transwells

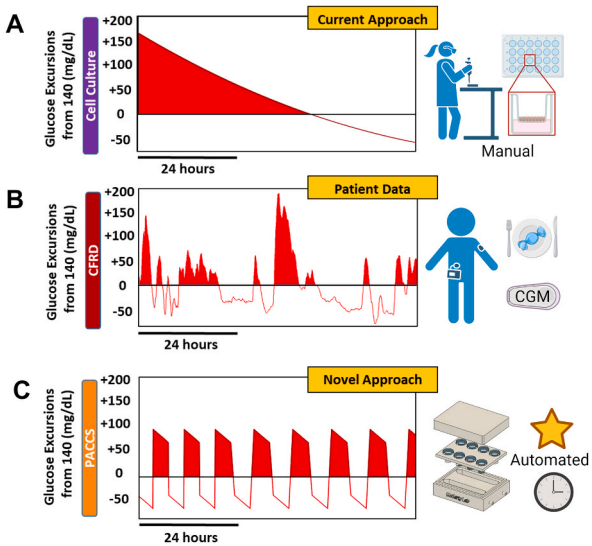

Fig. 1. Current cell culture protocols do not resemble CFRD physiology. A) Representative plot highlighting glucose depletion on the basolateral side of Transwells during traditional *in vitro* cell culture after receiving hyperglycemic media. Note that cells are exposed to hyperglycemic media for an extended period, with progressive depletion of glucose for 72 h. Manual media changes are required. B) Representative blood glucose excursions experienced by a CFRD patient over 72 h. Patterns of fluctuation in and out of the hyperglycemic region, in response to meals, are evident in the graph. Data were taken by continuous glucose monitoring (CGM). C) Representative glucose patterns that can be achieved using PACCS, better resembling patterns experienced by CFRD patients. This novel approach is automated and requires minimal user interaction. Note that in all plots zero represents 140 mg/dL glucose. Any measurements above are considered to fall in the hyperglycemia region. The area under the curve on hyperglycemia regions was highlighted (created with BioRender).

received manual media changes. Cells were exposed to either daily media changes or meal-like glucose fluctuations for 7 days. Selection agent was not added to cells undergoing experimentation.

2.2. Culture of primary cells

Primary human bronchial epithelial cells expressing either WT- (NhBE) or F508del-CFTR (CFhBE) were kindly provided by Dr. M. Koval (Emory). Cells were expanded and plated in Transwells (Corning, #3470) as previously described [18]. Briefly, previously expanded epithelial cells were plated onto Type IV collagen-coated Transwells at a density of 100,000 cells per well at liquid-liquid interface using E-ALI media. E-ALI is based on a 50:50 mixture of low-glucose DMEM containing 100 mg/dL glucose, without L-glutamine and with sodium pyruvate (Sigma, D5546), and LHC Basal Medium (ThermoFisher, 12677–019) containing additives. After 48 h of plating, the basolateral medium was replaced with fresh E-ALI and the apical medium was removed to bring the cells to ALI. Media was changed every 2–3 days. After 14 days, experimental Transwells were transferred to PACCS while control Transwells received manual media changes. Cells were exposed to either daily media changes or meal-like media changes for 7 days.

2.3. Programmable and automated control of PACCS

The ValveBank 8 Controller (Automate Scientific, #01–08) was used to make the PACCS system programmable and automated. This controller allows the user to make loop programs without the need of a computer. The controller was used to control peristaltic pumps (Adafruit, #1150) connected to media reservoirs containing normal or high glucose media (5.5 vs 17.5 mM glucose, respectively). We used autoclavable Nalgene bottles (Thermo, #2105-0016), but replaced the lids with filling/venting closures (Thermo, #2162-0531). Media bottles were connected to the pumps and the pumps were connected to the PACCS plate using autoclavable tubing (Cole-Parmer, #EW-96440-16).

2.4. Design iterations of PACCS cell culture plate components

There are no commercially available plates that could be used with our system. Thus, we designed and 3D-printed a cell culture plate that contained three main components: a main body to hold media, a tray to hold Transwells in place, and a lid to maintain sterility. Design iterations were made using Fusion 360 (AutoDESK). Variations between designs were made with the goal of reducing media consumption and ensuring good media exchange.

2.5. 3D-printing

All design iterations were printed at Emory's "Tech Lab". STL files were printed using a Form2 printer (FormLabs) and clear standard resin (FormLabs, Product #RS-F2-GPCL-04).

2.6. PACCS quality control

Chicago Sky Blue 6B (Sigma, C-8679) and Phenol Red (Sigma, P-5530) dyes were dissolved in 500 mL of water to a final concentration of 0.5 mg/mL in separate containers to simulate normal and high glucose media, respectively. These blue and red solutions were used to test solution exchange in PACCS plate iterations. The ratio of red versus blue absorbance (400 nm versus 620 nm) was compared at each Transwell location. Empty Transwells were used at the time of testing. A small amount of sample (100 μL) was collected from the basolateral side and transferred to a transparent 96-well plate for absorbance measurements. Absorbance measurements were performed using a SpectroMax M2 plate reader (Molecular Devices).

2.7. Running PACCS

Media bottles were cleaned and autoclaved. To sterilize the PACCS tubing, 70 % ethanol was run through the lines for 2 min, later closing the lines and allowing ethanol to sit inside for 15 min. The lines were then drained, and their openings covered with sterile foil. Media was added to media bottles and transferred to the incubator. Using the final design iteration, running PACCS for 7 days required 170 mL of normal glucose media (5.5 mM) and 170 mL of high glucose media (17.5 mM) for one full meal-like plate. An additional bottle with 340 mL of normal glucose media (5.5 mM) was required for the control plate. To sterilize the PACCS plate, all components were thoroughly sprayed down with 70 % ethanol and allowed to airdry inside a cell culture hood. Transwells were put at ALI and transferred to the PACCS plate inside the cell culture hood. The PACCS plate holding the Transwells was transferred to the cell culture incubator, and inputs and outputs were connected. Fasting media (5.5 mM glucose) was introduced into the lines using the manual run function on the controller. This ensures that all lines have media before starting the PACCS program. The lines were visually inspected for air bubbles, and then the PACCS meal-like program was started. This program was designed to change media three times a day for 7 days. The flowrate was ~285 μL/s. The inputs were opened for 28 s, dispensing ~8 mL of media divided between 2 reservoirs in each plate (~4 mL of media per reservoir). The vacuum was run for 20 s by itself, and then it overlapped with the inputs for 12 s to wash away any waste media left on the plate.

2.8. Ussing chamber analysis of cells cultured with PACCS and controls

The electrophysiological properties of airway epithelial cells were assessed by performing Ussing chamber analysis using standard protocols [19,20]. We report here only the resistance values.

2.9. Data analysis

Data were analyzed using both Microsoft Excel and GraphPad Prism.

3. Results

3.1. The PACCS design incorporates several unique components to create a reliable automated cell culture platform for mammalian cells plated on permeable Transwell supports

We developed a novel programmable and automated cell culture system by combining several essential components. The main components of PACCS are shown in Fig. 2. Hyperglycemic exposure patterns can be programmed using a commercially available and user-friendly controller, the ValveBank perfusion controller, which allows the user to create and store several programs to change media as often as desired. The controller powers peristaltic pumps that connect media storage bottles with the PACCS plate. The controller also powers a pinch valve that connects a waste container (fluid flow driven by vacuum) to the PACCS plate (Fig. 2A). The PACCS plate has a main body, a tray, and a lid. The main body of the plate serves as the media reservoir, and it has inputs and outputs to allow for fluid exchange (Fig. 2B). The tray holds up to eight Transwells in place (Fig. 2C), while the lid prevents contamination. The PACCS plate, pumps, valves, and media bottles are small and can be stored inside an incubator, with the perfusion controller outside.

3.2. PACCS plate design iterations to reduce media consumption and improve fluid exchange

An essential element of PACCS is its cell culture plate. There are no commercially available plates compatible with our needs. Thus, we designed and 3D-printed our own cell culture plate. The evolution of the main body of the plate design is shown in Fig. 3. The first design had a large media reservoir with a slanted surface to drive media flow from the inputs to the output. The plate had 2 inputs; one could be connected to a normal glucose media bottle and the other to a high glucose media bottle (Fig. 3A). Transwells cultured at ALI need the bottom of the permeable supports to be submerged in media, while the apical side of the Transwell can be left exposed to air. The first plate design required ~15 mL of media for Transwells to be submerged in media. This was not feasible for future experiments since we needed to perform multiple media changes each day for multiple days. Thus, the second plate design incorporated octagon-shaped columns to reduce unoccupied space and decrease media consumption. Channels between columns were added to allow for

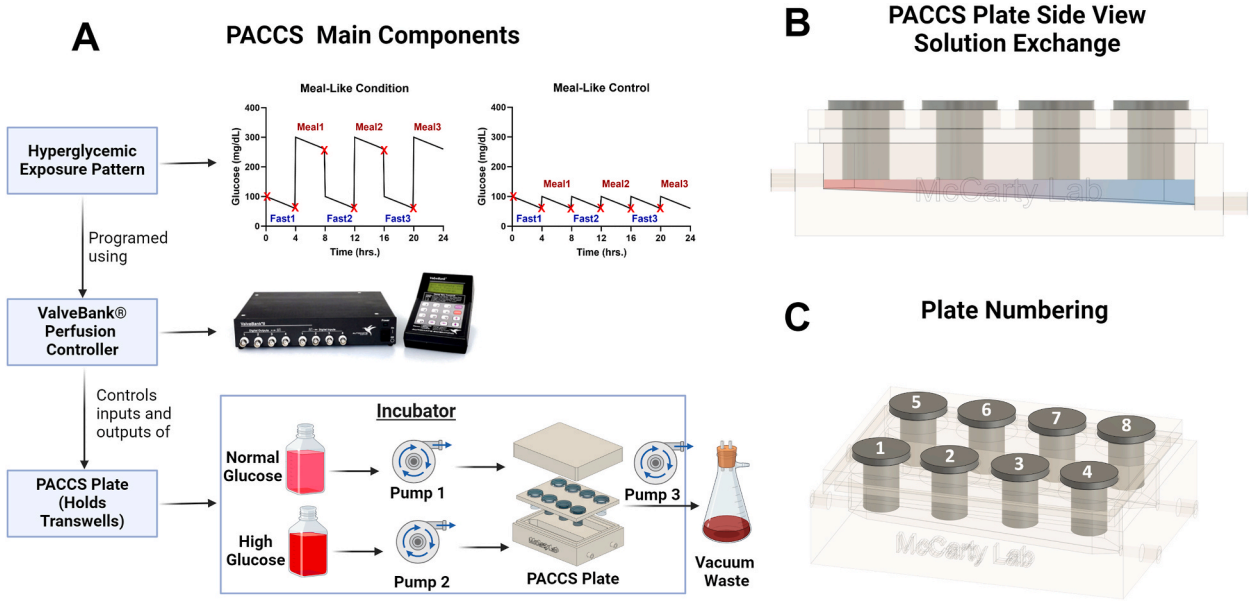

Fig. 2. Graphical representation of the PACCS design. A) Hyperglycemic exposure patterns (top) are programed using a commercially available perfusion controller (middle), which controls fluid flow from media bottles connected to a custom 3D printed cell culture plate (bottom). The red "Xs" shown on the top graphs represent automatic media changes performed by PACCS. B) Images of the PACCS cell culture plate highlighting fluid being exchange from fasting to a meal. C) Transwell location (created with BioRender).

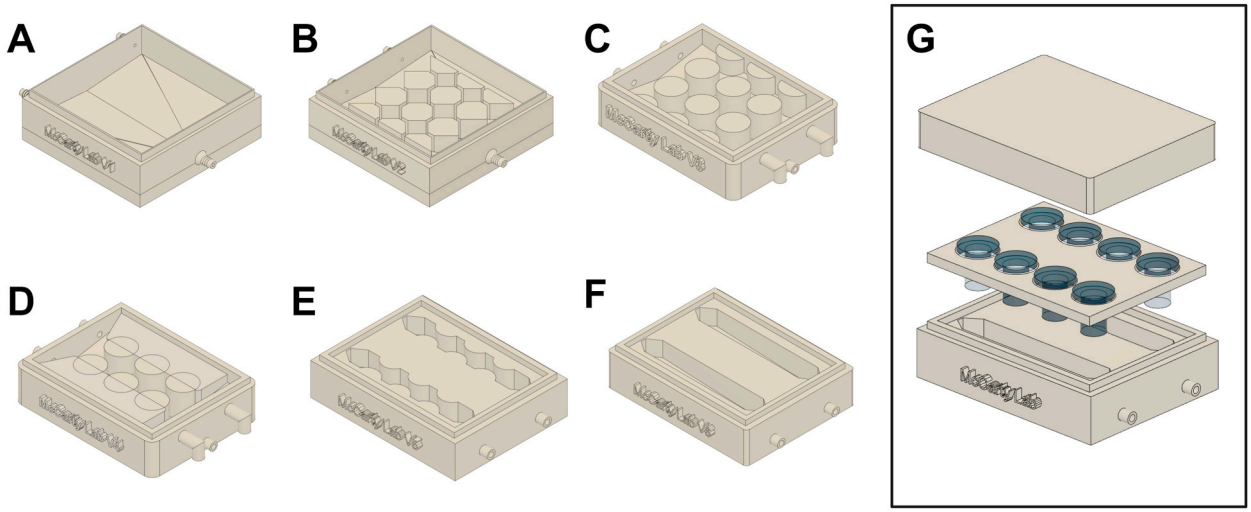

Fig. 3. Progression of the PACCS plate design. A) The initial design consisted of a large media reservoir with a slanted surface to drive fluid flow. This plate was able to accommodate 12 small Transwells, and it had two inputs and one output for media exchange. B) The second design incorporated octagon-shaped columns to reduce media consumption. C) The third design incorporated cylinder-shaped columns to aid with fluid exchange. This version also included a safety mechanism to prevent media overflow. D) The fourth design was able to accommodate 4 Transwells and incorporated large columns to the sides to minimize media consumption. E) The fifth design had two separated media reservoirs capable of accommodating 4 small Transwells each with their own inputs and outputs. F) The sixth design still consisted of two separated reservoirs, but columns were removed to aid with fluid exchange. G) Representation of the final design highlighting the main components of the PACCS plate: a lid (top), a tray capable of holding 8 small Transwells (middle), and the main body of the plate (created with BioRender).

fluid to flow (Fig. 3B). However, when this design was tested with mock solutions, media exchange was troublesome. The third plate design replaced the octagon-shaped columns with cylindrical pillars, increasing the fluid channel width to aid with fluid exchange. This design also incorporated overflow safety valves (Fig. 3C). The first three designs could hold twelve Transwells. However, all Transwells were exposed to the same media reservoir. Hence, WT and CF cells could not be cultured in the same plate. Thus, design 4 modified the main plate body to be able to hold only four Transwells, and it incorporated large side columns to reduce media consumption (Fig. 3D). The design required separate plates for WT and CF cells. To reduce the number of plates needed to run an

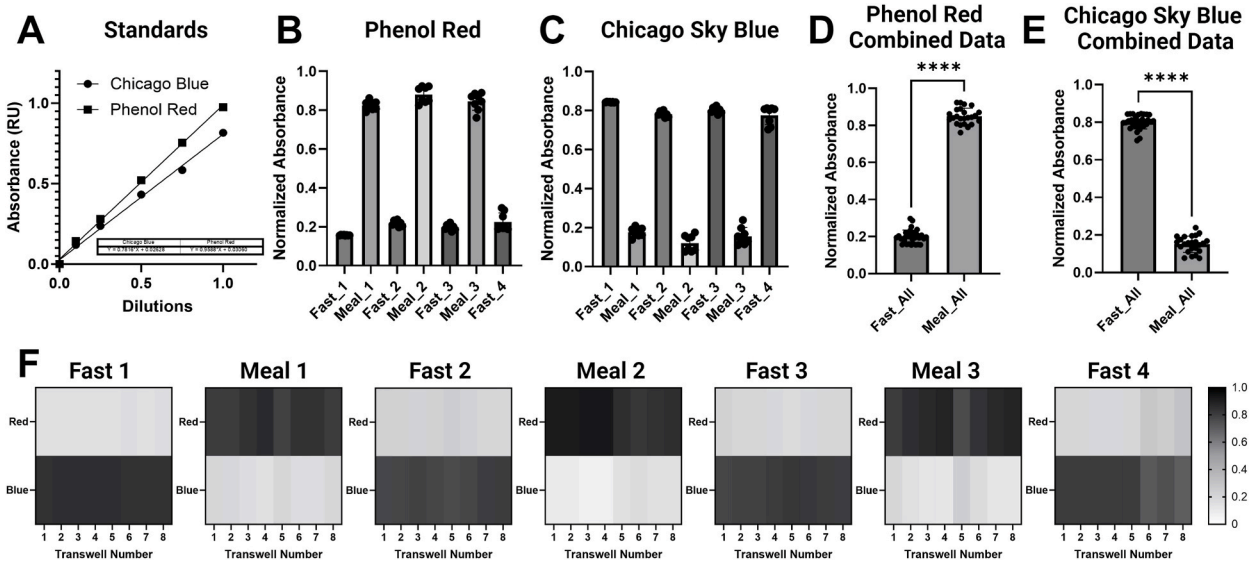

Fig. 4. Final design of the PACCS plate showed good solution exchange between fasting and meals. A) Standard curve for dyes used for fluid exchange tests: Phenol Red and Chicago Sky Blue. B) Phenol Red signal readings after each media change. C) Chicago Sky Blue signal readings after each media change. D) Summary of all Phenol Red data combined. E) Summary of all Chicago Sky Blue data combined. F) Heatmap showing the intensity readings at each time point for blue versus red signals. Data were normalized to the maximum absorbance intensity for the dye alone controls (n = 8, T-test, ****$p < 0.0001$) (created with BioRender).

experiment, design 5 had two separate media reservoirs in a single plate, each able to hold four Transwells (Fig. 3E). Since the media reservoirs are separate, WT and CF cells could now be cultured in the same plate. The safety valves were removed from the design, as they proved to be unnecessary in later models. However, this plate did not exhibit good fluid exchange. The final plate design removed cylindrical columns to optimize fluid flow (Fig. 3F). Each reservoir had its own input and output for fluid exchange. Upon testing with mock solutions, the final plate design showed good fluid exchange, and it was able to reduce media consumption requiring around 4 mL of media per reservoir. The graphical representation of the final PACCS plate design also shows the tray and lid components (Fig. 3G, Supplemental Fig. 2). This plate was used for further experiments.

3.3. *Quality control of the final PACCS plate design showed good media exchange from fasting to meal-like conditions*

PACCS was initially tested with mock solutions to check that its components were working correctly. Testing the system with mock solutions was also beneficial to assess fluid exchange on plate iterations. Mock solutions consisted of separate bottles containing either Phenol Red, to simulate a meal-like solution, or Chicago Sky Blue, to simulate a fast-like solution. The controller was used to create a program to mimic four fasting states and three meals, taking overall 24 min to run, simulating a whole day of meal-like media changes. The PACCS protocol used to run this experiment is shown in Supplemental Table 1. The program could be paused in-between media changes to collect samples from each Transwell location. Absorbance readings at 400 nm and 620 nm were collected to test red and blue dye abundance, respectively (Fig. 4). Standard curves showed that the absorbance of both dyes followed a linear trend, and that the data collected were within the linear range for absorbance (Fig. 4A). Phenol Red absorbance readings remained low during fasting and increased during meals (Fig. 4B). Chicago Sky Blue absorbances showed an opposite trend, remaining high during fasting and decreasing during meals (Fig. 4C). It is worth noting that the absorbance of either dye was never zero due to some spectral overlap between the two dyes. However, combining the data for all fasting and meal periods showed very significant differences in normalized absorbance, indicating good solution exchange between fasting and meals (Fig. 4D and E). Heatmaps graphing the normalized absorbance readings at each Transwell location show that fluid exchange is slightly different at each Transwell location (Fig. 4F). However, fluid exchange was sufficient to move forward and test PACCS with airway epithelial cells.

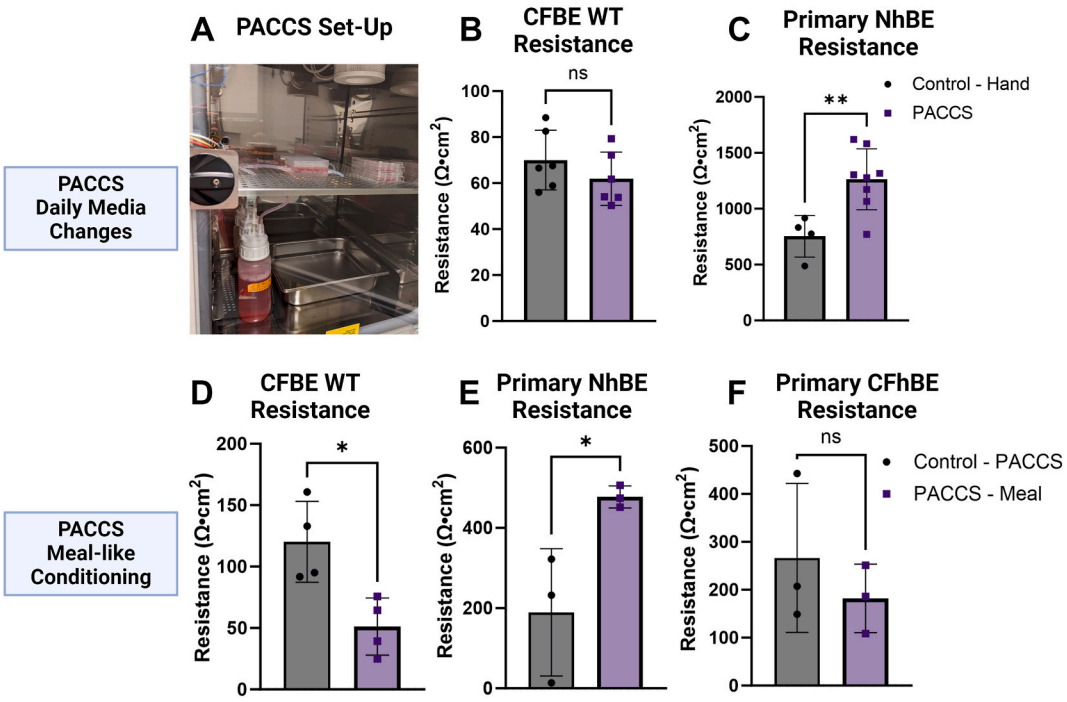

Fig. 5. Airway epithelial cells can be successfully cultured with PACCS using daily media changes or meal-like media changes. A) Representative image of PACCS components inside a cell culture incubator. Two media bottles can be seen connected to the 3D printed plate. Control plates (media changed by hand) shown on the incubator shelf at the right. B) Transepithelial electrical resistance (TEER) of CFBE WT and C) primary NhBE cells cultured with daily media changes via PACCS compared to control (daily manual media changes), using only media with 5.5 mM glucose. D) TEER of CFBE-WT, E) primary NhBE, and F) primary CFhBE cells cultured with meal-like media changes via PACCS (alternating between 5.5 mM and 17.5 mM glucose media) compared to PACCS control (media changes performed by PACCS at the same time points, but only receiving fresh 5.5 mM glucose media) (N = 3, T-test, *p < 0.05, **p < 0.01) (created with BioRender).

3.4. Immortalized cells and primary airway epithelial cells can be successfully cultured with PACCS

After initial testing of PACCS with mock solutions was completed, PACCS was used to culture airway epithelial cells. PACCS media bottles were stored inside the incubator, keeping the media at a constant temperature of 37 °C. Media bottles were connected to peristaltic pumps, which controlled media input into the 3D-printed plate. The PACCS plate was also connected to a vacuum waste container, with media suction out of the plate controlled by a pinch valve. These components fit inside a cell culture incubator as shown in Fig. 5A. Only the perfusion controller used to create and store the PACCS programs was stored outside of the incubator. Immortalized airway epithelial cells expressing WT-CFTR (CFBE-WT) were first used to test whether PACCS could be successfully used to culture airway epithelial cells. These cells were chosen because they can be grown at air-liquid interface, since only basolateral media can be changed using PACCS. Daily media changes were performed by PACCS or by hand (control) for 7 days, with all Transwells receiving only 5.5 mM glucose media during initial experiments. Results show that TEER was not significantly different between PACCS and control cultures (Fig. 5B). This result demonstrated that PACCS can be successfully used to culture airway epithelial cells, suggesting that using PACCS is not detrimental to cell health. We then moved forward to test whether primary airway epithelial cells expressing WT-CFTR (NhBEs) could be cultured with PACCS. Following a similar experimental approach, results show that NhBEs cultured with PACCS had greater TEER than NhBEs cultured by hand (Fig. 5C). The discrepancy seen between immortalized and primary cells might be due to primary cells being more sensitive to changes in temperature and carbon dioxide fluctuations that the cells experience when removed from the incubator for culture by traditional methods. We then tested both immortalized and primary cells with meal-like glucose fluctuation patterns using PACCS. The protocol used to run these experiment is shown in Supplemental Table 2. The control for this experiment was media changes performed by PACCS at the same time, but control plates only received fresh 5.5 mM glucose media at each solution change. TEER for CFBE-WT cells decreased (Fig. 5D), while TEER for NhBE cells was initially much higher and increased in response to meal-like glucose fluctuations compared to controls receiving fresh 5.5 mM glucose media (Fig. 5E). The difference in responses to PACCS meal-like glucose fluctuations between immortalized and primary cells might be due to differential expression of tight junction proteins in each model, and it highlights the importance of using primary cells when studying airway physiology. Primary cells expressing ΔF508-CFTR (CFhBEs) were also tested with meal-like glucose patterns. Results showed no significant differences in TEER in CFhBEs in response to meal-like glucose fluctuations compared to control (Fig. 5F). This result suggests that CFhBE lack the same adaptive response to glucose fluctuations as seen in NhBE cells, possibly due to CFTR dysfunction and dysregulation of tight junction proteins, and could begin to explain the mechanisms that play a role in CFRD airway pathophysiology. Overall, preliminary data shows that PACCS can be successfully used to culture airway epithelia, both immortalized and primary cells, and that exposure to meal-like glucose fluctuations leads to different responses in WT and CF cells.

4. Discussion

We developed a novel cell culture system, PACCS, that enables programed and automated media changes to culture airway epithelial cells plated on permeable Transwells. This is a valuable tool to study the pathophysiology of many diseases, such as CFRD. Patients with CFRD experience blood glucose fluctuations after meals. However, current cell culture protocols do not resemble CFRD physiology with respect to exposure to extracellular glucose levels. Thus, CFRD research is limited by the *in vitro* cell culture tools available to researchers. The system we have developed addresses this limitation by better mimicking blood glucose fluctuations. The system could also be used for other clinical applications, such as timed exposure to small molecules or other potential therapeutic agents.

To our knowledge, this is the first user-friendly, compact, and low-cost cell culture system of its kind. This system is an improvement from other existing devices in terms of being easily adaptable. Other groups have created automated cell culture systems to culture stem cells [21] and organoids [22]; however, their set-ups cannot be easily modified to culture airway epithelial cells on Transwells and simulate meal-like blood glucose fluctuations. One group was able to develop a miniaturized automated cell culture system for 96-well Transwells to culture airway epithelial cells [23]. However, their system is bulky and requires specialized equipment, such as robotic arms and a multi-plate media dispensing machine, both of which are not easily accessible by most research labs due to their high cost and complex operational requirements. In contrast, PACCS components are user-friendly, compact, and easily accessible. The PACCS plate has two separate media reservoirs, a tray that holds up to eight Transwells (with 0.33 cm^2 surface area), and a lid to protect from contamination. The inner surface of the plate reservoirs has a slight incline to drive fluid flow from the input to the outputs. This was a useful addition to our system that greatly improved fluid exchange, an improvement from commercially available plates with flat surfaces.

Even though PACCS offers many advantages over other existing devices, PACCS also has some limitations. Currently, the material used to 3D print the cell culture plate is not autoclavable. For future studies, we recommend using medical grade resin, such as BioMed Clear (FormLabs), which can be autoclaved for easy repeated use. Further, PACCS media consumption is high especially when running the meal-like glucose fluctuation protocol. It currently requires 4 mLs of media per reservoir per media change. This might be burdensome for labs that utilize cells that require expensive components in their media. To address this limitation, the plate can undergo further optimization to reduce media consumption.

To decrease media consumption while improving media exchange, the main body of the PACCS plate already underwent several design iterations. We found that having plain media reservoirs without columns/channels was best to optimize media exchange, even if it increased media consumption slightly. Further, we found that adding two separate reservoirs per plate was optimal to study two different genotypes (or treatments) in parallel without the possibility of cross-contamination between cell lines. After the plate design optimization was complete, the final PACCS plate iteration was used to culture airway epithelial cells. Both immortalized and primary

cells were used to test whether PACCS could be used to culture airway epithelial cells. The last plate iteration proved to have good fluid exchange and could be successfully used to culture both immortalized and primary airway epithelial cells with either daily media changes or meal-like glucose fluctuation media changes for up to a week.

When cells were tested with daily media changes, all at 5.5 mM glucose, there was no significant difference in CFBE TEER between the PACCS and control groups. However, NhBE cells cultured with PACCS had higher TEER compared to their control group. This observation was unexpected, but it provides further evidence of the utility of PACCS. We hypothesize that primary cells might be more sensitive to changes in temperature and carbon dioxide levels; thus, they might fare better when cultured with PACCS compared to manual media changes that include removal from the incubator. Further, discrepancies in results obtained when using immortalized cells versus primary cells highlight the need to always confirm findings in primary cells. Interestingly, NhBE cells increased their TEER in response to glucose fluctuations while CFhBE cells did not experience changes in TEER, indicating a lack of an adaptive response in CF primary cells challenged with meal-like glucose fluctuations.

Future studies will focus on using PACCS to condition immortalized and primary airway epithelial cells using CFRD-like glucose fluctuations. We will later test other endpoint measurements, besides TEER, that could help identify the mechanisms driving CFRD pathophysiology. Tight junction proteins, for example, play a role in barrier integrity and are believed to play a role in CFRD pathophysiology [24,25]. We will condition airway epithelial cells using PACCS and will then test gene expression changes of essential tight junction proteins, such as Claudins and Occludins, with the goal of identifying potential targets for therapeutic intervention. Overall, PACCS offers a novel and accessible platform to better mimic *in vivo* physiologically relevant conditions to study a range of diseases, such as CFRD.

5. Conclusions and future directions

We developed a programmable and automated cell culture system, PACCS, to better mimic physiologically relevant blood glucose fluctuations to study CFRD *in vitro*. This novel system has proven to be successful for cell culture, and it could be used for a wide range of applications. Future studies will focus on employing other quantitative techniques to measure differences in WT and CF airway epithelial monolayers in response to PACCS meal-like conditioning. Overall, understanding the pathophysiology of CFRD would allow us to develop therapies to combat this comorbidity, further improving quality of life and life expectancy for pwCF.

Data and code availability

Data and code included in the article/supplementary material is referenced in the article.

CRediT authorship contribution statement

Analia J. Vazquez Cegla: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Cameron Hedden:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Barry R. Imhoff:** Writing – review & editing, Data curation. **Guiying Cui:** Writing – review & editing, Investigation, Conceptualization. **Nael A. McCarty:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Research was supported by CF Foundation grants to N.A.M., numbers MCCART21G0 and MCCART22P0, and by the Marcus Professorship in Cystic Fibrosis, Emory University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37977.

References

[1] A. Granados, et al., Cystic fibrosis related diabetes: pathophysiology, screening and diagnosis, J. Cyst. Fibros. 18 (2019) S3–S9.
[2] H.A. Berger, et al., Identification and regulation of the cystic fibrosis transmembrane conductance regulator-generated chloride channel, J. Clin. Invest. 88 (1991) 1422–1431.
[3] J.S. Elborn, Cystic fibrosis, Lancet Lond. Engl. 388 (2016) 2519–2531.
[4] Cystic Fibrosis Foundation, Patient Registry Annual Report, 2022.
[5] M.C. Bierlaagh, D. Muilwijk, J.M. Beekman, C.K. van der Ent, A new era for people with cystic fibrosis, Eur. J. Pediatr. 180 (2021) 2731–2739.

[6] A.A. Stecenko, A. Moran, Update on cystic fibrosis-related diabetes, Curr. Opin. Pulm. Med. 16 (2010) 611–615.
[7] O. Breuer, D. Caudri, S. Stick, L. Turkovic, Predicting disease progression in cystic fibrosis, Expert Rev. Respir. Med. 12 (2018) 905–917.
[8] R.C. Boucher, An overview of the pathogenesis of cystic fibrosis lung disease, Adv. Drug Deliv. Rev. 54 (2002) 1359–1371.
[9] R. Kelsey, F.N. Manderson Koivula, N.H. McClenaghan, C. Kelly, Cystic fibrosis-related diabetes: pathophysiology and therapeutic challenges, Clin. Med. Insights Endocrinol. Diabetes 12 (2019) 1179551419851770.
[10] C.C.W. Hsia, P. Raskin, Lung function changes related to diabetes mellitus, Diabetes Technol. Ther. 9 (Suppl 1) (2007) S73–S82.
[11] 11965 - DMEM, high glucose - US. https://www.thermofisher.com/us/en/home/technical-resources/media-formulation.8.html.
[12] CDC, Diabetes Testing, Centers for Disease Control and Prevention, 2019. https://www.cdc.gov/diabetes/basics/getting-tested.html.
[13] H. Crow, C. Bengtson, X. Shi, L. Graves, A. Anabtawi, CGM patterns in adults with cystic fibrosis-related diabetes before and after elexacaftor-tezacaftor-ivacaftor therapy, J. Clin. Transl. Endocrinol. 30 (2022) 100307.
[14] K. Torimoto, et al., Glucose consumption of vascular cell types in culture: toward optimization of experimental conditions, Am. J. Physiol. Cell Physiol. 322 (2022) C73–C85.
[15] S. Winkler, J. Menke, K.V. Meyer, C. Kortmann, J. Bahnemann, Automation of cell culture assays using a 3D-printed servomotor-controlled microfluidic valve system, Lab Chip 22 (2022) 4656–4665.
[16] C.K. Byun, K. Abi-Samra, Y.-K. Cho, S. Takayama, Pumps for microfluidic cell culture, Electrophoresis 35 (2014) 245–257.
[17] D.S. Dkhar, R. Kumari, S.J. Malode, N.P. Shetti, P. Chandra, Integrated lab-on-a-chip devices: fabrication methodologies, transduction system for sensing purposes, J. Pharm. Biomed. Anal. 223 (2023) 115120.
[18] R. Morgan, C. Manfredi, K.F. Easley, L.D. Watkins, W.R. Hunt, S.L. Goudy, E.J. Sorscher, M. Koval, S.A. Molina, A Medium Composition Containing Normal Resting Glucose That Supports Differentiation of Primary Human Airway Cells, Sci Rep 12 (1) (2022) 1540. https://doi.org/10.1038/s41598-022-05446-x.
[19] G. Cui, et al., Chronic hyperglycemia aggravates lung function in a Scnn1b-Tg murine model, Am. J. Physiol. Lung Cell Mol. Physiol. (2024), https://doi.org/10.1152/ajplung.00279.2023.
[20] K.A. Cottrill, et al., Sphingomyelinase decreases transepithelial anion secretion in airway epithelial cells in part by inhibiting CFTR-mediated apical conductance, Phys. Rep. 9 (2021) e14928.
[21] A. Ohta, et al., Automated cell culture system for the production of cell aggregates with growth plate-like structure from induced pluripotent stem cells, SLAS Technol. 28 (2023) 433–441.
[22] S.T. Seiler, et al., Modular automated microfluidic cell culture platform reduces glycolytic stress in cerebral cortex organoids, Sci. Rep. 12 (2022) 20173.
[23] T. Bluhmki, et al., Development of a miniaturized 96-Transwell air–liquid interface human small airway epithelial model, Sci. Rep. 10 (2020) 13022.
[24] M. Koval, Claudin heterogeneity and control of lung tight junctions, Annu. Rev. Physiol. 75 (2013) 551–567.
[25] B. Schlingmann, S.A. Molina, M. Koval, Claudins: gatekeepers of lung epithelial function, Semin. Cell Dev. Biol. 42 (2015) 47–57.