Recapitulation of Particulate matter Effected Human Alveolus on Chip Inflammatory Responses

Faiza Jabbar
Jeju National University
Young Su Kim
Jeju National University
Afaque Manzoor Soomro
Jeju National University
Arun Asif
Jeju National University
Young Jae Cho
Jeju National University
Kyung-Hyun Choi (amm@jejunu.ac.kr)
Jeju National University Department of Law https://orcid.org/0000-0002-8988-4839
Lee Sang Ho
Bio PD Office of Strategic R&D Planning

Research

Keywords: COPD, TEER, ELISA, Real time monitoring, IL-13, IL-6, pH sensor, In-house built microscope, Particulate matter

Posted Date: January 29th, 2020

DOI: https://doi.org/10.21203/rs.2.22193/v1

Abstract

Background Particulate matter (PM10) pollution around the globe is a major risk factor for inducing respiratory diseases, How-ever such environmental conditions are a challenge to study in trans-well culture system. Micro-physiological system offers potential for mimicking these phenomena for better understanding of possible hazards to human respiratory health. In this study we are introducing an efficient alveolus on a chip for reconstituting this environmental condition in a microfluidic device by in house built TEER sensor, system embedded pH sensor and portable microscope for continuous monitoring of the alveolus on chip.

Results Three environmental conditions with respect to particulate matter in air Moderate 7.5 µg/ml , Poor 37.5 µg/ml and very poor 151 µg/ml of particulate matter exposure was executed in our microfluidic system. Our study provides physiological and toxicological data of the stimulated environmental condition by inflammatory markers of respiratory disease which leads to the identification of asthma and COPD, our claims were validated by confocal microscopy and ELISA. Significant increase in IL-13, IL-6 and Mucin advocated the incidence of asthma and COPD like conditions in our organ on chip.

Conclusion This study will lead to identification of potential therapeutics to study physiological and pathological conditions and will help in preventing chronic life-threatening toxicities. This device has provided the preliminary information for making this breakthrough in organ-on-chip technology. In future his device would help to make complex tissue mimetics of human for toxicity testing and drug discovery with system integrated pathological sensors for replacing time consuming molecular analysis of pathological conditions.

Background

Particulate matter have become a major concern owing to their increased level in air pollution correlating with higher respiratory diseases in metropolitan cities of the world, Human health is a challenge due to rapid advancement in cities and urbanization due to emission of hazardous gases from engines of heavy vehicles and burning of fossil fuels and mining (1). Coal burning power plants emit organic pollutants including polycyclic aromatic hydrocarbons, benzene, toluene and multitude of different gases. Weathering of soil turns clay particles into mineral dust composed of huge number of oxides and carbonates of metals. Dust storms in spring season carry the dust particles Flinches in Mongolia and Siberia moves dust particles to low-pressure areas such as eastern China, Korea, and Japan by south eastern to northwestern dust storm. Particulate matter stimulates clinical manifestations according to epidemiology and toxicology studies and contribute in developing lethal diseases such as respiratory disease cardiovascular disease, and mortality at an early age(3). Human lungs are the first organ to face airborne environmental stresses such as pollutants, toxicants and pathogens as they are involved in gaseous exchange (4). Environmental stress stimulants (particulate matter, cigarettes smoke and aerosols) are believed to be involved in the development of asthma, chronic obstructive pulmonary disease (COPD) and interstitial lung diseases (5). Particulate matter induces release of inflammatory and

allergic responsive cytokines in human airways effecting bronchiolar and alveolar passages, experimentally controlled human exposure studies suggest that interleukin-6 and tumor necrosis factor alpha (TNF-α) are key mediators of airway inflammation (6-8). Allergic airways release proinflammatory cytokines and cause Airway hyperresponsiveness (AHR). The immune response to allergen exposure in asthma is associated with TH2 inflammation and IL-13 is a key cytokine involved in directing allergen-induced airway inflammation and remodeling (9). Regrettably animal models of asthma and allergy have failed to offer therapeutics with high efficacy, so human lung physiological mimetics are required for thorough understanding of toxicological disease analysis because animal models of inhaled toxicants and pollutants express different molecular markers from that are being produced in human subsequently resulting data usually lead to erroneous therapeutic development (10). Animal models need to be replaced by physiologically relevant human mimetics therefore considerable advancements have been made in development of the substitutes of organs and tissues to study such stimulation based studies (11) Organ on chip technology helps in revealing physiologically relevant organs by providing technologically controlled biochemical, mechanical and dynamic fluid flow conditions for recapitulating cellular microenvironment to access diseased and stressed conditions (12) .

Many *in vitro* lung-on-a-chip models have been introduced in the past to study impacts of toxins under dynamic flow conditions almost all the previous models are PDMS based. PDMS absorbs hydrophobic biomolecules so PDMS based models fail to provide complete information of molecular markers and cytokine analysis in the culture medium, absence of real time monitoring or probe based off line monitored sample analysis provide vague information of stress response. To study acute or chronic stress response of the cells, real time microscopy is required to analyze the morphological changes in normal cells which is lacked by previous microfluidic models. Trans-epithelial electrical resistance (TEER) measurement is a rapid and conservative technique for the determination of integrity and differentiation of epithelial monolayers trans-well cultures because the electrical impedance across an epithelium is interrelated to the vigorous construction of tight junctions with neighboring cells (13). Cell monolayer integrity measurement by TEER has become a standard technique in trans-well culture system, technically TEER measurement of organ-on-chips is challenging because there is no valid and practical approach available. Micrometer sized closed microfluidic channels create hindrance in TEER measurements in organ-on-chips to contact epithelium. Therefore, measurement of permeability changes incessantly using TEER in microfluidic is almost impossible (14) . Printed TEER electrodes integrated into PDMS based microfluidic devices have been developed but only a few have been used to monitor in situ within organ chips epithelial barrier function (15). Conventional metal patterning techniques have been used in organ on chips microfluidic culture devices for construction of cell culture chambers around electrodes or integrating glass or polymeric substances (16-18). Previous studies with cells cultured in organ chips provide large variability in measurement, low sensitivity and they are affected by non-uniform cell cultures. TEER electrode location also significantly change in TEER values in these cultures, mathematical models can help to reduce these variations(14, 18, 19) Therefore successful fabrication of a robust on-chip TEER sensing capability enabled us to measure barrier function electrochemically., this

method measures electrical impedance it can also help to study other behaviors of the cells on a microfluidic chip such as cell proliferation, migration, ion channel activity, tissue conductivity and dissolved gases. pH sensors have been recently reported for real time monitoring by fabricating PDMS based organ chips with fully integrated electrodes. Embedded sensor in organ on chip platform are stringently required for future research applications for example many sensors such as strain sensor, humidity sensor for health monitoring have been developed for implantable applications (20, 21).

In an effort to address these problems, this study was designed to introduce human lung- alveolus-on-a-chip model with dynamic fluid flow conditions and multitasking sophisticated microfluidic platform with on chip printed ITO based TEER and pH sensors equipped with in-house built microscope for real time monitoring. Glass chip of size- with top and bottom channel printed with microfabrication techniques, TEER sensor is printed on the channel directly in contact with human Lung pulmonary alveolar epithelial type-I and type-II cells for continuously monitoring trans epithelial electrical resistance after every half an hour till end of the experiment. Impedance values provide complete information of monolayer membrane integrity in control chip with flow rate of 80µl/min and shear stress of 12dyne/cm^3, four chips were introduced to our organ on chip platform for physiological and mechanically controlled growing conditions. Particulate matter exposure was introduced after four days of stable culture on chip for eight hours daily for consecutive four days with three different concentrations to mimicking routine exposure to humans in daily life. Cell monolayer consisting of Human pulmonary alveolar type-I and type-II primary cells exhibited functions of properly differentiated epithelial cells. AT-I mucus secreting lung goblet cell and AT-II ciliated cells were confirmed by confocal microcopy. Particulate matter exposure induced inflammatory response in Human pulmonary alveolar type-I and type-II cells right after 12 hours showed sudden drop in impedance values correlates with cytokine secretion analysis and confocal microscopy ROS level also complements the dose dependent oxidative damage to the monolayer permeability.

Methods

Materials:

Particulate matter particles PM10 reference material organic components ERM CZ-100 and inorganic ERM CZ-120 were purchased from European Joint research commission JRC were characterized for their size with scanning electron microscopy (MIRA-3 TESCAN).Three concentrations of both materials were prepared according to guidelines provided by daily update of climate control department South Korea, According to these guidelines moderate, poor and very poor conditions are available Following these

concentrations particulate matter doses for exposure were determined for analysis. Stock solutions of particulate matter material were prepared in Class II biological safety cabinet to ensure the endotoxin free and sterile environment, 151*µg/ml* of particulate matter material was suspended in alveolar epithelial complete growth medium and was sonicated in probe sonicator for 60 minutes for disagglomeration, Further diluted for two less concentrated particulate matter solutions by adding more media with resulting concentrations of 37.5*µg/ml* and 7.5*µg/ml* respectively. Particulate matter was kept at 4°C either diluted or in original form.

Chip fabrication and sensor's development

Chip is made up of combination of two soda lime glasses each 1.1mm thick, 41 mm wide and 56mm long. Channel for cell culturing was printed on the bottom glass of the chip 15mm channel area was allocated for cell culture compartment. To launch a TEER sensor, indium tin oxide (ITO) coating was printed to make a square electrode of 4 mm length with a thickness of 500 nm. Screen printing technique was used for printing ITO 112 on the glass. 3D inkjet printer was used to print the pattern of silicon elastomer (Musil 113 medical grade silicon MED-6033) (900 nm width) to create channel and cell culture chamber on the chip. Top and bottom chip glasses were fixed in a chip holder at a place where ITO electrodes are exactly crossing each other to complete circuit for measuring electrical resistance. One ITO based TEER electrode is $4mm^{2.}$ The impedance was measured in Ohms (Ωmm^2). LabVIEW based software was used to monitor the TEER data from the chip and the connectors were coupled to the ITO electrodes.

A120 portable microscope was developed for real time monitoring of cell growth on transparent ITO based TEER sensor printed chip, A commercially available Plan Achromatic Objective 121 (AmScope TM) with 10X magnification power, a white LED for light source, and a SCMOS series 122 USB2.0 eyepiece camera (ToupTek TM) were assembled in a 3D printed assembly. A blue wavelength 469±17.5nm filter was used. A camera control software was used for high speed visualization of the images and video processing ToupView (ToupTek TM).

pH sensor was developed for real-time pH monitoring of media. A white LED was inserted in a 3D printed assembly with a photodiode and an optical filter. The pH measurement was calculated on the principle of change in light intensity. A media carrying biocompatible microfluidic, extremely transparent, tube was passed through the sensor. Sensor was programmed to measure the minor discrepancies in color of media with changes in phenol red color due to the acidification of media with time. An Arduino

microcontroller was used to quantify an optical signal. To calibrate and characterize the optical pH sensor standard pH media samples ranging from 6.0 to 8.0 were used , calibrations of real time microscope, pH and TEER sensors have mentioned in our previous paper (22). A peristaltic pump was connected to the chip for constant media circulation to mimic dynamic conditions. The shear stress on the cell monolayer induced by media was calibrated to mimic the alveolar environment. The fluid shear stress in in-vivo human lung physiology has been characterized as 8 dyne/cm2 (2). The media flow rate was regulated at 80µl/ml to maintain the 8 dyne/cm2 shear stress upon the monolayer of Alveolar epithelial cells. The media shear stress induced in the microchannel of the designed chip was calculated by the use of following equation (23);

$\tau = 6\mu Q / (wh^2)$

In this equation, µ represents the viscosity of the media, Q represents the media flow rate, w represents the width and h represents the height of the microfluidic channel.

Optical pH sensor was attached to measure real-time pH before the media inlet. Connectors were immobilized upon glass chip with epoxy raisin through which tubing was connected to chip for the media circulation controlled by peristaltic pump.

Microfluidic cell culture maintenance

Human pulmonary alveolar type-I and type-II cells (Cat#3200) were purchased from science cell and revived according to manufacturer's protocol, Poly-L lysine (Sigma Aldrich) was coated on T-25 flask (Corning) at concentration of 2µg/cm^3 incubated at 37°C overnight and flask was rinsed before adding culture medium containing Alveolar epithelial cells medium (AEpiCM, Cat. #3201) minimal eagle medium, Epithelial growth supplement(5ml), 10% FBS ,1%pencilin streptomycin solution, with 5% carbon dioxide at 37°C. Before seeding cells on chip bottom glass was sterilized for 1 hour in UV and ECM Collagen solution type-I from Rat tail (Sigma Cat # C3867) was coated at concentration of 10µg by using 0.01% solution in DPBS overnight. At reaching confluency <90 % at passage 2 Human pulmonary alveolar type-I and type-II cells at density of 3.27×10^5 were seeded on to the chip initially cells were allowed to adhere to the ECM coated channel of the chip for 4 hours then chip was introduced to the microfluidic platform for continuous circulation of media with help of peristaltic pump at 120µl/hour, TEER, pH sensors and microscope for real time monitoring were attached to the chip after assembling the chip components

after 12 hours media flow was increased to 80µl/min, after 48 hours fresh media was added to media reservoir.

Exposure scheme of particulate matter:

After 4 days of stable culture of Human pulmonary alveolar type-I and type-II cells on chip evaluated by impedance values of the TEER sensor, pH sensor and Real time microscope 3 chips representing moderate (7.5*µg/ml*), poor(37.5*µg/ml*) and very poor(151*µg/ml*) conditions of particulate matter were allowed to exposed to particulate matter containing culture medium, for consecutive 8 hours daily for 5 days, to mimic daily exposure of particulate matter to human lung, was then exchanged with normal medium after exposure. All the chips were kept in same culturing conditions except different concentrations of particulate matter excluding control chip Fig. 2a.

Determination of Membrane barrier integrity:

Epithelial cell tight junctions' integrity was characterized by impedance values provided by in-house developed TEER sensor for Real time monitoring. Alveolar type I-II cells were stained for immunofluorescence analysis by staining for tight junction's epithelial cell marker occludin conjugated Alexa flour 594 (Sigma (OC-3F10) For immunostaining cell were washed with PBS for 5 times and fixed with 4% paraformaldehyde for 15 minutes permeabilized with 0.2% triton X-100(Sigma) for 20 minutes and block with 5% BSA in PBS for 1 hour at room temperature and incubated for 4 hours with Occludin conjugated Alexa flour 594(1:200) after incubation viewed under confocal laser scanning microscope(24).

Immunofluorescence Analysis of compromised barrier integrity:

Alveolar type I-II goblet mucus producing epithelium and ciliated cells were stained for immunocytochemical analysis cells were washed with PBS and fixed with 4% paraformaldehyde

for 15 minutes washed with PBST for 3 time and permeabilized with 0.1% triton 100 -X for 20 minutes at room temperature and block with 1 % BSA for 30 min at room temperature followed by MUC5AC Monoclonal Antibody (45M1)1:1000 and Anti-β tubulin IV (ab11315) abcam (1:500) antibodies and secondary Alexa flour Goat anti mouse (H+L) 488 Green(A28175) and Goat anti-rabbit 555(H+L) (A-

21428) red respectively for proper morphological analysis and images were taken under laser scanning confocal microscope(Zeiss Germany) .

DCFDA assay for ROS analysis

As a consequence of cell damage and functional impairment activated defense mechanism release production of intracellular Reactive Oxygen Species (ROS) and anti-oxidant species, pro-and anti-inflammatory cytokines, and induce genotoxicity (25-27). ROS are responsible for mitochondrial damage and for making cellular environment more acidic environment which leads to the incidence of chronic inflammation and cancer long term exposure to the xenobiotic and air pollutants are responsible of many respiratory diseases such as COPD and asthma. For ROS evaluation on exposure to particulate matter we have analyzed three concentrations of particulate matter after four days of exposure along with normal healthy control and All the chips were washed with PBS and the cells were incubated at 37 °C, in 5 % CO2 with 50µM of a 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) (Sigma D6883) probe in PBS and incubated for half an hour. After incubation the chips were washed with PBS and incubated for 5 min in 90 % Dimethyl Sulfoxide (DMSO) (Sigma-Aldrich, D2438) in PBS. Cell were washed and then visualized under laser scanning confocal microscope.

Analysis of cytokines release and ROS representing diseased conditions

Our particulate matter model on chip mimicking in-vivo diseased conditions was characterized by complete information of cytokine signaling. Neutrophil uptake increases on the onset of inflammation in airway cells so here is the list of cytokines produced as consequence of allergy and asthma TNF-α, IL-6, IL-13, Mucin and Reactive oxygen species(ROS) detection is the indicator of incidence of asthma due to prolonged exposure of particulate matter to airway cells, Presence of IL-13 provide data about the incidence of COPD, TNF alpha , for oxidative stress and ROS production leading to apoptosis which ultimately cause cancer in the respective tissues. Mucus metaplasia and mucus hypersecretion in human lung airway are pathological changes which occurs on the onset of a severe respiratory disease asthma. These are associated with the $CD4^+$ Th2 type of immune response activation in the lung. This immune response is evaluated by by IL-13 secretion, with minimal production of the Th1 type of cytokines (e.g. IFNγ)(28). The destiny of effector $CD4^+$ T cells is decided by the cytokine environment, which is one of the most important factors, so that the local immune response is affected by cytokines production by lung physical foundations. Although, lung epithelial cells are not part of the immune system but also contribute to the type of immune response by secreting explicit cytokines. One of the cytokines that is

produced by lung epithelial cells is IL-6 (29, 30) IL -6 is the cytokine that is produced by lung epithelial cells and asthmatic patients data provide relatively increased secretion of IL-6 as compared to healthy controls (31),(32).In response to PM exposure IL-6 and tumor-necrosis-factor alpha (TNF-α) key intermediaries of the inflammatory response of lung epithelial cells in many in-vivo, in-vitro and human sample based studies(6-8).

In current study TNF-α, IL-13, IL-6 and Mucin were selected as biomarkers for asthma and COPD all the cytokines were detected in media samples collected after every 24 hours from all control and experimental media reservoirs, TNF-α, IL-13, IL-6 ELISA was performed by ELISA kits by standard protocol of manufacturer and ELISA for mucin was performed by sandwich method(33).

Results

Characterization of Particulate matter

Particulate matter (PM-10) include organic and inorganic components named ERM-CZ 100 and ERM-CZ120, purchased from European joint research commission Mainly contain PAH's, Dioxins, PCB's and Zinc, cadmium, mercury, cobalt, SiO2 respectively, particle size of Particulate matter is less than 10μm and characterized by Scanning electron microscope. Particle size detected in under scanning electron microscopy was less than 10μm Shown in fig. 3a.

Impedance data demonstrating barrier integrity:

According to impedance data of our TEER sensor, values in positive control chip started to increase gradually with increasing barrier junctions and increasing confluency of the cells on chip recorded after half an hour interval throughout the experiment. Impedance data demonstrated that the epithelial cells junctions increase with increasing confluency and stable culture for four days at fully confluent monolayer value of impedance was recorded 930Ωcm^2 average impedance values in previous studies of lung epithelial cells are 800-980 Ωcm^2 *Huh et al.,* 2010. Our lung on chip is providing more accurate data as chip is already printed with ITO based TEER sensor which is directly in contact with cell surface,

previously some studies reports TEER values in trans-well culture embedded sensor approach is first time implemented in a microfluidic platform shown in fig. 4a.

pH sensor data presenting comparison of control and treated chips

Our in-house built pH sensor measure pH starting from the day 1 of the experiment and provide data with minor fluctuations of the pH values Normal pH of the media was stabilized at 7.4 which is recapitulating human blood pH, media pH was showing a little drop when media was refreshed after 48 hours and media pH was dropped down in more acidic environment(22).In particulate matter treated chips minimum pH was recorded around 6.7 as shown in fig. 4b. Graph representing real time monitoring of media pH in fig. 4 b orange is showing control chip pH values stabilized at 7.4 as same as pH of human blood followed by a little drop after 48 hours and then stabilized in positive control chip treated with media only. Blue is presented as particulate matter concentration 7.5µg//ml showing less acidic environment as compared to green and red 37.5µg//ml and 151µg//ml respectively.

Monolayer Membrane integrity and differentiated epithelium

Immunofluorescence analysis by confocal microscopy revealed that this platform has provided ideal growth environment by making complete monolayer of Alveolar epithelial cells type I-II predominantly describing the formation of pseudostratified epithelium with goblet mucus producing and ciliated cells. characterized by epithelial cells marker and morphology specific markers MUC5AC for goblet cells and Antiβ tubulin IV for ciliated cells demonstrated in Fig 3 c. Tight junction's morphology of Alveolar epithelial cells type I-II was evaluated by staining the cells on microfluidic chip with Occludin conjugated Alex flour 594 (Sigma) can be visualize in Fig 3 b. Confocal images provide data that truly complements with impedance values of TEER sensor that complete monolayer integrity and differentiated morphology of Alveolar epithelium was achieved in our microfluidic platform.

Compromised barrier integrity upon particulate matter exposure

Particulate matter exposure at high concentrations significantly disrupt the barrier functions as confocal micrographs are showing in the Fig 5. At relatively low concentration of particulate matter epithelial cell junction marker expressed nearly in control chip but at high concentration of 37.5*µg/ml* and151*µg/ml*

barrier permeability was lost can be seen in Fig 5 b-d, which was also confirmed by TEER and cytokine analysis measurement.

Confocal imaging of Goblet cell hyperplasia and ciliated cells diminishing

Polymeric mucin MUC5AC is a low charge glycoform of MUC2 is major secretary protein in patients with chronic respiratory disease asthma, Mucins hypersecretion due to chronic inflammation results in airway diseases such as chronic obstructive pulmonary disease, and cystic fibrosis (CF). (34, 35) Goblet cell hyperplasia occurs when ciliated cells become unable to maintain airway homeostasis by shutting down mucociliary clearance and macrophage activation and this loss leads to chronic pulmonary disease Fig. 6 b-d. Results demonstrated that expression of mucin (MUC5AC) increased with increasing concentration of particulate matter. High mucin production and loss of mucociliary clearance is rational phenomena. Our results of control and particulate matter treated chips showed a dose dependent increase in mucin production and provide proof of incidence of asthma, COPD and hyperresponsiveness of airway whereas relatively decreased expression of anti-β tubulin IV with increasing concentration of particulate matter which provide proof of loss of mucociliary clearance and decreased cilia beating frequency shown in fig 6 h, which is the most important homeostatic function of alveolar epithelial goblet and ciliated cells.

ROS estimation

Cellular injury occurs in lung cells when exposed to particulate matter (PM) (36). Cr, Co, Ni, Mn, Zn, Cu, and, Fe are the most commonly found elements in airborne PM (37). Fe is designated as profoundly associated with the manufacture of oxidative stress and enabling superoxide anions ($O2^{-}$) and hydrogen peroxide (H_2O_2) alteration to hydroxyl ions (OH^{-}) (38). PM exposure results in impaired pulmonary function and surfactant dysfunction (39) inflammatory response leads to damage of epithelial cells, increased vascular permeability. PM includes PAH particularly benzo[a]anthracene, benzo[b] fluoranthene, benzo[k]fluoranthene, and benzo[a] pyrene, are potential carcinogenic risk, in accordance with IARC (40). The presence of PAH in PM is related to triggering of inflammation of alveolar epithelial and macrophages, ROS generation and lipid peroxidation (41) In this study DCFDA assay was performed for ROS measurement at the end of the experiment, In control chip healthy alive monolayer, level of ROS is very low as compared to the particulate matter treated chips and there is a relative increase in the alveolar epithelial monolayer chips with increasing concentration of particulate matter induced ROS data is presented in the fig 7.

Cytokine release analysis

IL-13 has a critical role in development of asthma (42). IL-13 directly affect human airway epithelium and involved in inflammation of respiratory tract, respiratory tract hyperresponsiveness, goblet cell hyperplasia and mucus hypersecretion eventually subepithelial fibrosis occurs as a consequence of airway inflammation similar to that observed in airway mucosa of the individuals with asthma (43, 44). In our experiment concentration of IL-13 found highest in media samples at 24 hours after exposure and the maximum concentration was observed in samples of particulate matter concentration 151µg//ml shown in fig 8 b. Whereas, at low particulate matter concentration of 7.5µg/ml interleukin IL-13 concentration was high throughout the experiment. Our data present highly significant values at 24 hour of particulate matter exposure and significant at 72 hours of particulate matter exposure. IL-13 release in cell culture media was analyzed after 12h of particulate matter exposure, bar graph showing significant value at 24h, IL-13 was highest and later on tends to decreased with time until 96h in fig 8a.

IL-6 is a cytokine has been considered as biomarker of inflammation rather than a regulatory cytokine with ability to modulate immune responses (45). In response to allergen stimulation to lung airway, IL-6 is released from lung epithelial cells as immune response.(30) Recently IL-6 is involved in adaptive immune response in the differentiation of effector CD4+T cells, Particularly it has function in suppressing Th1 and thus induce TH-2 differentiation of CD4+T cells via independent cytokine regulatory pathways (46). Many studies have provided the data for generation of IL-16 in combination with TGF- β for promotion of murine Th17 cells where as some studies relate it with IL-1β to promote Th-17(47) (48). Interleukin-16 showed damage dependent release from lung alveolar cells, IL-6 in media samples increased with time at higher concentration of damage inducing stimulus particulate matter. Maximum concentration of particulate matter was detected after 3 days of exposure and later it tends to decrease. Pattern of cytokines release in our Alveolar epithelial cell provide the significant data about inflammation of cells by particulate matter stress in acute conditions at 24 and 48 hours after exposure shown in fig 8 a. TNF-α is a cytokine and well-studied biomarker of PM stressed conditions in human and secreted as immune response, it is secreted by epithelial cells after ROS generation in mitochondria and leads to NF-Kappa-B pathway. TNF-α concentration was measured highest on increasing stress signal graph showed that concentration of this cytokine increased only at high concentration of particulate matter exposure, whereas lower concentration release of TNF-α is comparatively low this data presented significant value of the concentration after 24 and 72 hours of particulate matter exposure shown in fig. 8 d. Mucin is a secretory glycoprotein which is a potential biomarker observed in bronchioalveolar lavage and sputum samples of asthmatic patients, Mucin levels gradually increase in our particulate matter treated chips with increasing concentration of particulate matter. Mucin release in cell culture media was also noted as dose and time dependent because highly oxidative environment is the cause of bronchoconstriction and decreased mucociliary clearance. After 2 days of particulate matter exposure at high concentration leads to release of 400pg/ml mucin.

Discussion

Here in this study, we have established an efficient, and robust organ on chip system for mechanical, chemical and environmental toxicological analysis. Our organ on chip system is unique owing to its capacity to address previously reported limitations of the organs on chip we have introduced a reconfigurable glass based chip with transparent printed TEER electrode, pH sensor embedded in the system and real time microscope for real time analysis of different environmental conditions, PM pollution around the globe is responsible for causing fatal respiratory diseases i.e., asthma, COPD, and interstitial lung disease. Lack of appropriate models to study such real time conditions results in failure of effective and prompt drug development for the treatment of these harmful diseases, In past many studies have introduced their organ on chip devices for recapitulating real time phenomenon nevertheless there have been limitation such as embedded sensor and PDMS based chips (24, 49) In current attempt we're introducing our model with monolayer primary cell culture on chip, is an initial validation of our platform, sensors and microscope. We have tried to recapitulate environmental condition FD induced possible diseased conditions which is validated by presence of cytokines IL-13, TNF-α, IL-6 and mucin.

In order to achieve this goal, we select three levels of PM provided by daily climate control conditions of the country, Good, bad and worst and followed the exposure scheme that one individual can experience in a week. First of all, we characterized our monolayer chip permeability by TEER and confocal microscopy. Later we introduced PM stimulus to induce diseased conditions in our chip with three different concentrations for 5 days of daily exposure of 8 hours. For toxicity analysis media samples were collected from 12 hours after first treatment and then after every 24 hours, Impedance values were providing every change in the permeability of epithelium right after every half an hour. TEER, pH and microscope results were recorded automatically by software installed in computer attached to the organ on chip system.

Data from TEER software demonstrate that barrier permeability increased up to 80% for first four days in all chips, whereas in PM treated chips barrier permeability was started compromising after 12 hours of exposure, Cytokines analysis of the selected biomarkers followed the regular pattern of increasing toxicity in a dose dependent manner.

Immunofluorescence analysis of the control and PM treated chips provide proof of normal and diseased conditions. Expression of Occludin for permeability of tights junctions in control chips and in PM treated chip showed huge difference and pointed out the inflammatory conditions and morphology specific biomarkers such as Mucin and anti-β tubulin IV give the whole picture of the experiment. Mucin enriched alveolar epithelium is a known characteristic of asthmatic patients and because ciliated cells functions halt due and mucociliary movements almost shuts down as a consequence of pulmonary stress.IL-13 is the cytokines which promotes the mucin production by increasing eosinophils recruitment, in our ELISA for IL-13 PM induce increased production of this cytokine after 24 hours and the IL-13 release was significantly high in worst conditions of particulate matter.

Release of mucin is linked to IL-13, Increase in concentration of mucin release from alveolar cells is proof of defective function of the ciliated cells which are involved in the mucociliary clearance Mucin ELISA

graph is augmenting the confocal micrographs and validate our platform for recapitulating human physiology at alveolar level by showing increasing concentration of Mucin at high concentrations of particulate matter and for long time exposure, such biomarker could provide incidence of many diseases in our automated and sophisticated organ on chip platform. IL-6 is a proinflammatory cytokine produced in asthma and COPD conditions and is related to acute impaired lung function(50). In our data IL-6 concentration showed gradual increase in the production of this cytokines and this is associated with evidence of asthma patients sputum samples as published by a study(51) TNF- α is expressed by increasing ROS production in airway epithelium, Oxidative stress and free radical production occurs as inflammatory response of associated to PM exposure reported previously in many studies (52–54) Several evidences have provided information of validity of our platform for successful toxicological analysis and reconstituting organ pathophysiology, future target of our group is to study the complex tissue physiology with integration of embedded sensors. Graph representing mucin levels in Fig. 8c showing increase in mucin release in supernatant is also comparable with confocal micrographs.

Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM). For statistical analysis, the experimental data was compared to their controls. Using full-factorial ANOVA with Tukey's multiple comparison. All statistical analysis was performed by Microsoft Excel. A p value of < 0.05 was considered to be significant (*) and < 0.01 as highly significant (**). All the experiments were performed in triplicates.

Conclusion

Technological advancements in organ on chip technology is offering robust and efficient solutions for drug discovery and therapeutic evaluation. These solutions are in demand now a days. Our microfluidic alveoli on chip device have potential to mimic normal human physiology as well as environmental and pathological conditions. According to the results obtained from this study, have validated that this well-equipped system with reconfigurable microchip, portable microscope for Real time monitoring of cells, system integrated sensors pH and TEER sensors would help in the development of better human physiological and pathological mimetics. High metabolic activity of the alveolar epithelial cells was analyzed in this dynamic fluid flow environment as compared to the static culture conditions. High levels of cytokines were observed in this device due to optimized recirculation media conditions. The primary challenge of building an organ on chip is to duplicate in vivo environment for housing an organ with ease of handling and robustness. Second challenge of organ on chip technology is manufacturing of cost effective, toxicity evaluation with help of system embedded sensors. This device has provided the preliminary data for making this breakthrough in OOC technology. Future perspective of our group is to make complex tissue mimetics of human for toxicity testing and drug discovery with system integrated pathological sensors for replacing time consuming molecular analysis of pathological conditions.

Abbreviations

DCFDA
5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate
IL-13
Interlukin-13
TNFα
Tumor necrosis factor-α
IL-6
Interlukin-6
OOC
Organ on chip
TEER
Trans epithelial electrical resistance
AT- I, II
Alveolar type I, II
DMSO
Dimethyl sulfoxide
TH-2
T- Helper 2 cells
COPD
Chronic obstructive pulmonary disease
BSA
Bovine serum albumin
PAH’s
Polycyclic aromatic hydrocarbons
ELISA
Enzyme linked immunosorbent assay
PM
Particulate matter
PDMS
Polydimethylsiloxane
ROS
Reactive oxygen species
ITO
Indium tin oxide
$CD4^+$
T helper cells
SEM
Scanning electron microscopy

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

Not applicable

Availability of supporting data

Not applicable

Competing interests

Authors declare that they have no competing interests

Funding

This research was financially supported by the Ministry of Trade, Industry and Energy (MOTIE) and Korea Institute for Advancement of Technology (KIAT) through the international Cooperative R&D program. (Project No. P0006848)).

Authors' contributions

Faiza Jabbar and Kim Youngsu designed and performed the experiments, did the analysis of all obtained data and drafted the manuscript. Arun helped in characterizations, Afaque managed the implementation of sensors. Choi Kyung Hyun, Lee Sang Ho, Cho Young Jae supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable

Authors' information

Faiza Jabbar, Department of Mechatronics Engineering, Jeju National University, Jeju, South Korea

Kim Young Su, Department of Mechatronics Engineering, Jeju National University, Jeju, South Korea

Afaque Manzoor Soomro, Department of Mechatronics Engineering, Jeju National University, Jeju, South Korea

Arun Asif, Department of Mechatronics Engineering, Jeju National University, Jeju, South Korea

Cho Young Jae, 3Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul National University Bundang Hospital

Choi Kyung Hyun (Corresponding author), Department of Mechatronics Engineering, Jeju National University, Jeju, South Korea and BioSpero, Inc. Jeju–do, Republic of Korea

E-mail: amm@jejunu.ac.kr

Lee Sang Ho (Corresponding author) Bio PD, office of strategic R&D planning, 305, Teheran-ro, Gangnam-gu, Seoul, Republic of Korea

E-mail: biomedpd@keit.re.kr

References

1. Lee YG, Ho C-H, Kim J-H, Kim J. Quiescence of Asian dust events in South Korea and Japan during 2012 spring: dust outbreaks and transports. Atmospheric Environment. 2015;114:92-101. 2. Essig M, Friedlander G. Tubular shear stress and phenotype of renal proximal tubular cells. Journal of the American Society of Nephrology. 2003;14(suppl 1):S33-S5. 3. Lelieveld J, Evans JS, Fnais M, Giannadaki D, Pozzer A. The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature. 2015;525(7569):367. 4. Kurt OK, Zhang J, Pinkerton KE. Pulmonary health effects of air pollution. Current opinion in pulmonary medicine. 2016;22(2):138. 5. Gilmour MI, Daniels M, McCrillis RC, Winsett D, Selgrade MK. Air pollutant-enhanced respiratory disease in experimental animals. Environmental health perspectives. 2001;109(suppl 4):619-22. 6. Brook RD, Rajagopalan S, Pope III CA, Brook JR, Bhatnagar A, Diez-Roux AV, et al. Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association. Circulation. 2010;121(21):2331-78. 7. Woods A, Brull D, Humphries S, Montgomery H. Genetics of inflammation and risk of coronary artery disease: the central role of interleukin-6. European Heart Journal. 2000;21(19):1574-83. 8. Becker S, Mundandhara S, Devlin RB, Madden M. Regulation of cytokine production in human alveolar macrophages and airway epithelial cells in response to ambient air pollution particles: further mechanistic studies. Toxicology and applied pharmacology. 2005;207(2):269-75. 9. Ingram JL, Kraft M. IL-13 in asthma and allergic disease: asthma phenotypes and targeted therapies. Journal of Allergy and Clinical Immunology. 2012;130(4):829-42. 10. Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert opinion on drug metabolism & toxicology. 2006;2(6):875-94. 11. Davila JC, Rodriguez RJ, Melchert RB, Acosta Jr D. Predictive value of in vitro model systems in toxicology. Annual review of pharmacology and toxicology. 1998;38(1):63-96. 12. Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE. Soft lithography in biology and biochemistry. Annual review of biomedical engineering. 2001;3(1):335-73. 13. Thuenauer R, Rodriguez-Boulan E, Römer W. Microfluidic approaches for epithelial cell layer culture and characterisation. Analyst. 2014;139(13):3206-18. 14. Srinivasan B, Kolli AR, Esch MB, Abaci HE, Shuler ML, Hickman JJ. TEER measurement techniques for in vitro barrier model systems. Journal of laboratory automation. 2015;20(2):107-26. 15. Erickson D, Li D. Integrated microfluidic devices. Analytica chimica acta. 2004;507(1):11-26. 16. Booth R, Kim H. Characterization of a microfluidic in vitro model of the blood-brain barrier (μBBB). Lab on a Chip. 2012;12(10):1784-92. 17. Booth R, Noh S, Kim H. A multiple-channel, multiple-assay platform for characterization of full-range shear stress effects on vascular endothelial cells. Lab on a Chip. 2014;14(11):1880-90. 18. Yeste J, Illa X, Gutiérrez C, Solé M, Guimerà A, Villa R. Geometric correction factor for transepithelial electrical resistance measurements in transwell and microfluidic cell cultures. Journal of Physics D: Applied Physics. 2016;49(37):375401. 19. ávan der Meer AD, JungáKim H, ávan der Helm MW, den Berg A. Measuring direct current trans-epithelial electrical resistance in organ-on-a-chip microsystems. Lab on a Chip. 2015;15(3):745-52. 20. Soomro AM, Khalid MAU, Shah I, Choi KH. Highly stable soft strain sensor based on Gly-KCl filled sinusoidal fluidic channel for wearable and water-proof robotic applications. Smart Materials and Structures. 2019. 21. Soomro AM, Jabbar F, Ali M, Lee J-W, Mun SW, Choi KH. All-range flexible and biocompatible humidity sensor based on

poly lactic glycolic acid (PLGA) and its application in human breathing for wearable health monitoring. Journal of Materials Science: Materials in Electronics. 2019;30(10):9455- 65. 22. Khalid MAU, Kim YS, Ali M, Lee BG, Cho Y-J, Choi KH. A lung cancer-on-chip platform with integrated biosensors for physiological monitoring and toxicity assessment. Biochemical Engineering Journal. 2019:107469. 23. Vormann MK, Gijzen L, Hutter S, Boot L, Nicolas A, van den Heuvel A, et al. Nephrotoxicity and kidney transport assessment on 3D perfused proximal tubules. The AAPS journal. 2018;20(5):90. 24. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting organ-level lung functions on a chip. Science. 2010;328(5986):1662-8. 25. Johnston H, Pojana G, Zuin S, Jacobsen NR, Møller P, Loft S, et al. Engineered nanomaterial risk. Lessons learnt from completed nanotoxicology studies: potential solutions to current and future challenges. Critical reviews in toxicology. 2013;43(1):1-20. 26. Bakand S, Hayes A, Dechsakulthorn F. Nanoparticles: a review of particle toxicology following inhalation exposure. Inhalation toxicology. 2012;24(2):125-35. 27. Stone V, Johnston H, Schins RP. Development of in vitro systems for nanotoxicology: methodological considerations. Critical reviews in toxicology. 2009;39(7):613-26. 28. Georas S, Guo J, De Fanis U, Casolaro V. T-helper cell type-2 regulation in allergic disease. European respiratory journal. 2005;26(6):1119-37. 29. Cromwell O, Hamid Q, Corrigan C, Barkans J, Meng Q, Collins P, et al. Expression and generation of interleukin-8, IL-6 and granulocyte-macrophage colony-stimulating factor by bronchial epithelial cells and enhancement by IL-1 beta and tumour necrosis factor-alpha. Immunology. 1992;77(3):330. 30. King C, Brennan S, Thompson PJ, Stewart GA. Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. The Journal of Immunology. 1998;161(7):3645-51. 31. Marini M, Vittori E, Hollemborg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. Journal of Allergy and Clinical Immunology. 1992;89(5):1001-9. 32. Kicic A, Sutanto EN, Stevens PT, Knight DA, Stick SM. Intrinsic biochemical and functional differences in bronchial epithelial cells of children with asthma. American journal of respiratory and critical care medicine. 2006;174(10):1110-8. 33. Lee HJ, Ryu J, Park SH, Seo E-K, Han A-R, Lee SK, et al. Suppressive effects of coixol, glyceryl trilinoleate and natural products derived from CoixLachryma-Jobi var. ma-yuen on gene expression, production and secretion of airway MUC5AC mucin. Archives of pharmacal research. 2015;38(5):620-7. 34. Morcillo EJ, Cortijo J. Mucus and MUC in asthma. Current opinion in pulmonary medicine. 2006;12(1):1-6. 35. Kirkham S, Sheehan JK, Knight D, Richardson PS, Thornton DJ. Heterogeneity of airways mucus: variations in the amounts and glycoforms of the major oligomeric mucins MUC5AC and MUC5B. Biochemical Journal. 2002;361(3):537-46. 36. Prahalad S, Bove KE, Dickens D, Lovell DJ, Grom AA. Etanercept in the treatment of macrophage activation syndrome. The Journal of rheumatology. 2001;28(9):2120-4. 37. Billet S. The theologies of transparency in Europe: the limits and possibilities of ‘fusion’in the EU transparency regime. Journal of Public Affairs: An International Journal. 2007;7(4):319-30. 38. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free radical biology and medicine. 1995;18(2):321-36. 39. Chauhan SS, Misra UK. Elevation of rat pulmonary, hepatic and lung surfactant lipids by fly ash inhalation. Biochemical pharmacology. 1991;41(2):191-8. 40. Castano-Vinyals G, D’errico A, Malats N, Kogevinas M. Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. Occupational and Environmental Medicine. 2004;61(4):e12-e. 41. Thomsen AE, Friedrichsen GM,

Sørensen AH, Andersen R, Nielsen CU, Brodin B, et al. Erratum: Prodrugs of purine and pyrimidine analogues for the intestinal di/tri-peptide transporter PepT1: Affinity for hPepT1 in Caco-2 cells, drug release in aqueous media and in vitro metabolism (Journal of Controlled Release (2003) 86 (279-292) PII: S0168365902004133). Journal of Controlled Release. 2003;88(2). 42. Kelly-Welch A, Hanson EM, Keegan AD. Interleukin-13 (IL-13) pathway. Sci STKE. 2005;2005(293):cm8-cm. 43. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, Zhu Z, et al. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. Nature medicine. 2002;8(8):885. 44. Bafadhel M, McKenna S, Terry S, Mistry V, Reid C, Haldar P, et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. American journal of respiratory and critical care medicine. 2011;184(6):662-71. 45. Kamimura D, Ishihara K, Hirano T. IL-6 signal transduction and its physiological roles: the signal orchestration model. Reviews of physiology, biochemistry and pharmacology: Springer; 2003. p. 1-38. 46. Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. Clinical immunology. 2009;130(1):27-33. 47. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector T H 17 and regulatory T cells. Nature. 2006;441(7090):235. 48. Acosta-Rodriguez EV, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1β and 6 but not transforming growth factor-β are essential for the differentiation of interleukin 17–producing human T helper cells. Nature immunology. 2007;8(9):942. 49. Benam KH, Novak R, Nawroth J, Hirano-Kobayashi M, Ferrante TC, Choe Y, et al. Matched-comparative modeling of normal and diseased human airway responses using a microengineered breathing lung chip. Cell systems. 2016;3(5):456-66. e4. 50. Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. The Journal of clinical investigation. 2008;118(11):3546-56. 51. Grubek-Jaworska H, Paplińska M, Hermanowicz-Salamon J, Białek-Gosk K, Dąbrowska M, Grabczak E, et al. IL-6 and IL-13 in induced sputum of COPD and asthma patients: correlation with respiratory tests. Respiration. 2012;84(2):101-7. 52. Nam KH, Li J. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. Cell. 2002;110(2):203-12. 53. González-Flecha B. Oxidant mechanisms in response to ambient air particles. Molecular aspects of medicine. 2004;25(1-2):169-82. 54. Ootsubo T, Usui F, Kawakita H, Ishiguro M, Furusho R, Hasegawa S, et al. Detection of parent H2O and CO2 molecules in the 2.5–5 μm spectrum of comet C/2007 N3 (Lulin) observed with AKARI. The Astrophysical Journal Letters. 2010;717(1):L66.