Science of the Total Environment 1025 (2026) 181645

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

Innovative methodologies for elucidating bushfire smoke-induced pathophysiological mechanism

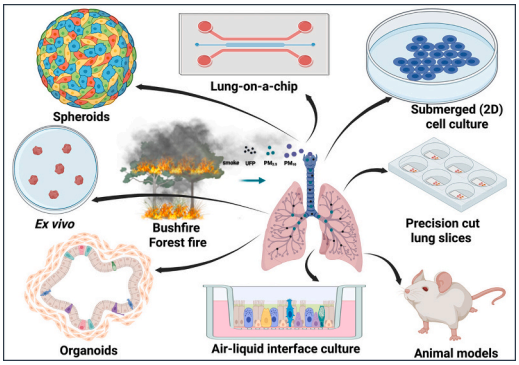
Keshav R. Paudel [a,b,*], Karl J. Hegarty [b], Jesus Shrestha [c], Kurtis F. Budden [d], Nikhil T. Awatade [e,f], Tayyaba Sadaf [b], Vamshikrishna Malyla [b], Shafagh Waters [g,h], Paris C. Papagianis [i], Jane E. Bourke [i], Peter A. Wark [j,k], Philip M. Hansbro [b]

[a] NICM Health Research Institute, Western Sydney University, Westmead, NSW, 2145, Australia
[b] Centre for Inflammation, Centenary Institute & University of Technology Sydney, Faculty of Science, School of Life Sciences, Sydney, New South Wales, 2050 & 2007, Australia
[c] School of Biomedical Engineering, University of Technology Sydney, Sydney, New South Wales, 2007, Australia
[d] Priority Research Centre for Healthy Lungs and Immune Health Research Program, School of Biomedical Sciences and Pharmacy, The University of Newcastle and Hunter Medical Research Institute, Newcastle, NSW, Australia
[e] Immune Health Program, Hunter Medical Research Institute, New Lambton Heights, New South Wales, Australia
[f] School of Medicine and Public Health, University of Newcastle, Newcastle, New South Wales, Australia
[g] School of Biomedical Sciences, Faculty of Medicine and Health, UNSW Sydney, Sydney, Australia
[h] Sydney Children's Hospital, Department of Respiratory Medicine, Sydney, Australia
[i] Department of Pharmacology, Biomedicine Discovery Institute, Monash University, Clayton, Australia
[j] Cystic Fibrosis Service, Department of Respiratory Medicine, Alfred Health, Melbourne, Australia.
[k] Central Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, Australia

HIGHLIGHTS

- Bushfire events are increasing globally & exposure to smoke can impact our health.
- Bushfire smoke particulate matter of various size deposit at different area of lung
- Bushfire smoke exposure worsens pre-existing respiratory disease.
- Bushfire smoke inhalation can trigger oxidative stress, inflammation, & senescence.
- Experimental models can elucidate the damage of bushfire smoke & study therapeutics.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Bushfire
In vitro
In vivo

ABSTRACT

Over the last century, the awareness of air pollution awareness and its harm to public health have resulted in the implementation of new protective measures to try and limit exposure. Bushfires generate waves of air pollution with orders of magnitudes higher than normal background pollution, necessitating studies to understand the physiological impact. Previous work has strongly linked bushfire smoke to respiratory disease (chronic

* Corresponding author at: NICM Health Research Institute, Western Sydney University, Westmead, NSW, 2145, Australia.
E-mail address: K.Paudel@westernsydney.edu.au (K.R. Paudel).

https://doi.org/10.1016/j.scitotenv.2026.181645
Received 7 October 2025; Received in revised form 27 February 2026; Accepted 1 March 2026

Air-liquid interface culture
Lung-on-a-chip
Organoids

obstructive pulmonary disease COPD, asthma, lung cancer) cardiovascular disease (myocardial infarction, stroke), and increased susceptibility to infection (COVID-19). Nevertheless, the underlying mechanisms of pathogenesis remain unclear. There is little consensus on what *in vitro* and *in vivo* techniques are best used to examine disease mechanisms. Individual investigations are useful but a lack of standard methods creates variability and makes comparisons difficult. Developing adaptable *in vitro* and *in vivo* models that are replicable, physiologically relevant, and affordable may reduce variability enabling comparisons. These models should also be capable of integrating multiple types of pollutants or reference materials to develop standards that other studies can follow, facilitating comparisons. Here, we discuss advance *in vitro* and *in vivo* experimental models to study the impact of bushfire smoke exposure induced pathophysiology. The goal is to improve comparison and translation across studies, and to lay the groundwork for future research into the mechanisms that underpin bushfire smoke-induced pathogenesis to enable the development of preventative measures and effective therapies.

1. Introduction

Bushfires are a regular phenomenon in temperate regions across the world, occurring in different regions throughout the year. As a prime example, between September 2019 and February 2020, known as the "Black Summer", devastating bushfires ravaged ~1.8 million hectares of forests in Australia, making it the second largest area burnt by a bushfire in the 21st century. Highly populated cities on the East Coast were blanketed with smoke, and particulate matter (PM)-2.5 levels regularly exceeded the 95th percentile of the historical daily average (125 of 133 days at one monitoring station), with the maximum 24 h national average of PM2.5 reaching 98.5 μg/m^3 (Borchers Arriagada et al., 2020). The fires were triggered as 2019 was the hottest year recorded in Australia, which has the highest impact of climate change with a mean temperature rise of 1.52 °C since 1910, approximately 1.4–1.5 x the global average warming. The 2019–2020 bushfire season led to >3000 hospitalisations and 400 excess deaths (MacIntyre et al., 2021). People with pre-existing respiratory conditions like asthma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) are at the highest risk of adverse effects from bushfire smoke (BFS) along with vulnerable populations like pregnant women, infants/children and elderly people. A 2007 study predicted that the average global surface temperature in the summer of 2020 would be around 1 °C, which would trigger bushfires (Lucas et al., 2007). It follows that by 2050 the surface temperature potentially reaching 2.9 °C under high-emission scenarios, further increasing bushfires risk globally. Despite the prevalence of bushfires in many countries and although people with pre-existing chronic diseases are disproportionately affected, there is a lack of studies aiming to develop targeted preventative therapies or treatments or investigating the effects of BFS exposure on vulnerable individuals (Dharwal et al., 2020).

Current public health advice concerning BFS exposure exists (Vardoulakis et al., 2020), however, current prevention and treatment strategies are insufficient and not broadly applicable, and there is a need to develop new strategies. As BFS exposure becomes more frequent, and as our understanding expands, it is crucial that these strategies incorporate new information to avoid becoming obsolete, redundant and potentially detrimental.

In studies examining short-term exposure to BFS, there are consistent associations with chronic respiratory diseases such as COPD and asthma, as well as less consistent associations with cardiovascular disease (MacIntyre et al., 2021). The Black Summer bushfires increased hospital visits from patients with respiratory and (2027, 95% CI, 0–4252), cardiovascular (1124, 95% CI, 211–2047) problems, and increased emergency department visits for those with asthma (1305, 95% CI, 705–1908). Inhaled BFS was responsible for ~417 (95% CI, 153–680) deaths (Borchers Arriagada et al., 2020). Analysis of the Flutracking surveillance system showed that those with chronic respiratory disease reported higher overall symptoms (83%) than those without (60%), which included breathlessness, wheezing, and chest pain (Howard et al., 2020). An Asthma Australia survey found that people with asthma had a higher incidence of hospitalisations, emergency department visits, corticosteroid medication, financial burden, and lower quality-of-life than those who did not. Healthcare costs and hospital visits, as well as lost or reduced income increased financial burden and reduced quality-of-life (Bui et al., 2021). This was despite people with asthma taking more precautions to protect themselves before and during bushfires, such as corticosteroid medication, wearing N95 masks, staying indoors, using recirculating air conditioners and continuing to take their medication (Bui et al., 2021).

Mounting evidence suggests that BFS exposure poses short- and long-term threats to public health. Understanding the physiological impacts and developing targeted preventative and therapeutic strategies could suppress the adverse effects of BFS. This review outlines the known physiological impacts of BFS exposure and the techniques best employed to better understand these impacts.

2. BFS source and composition

'Bushfire' is a distinct term mainly used in reference to uncontrolled forest fires in Australia. Elsewhere they are termed wildfires, forest fires, unplanned fires, biomass burning or have vegetation specific terms (Clarke et al., 2015). Here, the term 'bushfire' will be used as a blanket term. There are differences with the use of these terms, which are typically regional or refer to the fuel source, resulting in differences in smoke composition. Indeed, regional fuel source, weather, burning temperature all influence smoke composition. Therefore, a bushfire moving through dry scrub in one area has a unique smoke composition compared to a bushfire in a pine forest elsewhere. Nevertheless, BFS from different sources do share compositional similarities, where burning of organic fuel sources always produces carbon dioxide and poly aromatic hydrocarbons (PAHs), making examining and comparing the effects of BFS components from different sources instructive in understanding their physiological impact.

Physiochemical analyses are commonly carried out on PM from urban or industrial sources such as diesel exhaust particles and BFS. For both sets of PM that their composition is complex and requires extensive time and expertise to develop an understanding of their makeup. To begin with, most studies separate their particles based on diameter. This can differ between studies depending on the equipment available, or a specific PM size may be used (typically PM_{1}, $_{2.5}$ or $_{10}$ μM).

2.1. Particle size analysis

Particle size analysis is commonly performed as a part of PM characterisation, and there is a range of techniques that are used to analyze and recover desired PM sizes (*e.g.*, sieve analysis, sedimentation, acoustic spectroscopy, electro-resistance counting, laser diffraction, optical counting using EM). This review covers the most commonly used methods; however, the optimal techniques are often case-specific and are chosen based on experimental needs. For example, if a particular PM is scarce or was collected and cannot be re-collected, techniques are often used that will allow the PM to be recovered.

The Andersen cascade impactor (ACI) is a commonly used apparatus

for size fractionating particles. ACI is a portal apparatus that has several stages (*e.g.*, seven or eight stage ACI), and each stage has a nozzle of different size that get progressively smaller. The bottom stage of the ACI is connected to vacuum at particular flow rate. For separation, the PM sample is first placed into an empty gelatin capsule with the mostly commonly used size 000 that can accommodate ~50 mg of PM. This capsule is loaded into the device and sharp needles on both sides pierce the capsule, and PM is suctioned by vacuum as it passes through different stages of ACI and is deposited accordingly (Fig. 1). For example, an eight stage ACI at a flow rate of 28.3 L/min gives PM sizes of 9 μm at stage 0, 5.8 μm at stage 1, 4.7 μm at stage 2, 3.3 μm at stage 3, 2.1 μm at stage 4, 1.1 μm at stage 5, 0.7 μm at stage, and 0.4 μm at stage 7. The cutoff for $PM_{2.5}$ size is, 2.1 μm (stage 3)-3.3 μm (stage 4), and similar cut-off applies to different size of PM at different stage of ACI.

2.2. Particle composition

Liu et al., assessed population-based exposure to wildfire-related PM by integrating monitor measurements on 29 $PM_{2.5}$ species and previous findings on smoke waves in 51 Western US counties across six ecoregions from 2004 to 09 (Liu and Peng, 2019). They discovered that, smoke waves were associated with 20% increases in the fraction of organic carbon of total $PM_{2.5}$ (95% confidence interval (CI): 17, 23), a 1% increase in the fraction of elemental carbon (95% CI: 0.43, 1.6), and decreases in sulfate and crustal species fractions across all ecoregions. While the $PM_{2.5}$ mixtures were dominated by the same source (wildfires), the compositions in North American Deserts and the Great Plains during smoke waves were distinct. That study has implications beyond wildfires and could aid future population-based epidemiological research on $PM_{2.5}$ mixtures by source and region. Apart from organic and elemental carbon, other elements (aluminium, arsenic, bromine, calcium, chloride, chlorine, chromium, copper, iron, lead, magnesium, manganese, nickel, phosphorus, potassium, rubidium, selenium, silicon, sodium, strontium, sulfate, sulfur, titanium, total nitrate, zinc, zirconium) were also detected in the $PM_{2.5}$.

2.3. PM and BFS extract

There is little consensus over the methods used to investigate the effects of BFS. Before achieving consensus on the best methods to use, it will be difficult to understand the effects of BFS beyond the scope of a single study. For example, the use of PM suspensions or BFS extract is a significant difference between study methods. Both are used to study the effects of BFS *in vitro*, however there are key differences in their production and composition.

There are two common methods for collecting PM: filtering outside air during BFS events to capture particles, or by burning relevant vegetation in a furnace and collecting the PM produced. The raw PM collected is separated by size and can be used to make a PM suspension. Other studies produce BFS extract (100%) by burning vegetation and bubbling the smoke through a solvent (*e.g.* PBS) or cell culture medium. This technique is commonly used to study the effects of cigarette smoke *in vitro*.

The size of the PM examined varies between studies, but PM_{10}/coarse, <2.5 μm $PM_{2.5}$/fine, <1 μm PM_1/fine, and < 0.1 μm $PM_{0.1}$/ultrafine are used. $PM_{2.5}$ and PM_{10} are commonly measured pollutants at weather stations (average 24 h/7d/annual concentration μg/m^3) and relevant concentrations based on these parameters are often used.

When using either PM suspensions or BFS extract, the relevant fuel source (*e.g.*, vegetation from a bushfire region) and burning temperature

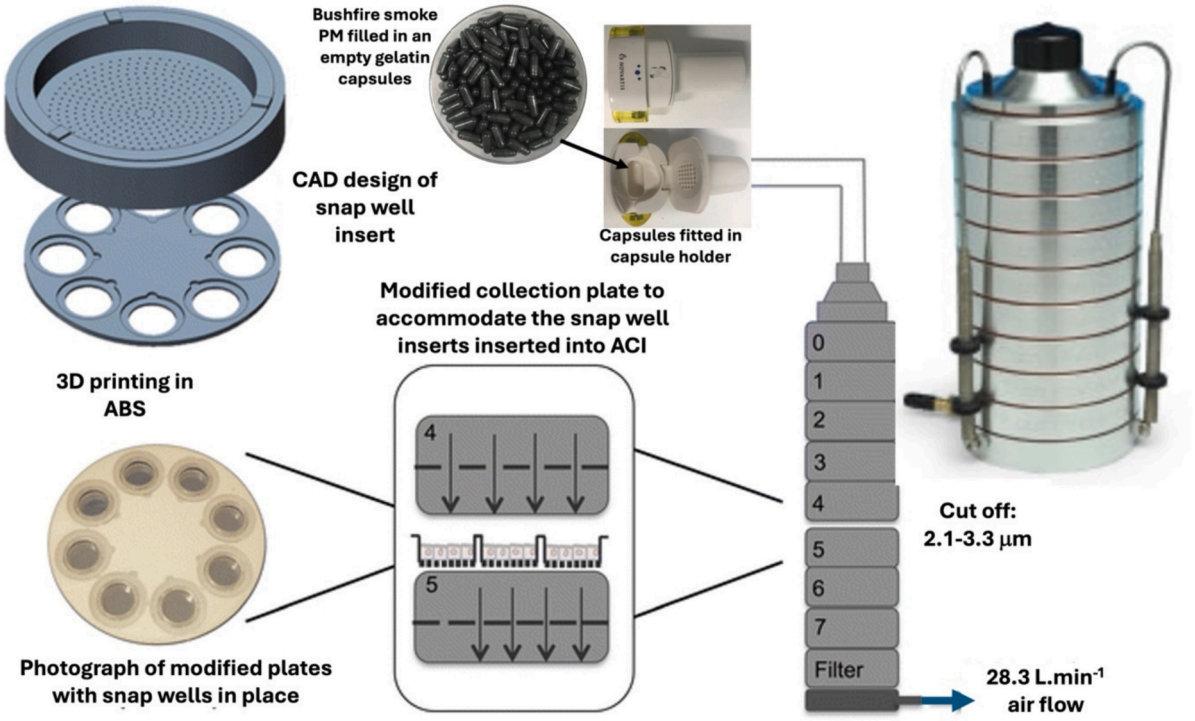


Fig. 1. Eight stage Anderson cascade impactor (ACI). To sieve bushfire smoke PM into various size particle, bushfire ash can be filled into empty gelatin capsule (Size 000 has capacity to filled approximately 50 mg of bushfire ash per capsule). The capsule is fitted into a capsule holder and then to ACI. The various stages of this impactor have nozzle of various size and progressively gets smaller from stage 0 to stage 7. The lower end of ACI (after filter) is connected to vacuum with flow rate of 28.3 L·min^{-1} air flow. Based on the cut point for stage, example: the bushfire PM of size 2.1–3.3 μm can be collected from stage 4 and referred as $PM_{2.5}$. Custom-made ACI plates that can accommodate the snapwells can be designed using computer-aided design, and a series of modified plates produced in acrylonitrile butadiene styrene (ABS) using a 3D printer. The plates are designed to hold eight snapwells, equally spaced at a 29 mm radius from the centre of the plate to the centre of each snapwell.
Reproduced from Haghi et al. (2014) with permission and minor modification.

can be controlled to mimic real-life pyrolysis conditions. PM suspensions can be made up to specific concentrations (w/v) of PM and separated by PM size, and since PM is the primary pollutant during bushfire events, it is highly relevant when studying the physiological consequences of exposure. PM size has been shown to be important in studies that have demonstrated different physiological effects *in vitro* and *in vivo*. Smaller particles (*e.g.* PM_1), can enter the lower respiratory tract, circulatory system and nervous system, whereas larger particles (PM_{10}) settle in the upper respiratory tract (Patton and Byron, 2007). Using PM suspensions has the disadvantage of causing particle agglomeration especially with hydrophobic constituents, which reduces the surface area of particles. To avoid this, PM stock suspensions must be thoroughly mixed before use *in vitro* or *in vivo*. Supplementing PBS with low concentration of DMSO or surfactants (*e.g.* Tween 80) may help reduce agglomeration, however care must be taken not to completely dissolve the PM. PM suspensions also overlook the gaseous component of BFS. BFS extract can contain gaseous components of BFS that would otherwise be lost with PM alone, however, it contains minimal or negligible PM, the primary pollutant emitted by bushfires.

To best understand the effects of BFS exposure, studies should use compositions and concentrations of BFS that are most representative of real-life exposures. Ideal studies use both BFS extract and PM suspensions in their studies.

3. Experimental models examining the effects of BFS *in vitro*

The optimal *in vitro* techniques mimic what is observed in disease settings and can be used to create a replicable toxicity profile. In toxicology studies, they are used to investigate the molecular mechanisms by which a substance acts as well as to obtain cellular readouts of cytotoxicity, gene and protein expression related to oxidative stress, inflammation, senescence, and various other processes. When investigating the effects of different airborne pollutants, this becomes complicated since pollutants are composed of complex mixtures of hundreds to thousands of chemical species including particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), metals, and oxidant gases. *In vitro* techniques and assays have been reviewed for use in air pollution toxicology studies (Zavala, 2020). Here we will discuss additional state-of-the-art models with increasing physiological relevance.

3.1. Submerged culture (extracts versus PM, cell lines, primary human cells)

Submerged culture remains one of the most widely used and accessible *in vitro* platforms to assess the toxicological effect of pollutants/PM particles (Aydin et al., 2021). In this approach, airway relevant cell types – including immortalized bronchial epithelial lines (*e.g.* BEAS-2B, Calu-3), alveolar epithelial cells (e.g. A549), macrophage models (e.g. THP-1), or primary human airway epithelial cells are expanded as monolayer under submerged conditions, typically in multi-well plates or tissue culture flasks (Shrestha et al., 2023). Following adherence and expansion to confluence, cells are exposed to bushfire-relevant PM suspensions or smoke extracts diluted directly into the culture medium in an acute or chronic exposure timelines.

While submerged models offer simplicity, cost-effectiveness and scalability for high-throughput screening, they lack several critical features of physiological inhalation exposure. Cells are exposed to liquid-phase media rather than air, which alters particle dispersion, agglomeration, and chemical reactivity compared to airborne deposition. The lack of air-liquid interface exposure precludes accurate modeling of mucociliary clearance, aerosol deposition mechanics, or interaction with airway lining fluids, all of which critically modulate inhaled toxicant behavior *in vivo* (Shrestha et al., 2023). 2D submerged airway cell cultures deviate most when the research question depends on realistic exposure, epithelial differentiation, barrier function, or immune metabolic coupling. As 2D cell culture represent the non-physiological exposure conditions, cells are submerged entirely in media, not air, therefore, bushfire PM is delivered as a bolus suspension and bushfire PM agglomeration and sedimentation dominate, soluble components are overrepresented, insoluble fractions underrepresented leading to consequences such as artificially high intracellular dose, overestimated cytotoxicity and oxidative stress, loss of PM size- and composition-dependent effects. Moreover, there is no ciliation, mucus layer, or tight junction maturation, flat, squamous-like morphology, altered receptor localization (apical *vs* basolateral) that results in circumstances such as exaggerated barrier dysfunction, distortion of pathogen–pollutant interactions, and absence of mucociliary clearance mechanisms.

Despite these limitations, submerged models remain useful for delineating cellular stress responses, including cytotoxicity, oxidative stress, DNA damage, apoptosis, and pro-inflammatory signalling pathways activated by BFS constituents. Numerous studies have utilized submerged cultures to characterize dose-dependent effects of smoke PM on inflammatory mediator release (*e.g.*, IL-6, IL-8, TNF-α), ROS generation, mitochondrial dysfunction, and alterations in epithelial barrier integrity (Ortiz-Quintero et al., 2022). Moreover, chronic exposure protocols spanning several days allow examination of adaptive or cumulative cellular responses relevant to repeated exposure scenarios.

Importantly, the choice of PM preparation strongly influences submerged culture outcomes. PM suspensions prepared from bushfire smoke may exhibit particle aggregation, particularly among hydrophobic components, leading to altered bioavailability and cellular uptake. Techniques such as ultrasonication, careful stock preparation, and use of dispersants (*e.g.*, low-dose surfactants like Tween 80) have been employed to improve suspension homogeneity. Nonetheless, submerged models inherently omit the gaseous phase of BFS, thereby failing to capture combined particle-gas interactions that occur during real-world exposures. While submerged mono layer cell culture provides platform to assess the impact of bushfire PM exposure *in vitro*, there are more advanced human lung cells model such air-liquid interface, co-culture techniques, organoids, lung-on-a-chip gaining popularity these days (Rothen-Rutishauser et al., 2023). The newly developed dynamic *in vitro* systems incorporate breathing motion or mechanical stretching under exposure conditions such as Acini-on-Chips (Artzy-Schnirman et al., 2019), fluidically coupled vascularized organ chips, and human-airway-on-a-chip (Si et al., 2021). These advanced *in vitro* systems for aerosol or PM delivery are discussed in detail in the following sections.

3.2. Air-liquid interface (ALI) cultures

The ALI model represents a significant advancement in the *in vitro* modeling of inhalation toxicology, providing a physiologically relevant system to study airway epithelial responses to airborne pollutants such as BFS. In ALI cultures primary human airway basal stem/progenitor cells or immortalized bronchial epithelial cell lines are expanded on permeable membrane supports until confluent, after which apical medium is removed to expose the epithelial surface to air, initiating differentiation under the influence of basolateral nutrients and growth factors.

The success of ALI culture depends upon many factors such as passage of cells (early passage is preferable), selection of polyester or polycarbonate inserts. (0.4 μm pore size), collagen IV or human placental collagen coating, seeding density ($1.0–2.5 \times 10^5$ cells/cm^2), uniform distribution, additional of retinoic acid in culture media is absolutely essential for mucocillary differentiation.

Under optimised conditions, ALI cultures differentiate to a complex, pseudostratified epithelium that recapitulates many key features of the human conducting airway, including up to 11 cell types representative of a human airway in a dish in a physiological environment where these cells are exposed to breathing air on one side and blood circulatory factors on the other (Moheimani et al., 2022). This cellular

heterogeneity supports the formation of critical airway defense mechanisms such as mucus production, mucociliary clearance, tight junction barrier integrity, and innate immune responses, which are absent or poorly developed in submerged culture models (Kesimer et al., 2009). Pezzulo et al. (2011) observed that the transcriptional profile of differentiated primary cultures grown at ALI closely mirrors that of the *in vivo* airway epithelium. Therefore, the ALI cultures serves as a more reliable model of the human airways compared to submerged (2D) culture (Pezzulo et al., 2011), as ALI systems allow for apical exposure to airborne BFS particles and gases delivered *via* aerosolization systems or controlled environmental chambers, closely mimicking real-world inhalation exposure routes. ALI has proven to be complementary to *in vivo* experimentation (example: mice model of asthma, COPD, idiopathic pulmonary fibrosis).

ALI has been extensively used to investigate the impact of BFS. For instance, Roscioli et al. demonstrated that wildfire smoke extract inhibits autophagic flux and induces barrier dysfunction in ALI-differentiated human bronchial epithelial cells (Roscioli et al., 2018). Impaired autophagy may ameliorate the consequences of BFS exposure in individuals with pre-existing respiratory conditions. Similarly, recent studies utilising human nasal epithelial ALI cultures have shown that BFS exposure rapidly impair muco-ciliary function, with ciliary dyskinesia, mucus hypersecretion, and PM entrapment observed over hours of exposure. Such impairments are directly relevant to the pathophysiology of acute exacerbations seen in asthma, COPD, and bronchiectasis during bushfire episodes.

Despite their advantages, ALI models also have limitations. They primarily model proximal airways and do not replicate alveolar or small airway regions where ultrafine PM (PM0.1) deposits. Moreover, ALI monocultures lack immune, endothelial, fibroblast, and neuronal components that modulate epithelial responses *in vivo*. Efforts to incorporate co-culture systems with macrophages, dendritic cells, fibroblasts, or endothelial cells are advancing, offering increasingly sophisticated models of the lung microenvironment (Allouche et al., 2025).

Emerging developments in dynamic ALI platforms include integration of cyclic mechanical stretch, microfluidic shear forces, and controlled breathing simulations to better reproduce biomechanical cues encountered during respiration. These innovations hold promise for improving the predictive power of ALI systems in modeling complex inhalation toxicology relevant to BFS exposure (Graf et al., 2023). While ALI cultures recreate the structural and functional features of the airway epithelium, meaningful inhalation toxicology also depends on how airborne material is delivered to the epithelial surface. Submerged dosing exposes cells to particles suspended in liquid and does not reproduce aerosol deposition kinetics, gas–particle interactions, or realistic delivered dose. Therefore, ALI cultures are commonly coupled to dedicated aerosol exposure systems that control particle generation, transport, and deposition onto the epithelial surface. These platforms standardise exposure conditions independently of the biological model and enable comparison between studies using different cell types or levels of tissue complexity.

3.2.1. Aerosol exposure platforms for in vitro airway models

To expose the cells grown in ALI culture with bushfire PM, a technique called Vitrocell can be used as an aerosol-cell exposure platform (Bannuscher et al., 2022). Several aerosol delivery platforms have been developed for ALI exposure (*e.g.*, Vitrocell ALICE-Cloud, Cultex RFS, VITROCELL® modules, NACIVT and custom whole-smoke exposure chambers). Among these, the ALICE-Cloud (Vitrocell Systems GmbH) system is widely used for controlled nebulised particle exposure and is discussed here as a representative example.

Vitrocells platform allow the bushfire PM to be aerosolized over the cells layer grown as ALI. In this system, the test suspension is nebulised using a vibrating mesh nebuliser generating droplets with a defined aerodynamic size distribution. The aerosol is transported into a temperature- and humidity-controlled exposure chamber where particles deposit onto the cell surface primarily through gravitational settling and diffusion.

For this bushfire PM of desired size can be mixed with cell culture media to achieve the physiologically relevant human exposure dose and aerosolized using vitrocell platform while maintaining temperature and humidity (Steiner et al., 2017). Importantly, the delivered dose is measured rather than assumed: integrated quartz crystal microbalance (QCM) sensors provide real-time quantification of deposited particle mass (μg/cm^2), enabling accurate and reproducible exposure assessment across experiments.

Vitrocell®Cloud are also used for nanotoxicological studies using dose-controlled ALI aerosol-cell exposure experiments (Ding et al., 2020). According to Bannuscher et al. (2022), an inter-laboratory comparison lab of the Vitrocell®Cloud device-specific performance was conducted in seven different laboratories using same standard operating procedure. All laboratories were able to demonstrate dose-controlled aerosolization of material suspensions using the VITROCELL® Cloud12 exposure system at dose levels relevant for observing *in vitro* hazard responses suggesting VITROCELL® Cloud12 could be great platform for ALI lung cell cultures for *in vitro* hazard assessment of aerosolized materials (Bannuscher et al., 2022).

However, this system has some complexity and limitation. The processes governing aerosol transport and deposition are well characterised, and exposure conditions (temperature, humidity, and deposited mass) can be tightly controlled. The exposure system standardises aerosol delivery independently of the biological model used. Importantly, ALICE-Cloud is compatible with multiple biological systems, including epithelial monocultures, epithelial-immune co-cultures, fibroblast co-cultures, tissue slices, and organoid-derived epithelial layers grown on permeable membranes. The exposure platform therefore complements complex biological models by providing a consistent and quantifiable aerosol delivery method across increasing levels of tissue complexity.

3.3. Lung organoids

Lung organoids represent one of the most advanced *in vitro* platforms for modeling human lung development, injury, and disease, including responses to inhaled pollutants such as BSF. Organoids are self-organizing three-dimensional structures derived from primary tissue-resident stem/progenitor cells or induced pluripotent stem cells (iPSCs) that recapitulate key aspects of lung architecture, cellular diversity, and function (van der Vaart et al., 2021). Depending on the differentiation protocols employed, lung organoids can model distinct anatomical compartments of the respiratory system, including proximal airways, distal alveoli, and bronchioalveolar transition zones. Advanced protocols yield organoids containing multiple relevant epithelial cell types (*e.g.*, basal cells, secretory cells, ciliated cells, type I and type II alveolar epithelial cells), as well as supporting mesenchymal, endothelial, and in some cases even early immune-like elements.

Lung organoids offer several advantages over traditional 2D or ALI systems for modeling BFS exposure: (a) Complex 3D architecture allows for cell-cell and cell-matrix interactions that govern tissue homeostasis, repair, and remodelling. (b) Stem cell-driven self-renewal capacity enables long-term expansion, repeated exposures, and modeling of chronic injury-repair cycles relevant to repetitive bushfire seasons. (c) Patient-specific organoids derived from primary lung biopsies or iPSCs allow personalized modeling of inter-individual susceptibility to BFS, including genetic predispositions and pre-existing comorbidities such as asthma, COPD, cystic fibrosis, and idiopathic pulmonary fibrosis (Shrestha et al., 2023).

Emerging applications of lung organoids in BFS research include modeling epithelial regeneration following PM-induced injury, dissecting pathways of epithelial-mesenchymal transition (EMT), studying particulate-induced fibrotic remodelling, and screening candidate therapeutics aimed at restoring epithelial barrier function or mitigating

oxidative injury (Kong et al., 2021).

However, significant technical challenges remain for applying lung organoids to inhalation toxicology. Organoids are typically maintained in submerged conditions, limiting direct exposure of the epithelial surface to airborne BFS particles and gases. Adaptations for aerosol delivery or microfluidic perfusion are still under development. The absence of resident immune cells, vascular compartments, and functional air-liquid interfaces limits full recapitulation of *in vivo* BFS exposure and systemic responses. Standardization of organoid differentiation protocols and BFS exposure metrics remains an ongoing challenge, hindering direct comparison across studies (Kong et al., 2021). To overcome these limitations, integration of lung organoids into lung-on-chip microfluidic platforms is gaining momentum. These hybrid systems combine the self-organizing complexity of organoids with dynamic air exposure, vascular perfusion, and mechanical cues, offering an unprecedented level of physiological realism for modeling inhalation injury.

In the context of BFS research, such integrated models may provide unparalleled insight into long-term consequences of repeated wildfire smoke exposure, mechanisms of airway remodelling, fibrosis progression, and discovery of targeted interventions aimed at protecting vulnerable populations. While lung organoids are better than 2D and single cell monolayer system, it is still an *in vitro* system which lack advantages of other advance *ex vivo* system such as precision-cut lung slices (PCLS) where we get cells' native biological environment and preserving the cell-to-cell and cell-to-matrix interactions.

3.4. Precision-cut lung slices (PCLS)

Animal models can provide important insights into the pathophysiology of chronic lung diseases and facilitate the identification of therapeutic targets. However, their biology and physiology differ significantly from that of humans, limiting potential translational relevance. Human PCLS retain the native structure and extracellular matrix (ECM) composition of the intact lung, providing an *ex vivo* model of living tissue to bridge the gap between cell-based and *in vivo* studies (Lehmann et al., 2024) (Fig. 2). PCLS contain all resident lung structural and immune cells *in situ* (Crue et al., 2023), closely representing the cells' native biological environment and preserving the cell-to-cell and cell-to-matrix interactions which are lacking in conventional 2D models. Multiple PCLS that can be prepared from tissue samples from patients with asthma, COPD, lung cancer, lung fibrosis and viral infections, allowing researchers to study disease at the molecular level (Alsafadi et al., 2020), and providing a particularly useful approach for capturing clinical heterogeneity (Stegmayr and Wagner, 2021). Chemically-induced changes in airway and ciliary dynamics and vasoconstriction can be observed in PCLS (Studley et al., 2024). For example, exposure of PCLS to menthol-containing e-cigarette condensates impaired contractile responsiveness of airways to methacholine and dampened ciliary beating (Herbert et al., 2023). In addition, single cell RNA-sequencing of human PCLS has shown that vaping extract augmented pro-inflammatory responses in lung epithelial cells and fibroblasts (Crue et al., 2023). Recently, PCLS were used to assess the harmful effects of bushfire smoke exposure in PCLS where authors showed that exposure to bushfire smoke particulate matter (PM)-2.5 and PM10, but not PM1, increased senescence >2-fold in human PCLS at 72 h ($p < 0.05$ *cf.* untreated) (Papagianis et al., 2025). Although most PCLS studies have been conducted under static conditions, cyclic stretching of PCLS can be incorporated to mimic breathing, resulting in an amplified inflammatory response without altering airway contraction following exposure to cigarette smoke extract (Mondonedo et al., 2020). The lack of an intact immune system in *ex vivo* PCLS is also being addressed, with co-culture models of PCLS with inflammatory cells such as macrophages becoming more widely adopted. These novel approaches provide further support for the relevance of PCLS models to investigate the effects of pollutants, permitting higher throughput measurement of outputs complementary to those obtained from *in vivo* studies.

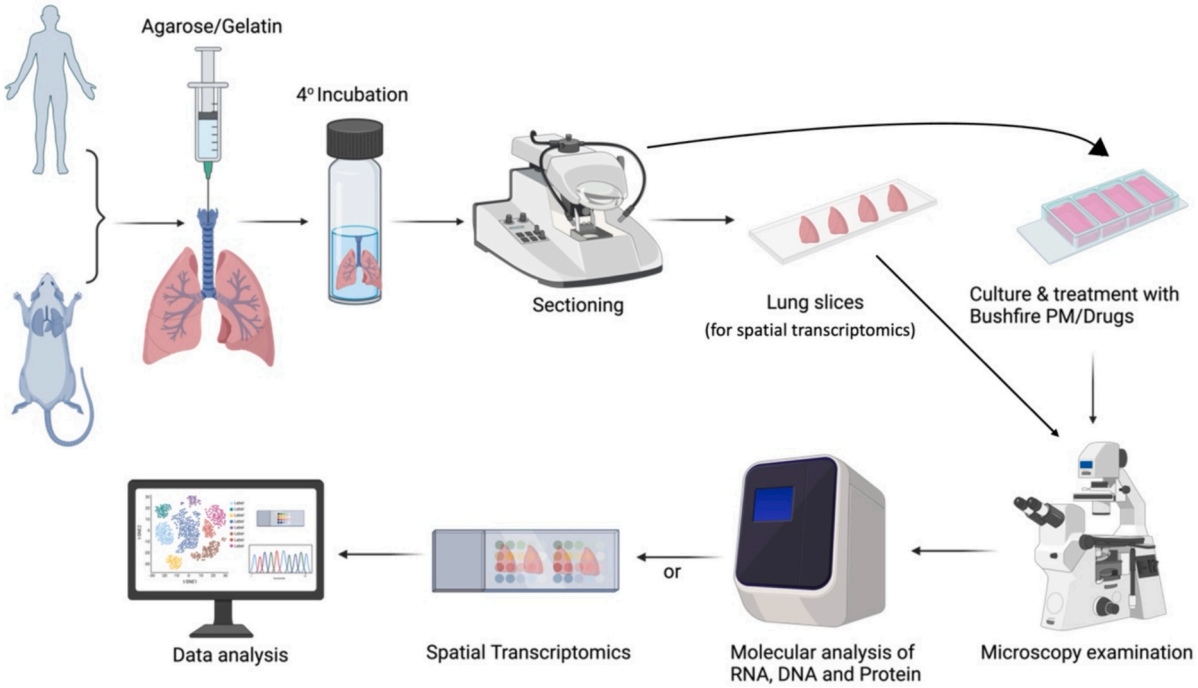


Fig. 2. Precision-cut lung slices (PCLS). Human/animal lungs are inflated with melted agarose/gelatin which solidifies when cooled to 4° C thereby retaining inflated lung architecture. Lungs are sectioned into slices and cultured in media followed by challenge with stimuli such as pollutants and/or treatment with drugs of choice for several days without loss of viability. While functional responses such as airway contraction and cilia beating can be visualized in living PCLS, additional molecular and biochemical analyses such as immunohistochemistry or spatial transcriptomics/proteomics and RNA can be performed on fixed or frozen samples, while DNA and protein extracted from PCLS and conditioned PCLS media can be used for other analyses. Figure was drawn in BioRender.com.

3.5. Microfluidics and lung-on-a-chip

Pre-clinical drug development studies are currently conducted on animals because conventional cell culture models fail to mimic the functional and structural complexity of human disease processes and physiological response at an organ level. Despite the ability to induce differentiated tissue-specific phenotypes using 3D extracellular matrices, this approach still lacks the ability to mimic the spatial arrangement and architecture of multiple tissues (Huh, 2015). Cells in conventional cell culture are not exposed to physiological mechanical signals, such as fluid shear stress, strain, compression, or specific dimensional and geometric nanostructures. Recently, researchers developed advanced microfabricated microfluidic biosystems known as organ-on-chips, which contain continuously perfused channels with living cells to mimic the physiological functions of tissues and organs (Francis et al., 2022). LOC models are microfluidic cell culture models (Fig. 3) that reproduce these crucial physiological functions and dynamic responses by mimicking the cellular environment and 3D lung architecture of an *in vivo* lung. LOC can mimic different functional units of the lungs by simulating different cell types, structural organization, and biochemical and biophysical microenvironments (Zhu et al., 2022).

They are designed to model specific diseases such as asthma, COPD, lung cancer, pulmonary embolism, fibrosis, obstructive sleep apnoea; infections (tuberculosis, pneumonia, COVID-19, RSV), toxicological analyses for cigarette smoke, nanoparticles exposure and BFS, air pollutants and allergens as well as drug screening studies to identify effective therapeutics and diagnostic biomarkers (Bovard et al., 2018). With further technological and optimization advances, personalized LOC models can be developed to study complex diseases and identify the most effective therapies for personalized medicine. LOC still lack an immune system or systemic effects and should ideally be performed complementary with *in vivo* animal models.

3.6. Bridging epidemiological evidence and in vitro mechanisms in bushfire smoke inhalation research

The bridge between epidemiology and *in vitro* mechanisms in bushfire inhalation studies is built by exposure science plus biologically informed dose extrapolation plus intermediate biomarkers that are measurable *both* in populations and in cell/organ models. While epidemiology focusses on ambient concentrations (*e.g.* $PM_{2.5}$ $\mu g/m^3$ over time), *in vitro* work uses delivered cellular dose ($\mu g/cm^2$ of bushfire PM) and its pathological outcomes. A few important factor to consider while linking epidemiology and *in vitro* mechanisms includes the epidemiology parameters such as linear or non-linear increases in hospital emergency room visits, asthma exacerbations, COPD admissions, mortality per 10 $\mu g/m^3$ bushfire $PM_{2.5}/PM_{10}$, the bushfire $PM_{2.5}/PM_{10}$ concentrations in the environment (air quality index), breathing rate (rest *vs* exercise, infant *vs* adult, healthy *vs* disease), exposure duration (acute bushfire exposure *versus* prolonged exposure over time), bushfire PM deposited mass per airway region (tracheobronchial *vs* alveolar).

The toxicity profile of bushfire PM may vary in different country. For example, Australia is predominantly covered by Eucalyptus species that gets burnt during bushfire event. Bushfire PM is not generic $PM_{2.5}$, it content high organic carbon, redox-active compounds, PAHs and quinones, heavy metals. Epidemiology can stratify by fire type (forest *vs*

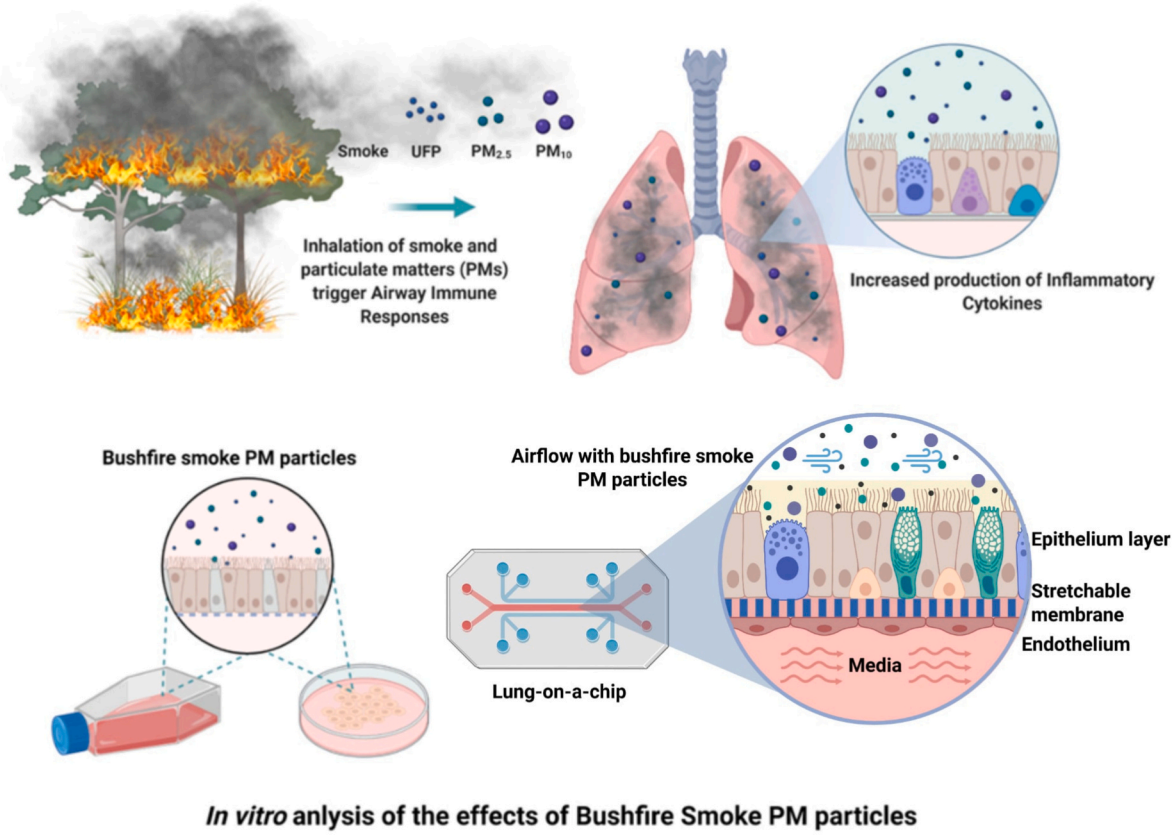


Fig. 3. Exposure of bushfire particulate matter to lung-on-a-chip in a microfluidics device. The lung-on-a-chip model consists of two chambers separated by a thin, porous, flexible membrane. The upper chamber is lined by airway epithelium exposed to air flow to mimic the alveolar environment and is connected to the side vacuum channels to replicate the physiological inspiration and expiration movements. The lower chamber is the endothelium chamber, perfused with pumps to have a continuous flow of medium to represent the *in vivo* blood circulation. This set-up closely mimics the *in vivo* alveolar-capillary interaction found in human lungs at the air-liquid interface. Both chambers can be used to introduce drugs, aerosols, toxins, nanoparticles, or supplements, depending on the aim of the study. In this context, the upper chamber is infused with bushfire smoke PM particles in the upper chamber. The figure was drawn using Biorender.com

grassland), smoke age (fresh *vs* aged), distance from source and *in vitro* can match chemical profiles, compare toxicity (based on vegetation burnt), pathological changes on health.

A major translational failure point is ignoring what actually reaches the cells. For this, the use of appropriate lung deposition model is essential. The multiple-path particle dosimetry model assessing the health impact of inhaled bushfire PM aerosol could be used as suggested by Mori et al. for tobacco product aerosol dynamics (Mori et al., 2024). Another computer based model; LUDEP (Lung Dose Evaluation Program) is used to estimate the contribution of ultrafine particles to lung deposition. This model used by Kawanaka showed that about 95% of the lung deposition of inhaled mutagens is caused by fine particles for both roadside and suburban atmospheres (Kawanaka et al., 2011).

4. *In vivo* models

4.1. Exposure techniques

BFS-derived PM can be exposed to animal models *via* intratracheal, intranasal, oropharyngeal, insufflation, inhalation (whole-body inhalation in exposure systems, nose-only inhalation) methods. The most recommended techniques of intranasal and intratracheal administration of coarse PM *in vivo* has been discussed (Linderholm et al., 2015). Recent advanced techniques such as the InExpose system to expose small animal (rats, mice) to re-aerosolized PM are gaining popularity (Lechasseur et al., 2019).

4.2. inExpose system

The inExpose system (SciReq, Canada) is portable and can operate inside a fume hood. It enables acute and chronic sample exposures (pollutants, PM, smoke, e-cigarette) *via* nose-only and/or whole-body exposure of mice and rats (Fig. 4) (Cui et al., 2020). The whole-body exposure chamber can be assembled for *in vitro* (cell culture, organoids, LOC) exposure of aerosols, BFS and drugs. The small form factor and low internal volumes reduce costs, required sample sizes an drug amounts. Although, the use of inExpose system to study BFS has not yet been published, it has been applied to studying the effects of cigarette smoke (Cui et al., 2020) and other PM (*e.g.*, silica dust). Tobacco cigarettes or cigarettes made from bushfire material, are placed in the integrated cigarette smoking robot, which electronically lights the cigarette and smoke passes through the nose-only or whole-body chamber. For exposure to BFS extract, samples are diluted (*e.g.*, PBS) and placed in the aeronab (up to 2 ml). Once the aeronab is started, a PM aerosol is generated that passes through the tiny mesh that governs particle size for exposure to animals or cells. For *in vitro* studies, it is recommended to expose the cells for <30 min as the system does not create ideal condition of temperature, humidity and 5% CO_2 as in a cell culture incubator.

4.3. Intranasal exposure of bushfire PM to small animals (mice, rats)

Although BFS has great impact throughout the respiratory system, what affects the lungs in the long term is the deposition of smoke PM that is difficult to clear (Peters et al., 2021). This can be studied by

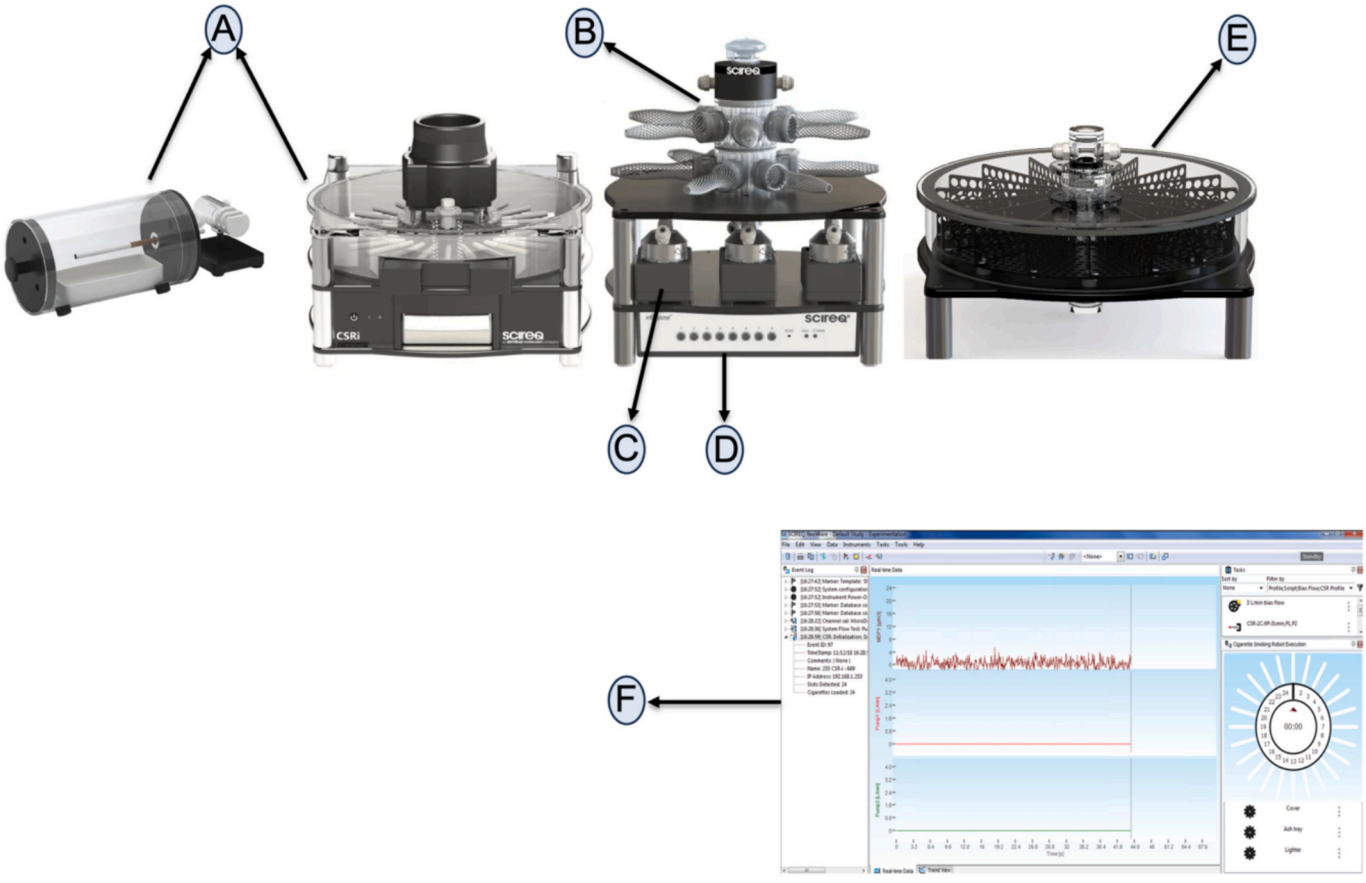


Fig. 4. InExpose system (Alasmari et al., 2018). Exposure of aerosolized particles or smoke using the InExpose system involves six functioning components. (A) A manual single cigarette unit chamber or robotic multiple cigarette unit chamber where cigarettes can be burnt and flow for either (B) Nose-only or (E) whole body exposure. (C) Pumps generate smoking and flow to (B) or (E). (D) The base unit has various ports to connect the system to the (F) flexiware software that controls the whole process of smoke or PM exposure.

isolation of bushfire PM into different sizes. PM of the desired size can be dissolved in a suitable vehicle (*e.g.* PBS). Mice are placed in a box supplemented with oxygen and anesthesized with 3–5% isoflurane. Then 20–50 μl (volume depends on animal age/size) of PM is administered dropwise into the opening of the nares. Mice are held upright for >20 s and then returned to their cages (Seo et al., 2023).

4.4. Intratracheal instillation of bushfire PM in small animals (rat, mice)

Intratracheal instillation of bushfire PM can be performed similar to published studies where authors have intratracheally administered environmental and traffic PM (Alewel et al., 2023). To avoid stress to animals, non-invasive intratracheal instillation should be practice as elaborated by Ortiz-Munoz and Looney (2015). Animals are anesthesized using ketamine and xylazine intraperitoneal injection and placed in the intubation platform. Using curved blunt-ended forceps, the tongue is moved to visualize the larynx using a light source. Polyethylene tubing attached to an insulin needle and syringe is inserted 0.5–1 cm into the trachea and bushfire PM solution is instilled (in 50 μl of PBS). To avoid the instillate from escaping the trachea, the tubing is held in place for >5 s after instillation and the animals are maintained in the same position for >30 s. Animals are then placed on a heating pad for recovery before return to their cages (Ortiz-Munoz and Looney, 2015).

5. Assays/readouts

Various pathological changes in response to BFS/extract or PM can be assessed *ex vivo/in vitro* using primary or immortalized cells. Inhaled BFS travels deep into the lungs, while PM deposition along the respiratory tract is proportional to its size. Larger particles (*e.g.*, PM_{10}) deposit in the mouth and throat and upper airways, whereas smaller particles ($PM_{1,\ 2.5}$) reach the alveoli. Particles <1 μm can be exhaled, reducing deep-lung deposition. As the lungs are the important organ exposed to inhalation, airway epithelial cells, alveolar macrophages and lungs fibroblasts can be used to assess various cellular responses to understand pathophysiology associated with BFS exposure.

5.1. Oxidative stress

Assessment of oxidative stress uses various fluorescence-based assays to quantify total cellular reactive oxygen species (ROS) or the levels of gene expression and proteins involved in oxidative stress or antioxidant defense. Measurement of total ROS generation by cells after exposure to BFS or PM, uses fluorescence quantification with 2′,7′-Dichlorofluorescein diacetate (DCF-DA) like other have used for *in vitro* model such as cigarette smoke extract induced COPD (Paudel et al., 2022). This is a cell permeable probe that upon oxidation undergoes deacetylation to 2′,7′-dichlorofluorescein to form fluorescence DCF measured spectrophotometrically or by imaging (Paudel et al., 2024). Similarly, other fluorescence staining dyes, such as dihydrorhodamine 123, C11-BODIPY$^{581/591}$ are commonly used to measure ROS (Forkink et al., 2010).

To measure mitochondrial specific oxidative stress and function, the Agilent seahorse analyser is used to assess oxygen consumption rate (24 or 96 well format) or staining cells with mitochondrial specific fluorescence stains such as MitoSox-red, cpYFP, HyPer, and roGFPs (Forkink et al., 2010). To analyze oxidative stress/antioxidant protein levels, immunoblotting or immunocytochemistry is ideal while for gene expression qPCR is standard. The levels of gene expression of antioxidants NADPH quinine oxidoreductase-1 (*NQO1*); γ-glutamyl cysteine synthetase catalytic subunit (*GCLC*), glutathione peroxidase 2 (*GPX2*) and pro-oxidant NADPH oxidase *(Nox)-2B* and *-4* (among many others) in human bronchial epithelial cells are often assessed as indicators.

5.2. Inflammation

In vitro airway inflammation is examined in BFS/PM-exposed bronchoepithelial cells or alveolar macrophages by quantifying the levels of inflammatory cytokines in culture supernatants using ELISA or cytometric bead array. Protein assays can also be performed measuring many inflammatory proteins in the same sample, including cytokines (interleukin-6, IL-8, IL-1β, IL-10, IL-4, IL-13, TNF), inducible nitric oxide (NO) synthase (iNOS), cyclooxygenase (COX)-2, 5-lipoxygenase (5-LOX), prostaglandins, while gene expression is easily assessed by qPCR. The massive production of NO by activated macrophages and airway epithelial cells is another source of airway inflammation, which is converted to peroxy nitrate that leads to further cellular damage/inflammation (Paudel et al., 2020). The Griess reagent colorimetric assay is commonly used to examine the level of NO in terms of nitrate in cell culture supernatants. For confirmation, these data should be correlated with high protein levels of iNOS. One study investigated the potential of coarse ($PM_{10–2.5}$), intermodal size range; coarse ($PM_{10–2.5}$), intermodal size ($PM_{2.5–1}$), fine ($PM_{1–0.2}$), and ultrafine ($PM_{0.2}$) particles from wildfire smoke to cause cytokine production (TNF, IL-6, MIP-2) using ELISA and NO production using Griess reagent in a murine macrophage cell line (RAW264.7). PM-induced cytokine production reduced along with particle size, but the size range had a much smaller impact on NO levels (Jalava et al., 2006).

5.3. Mucus production

Cigarette smoke is well-known to caused excessive mucus production that limits the lungs' ability to function (Liu et al., 2022). Mucus is produced by airway epithelial cells, and is regulated by mucus producing genes (MUC1–4, MUC5AC, MUC5B, MUC6–8, MUC13, MUC16, MUC19) (Thai et al., 2008). Since the expression of these genes is regulated by cytokines and cell signalling pathways, a good approach is to validate the mechanisms of induction of gene expression by these factors. For example, MUC2 and MUC5AC expression/activation is regulated by the inflammatory cytokines IL-1β, TNFα and IL-4, and mitogen-activated protein kinase, nuclear factor-κB, MyD88 and epidermal growth factor receptor signalling pathways (Thai et al., 2008). In regard to BFS/PM exposure, the potential of these factors to induce the expression of mucus production genes should be explored. In ALI culture of airway epithelial cells, BFS/PM increased the production of mucus, in an attempt to clear noxious agents from the airways, however, excessive mucus production blocks the airways and limits lung function (Roscioli et al., 2018).

5.4. Airway remodelling

Airway remodelling is the gradual disruption and modification of cellular and structural changes in airways. This includes ECM deposition like collagen, increased smooth muscle mass, disorganisation of tissue architecture, increased airway wall thickness and subepithelial fibrosis leading to severe airway obstruction (Bergeron et al., 2007). In COPD, Asthma, COVID-19 and IPF airway remodelling is an established key driver of chronic disease features (CLD). People with these existing lung diseases are at highest risk of the detrimental effects of BFS/PM exposure, and smoke and air pollution are known inducers of airway remodelling (Cui et al., 2023). Therefore, studying airway remodelling can be beneficial in understanding the effects of BFS exposure. It can be measured in bodily fluids, and by radiological, histological and lung function assessment. Assessing bodily fluids such as broncho-alveolar lavage (BAL) can be used to define remodelling, as well as the inflammatory cell composition of the lungs mainly immune and some bronchoepithelial cells. Remodelling markers like collagen, profibrotic cytokines/chemokines, matrix metalloproteases (MMP) like MMP-9 and inhibitors of metalloproteases (TIMP) can be assessed in BAL (Camargo et al., 2023). ECM or remodelling makers can also be assessed in BALF, sputum, blood and urine by ELISA or activity assays (Bergeron et al., 2007). Histological examination of lung tissues is highly invasive but effective in determining remodelling by Picrosirius Red Staining and

Masson's trichrome, which detect collagen fibers and can be quantified by image analysis (Courtoy et al., 2020). In terms of lung function, a longitudinal study from adolescents to young adults showed that a low post-bronchodilator forced expiratory volume in 1 s (FEV_1):vital capacity (VC) ratio indicated airway remodelling in asthma (Rasmussen et al., 2002). Recently emerged techniques like single-cell and spatial transcriptomics will be valuable in defining entire cell states and entire disease pathways and networks during remodelling (Lang et al., 2023).

5.5. Cellular senescence

Cellular senescence is established as a key driver of chronic lung disease (Jha et al., 2024). There are various markers of senescence and anti-aging molecules that can be studied for roles in pathogenesis and therapeutic targeting. The mostly commonly used senescence markers includes senescence associated secretory phenotype, senescence-associated β-galactosidase (SA-β-Gal) protein activity (the archetypal senescence marker), cyclin-dependent kinase inhibitor 2A (CDKN2A, aka p16), and CDKN1A (p21), and anti-aging molecules includes SIRT1 and Klotho.

Senescence is a mechanism by which healthy cells undergo cell cycle arrest due to injury, aging or stress due to various genetic and external factors like cigarette smoking, air pollution and BFS. Cellular senescence leads to reduced tissue function and increased susceptibility to various pathologies (Scieszka et al., 2023). It is well known that cigarette smoking, and air pollution increase oxidative stress, cellular inflammation, mitochondrial dysfunction, genomic instability, and epigenetic modifications that promote cellular senescence (Scieszka et al., 2023). Early studies indicate that BFS/PM has a similar impact on cellular senescence contributing to accelerated aging in humans (Scieszka et al., 2023). Cellular senescence can be assessed by various *in vitro* and *in vivo* techniques (Fig. 5). The most indicative is SA-β-Gal protein activity assessed by simple colorimetric assay that stains for breakdown of the substrate β-Gal (Gonzalez-Gualda et al., 2021). DNA synthesis can be assessed using a modified DNA nucleotide dye like 5-bromo-2-deoxyuridine (BrdU), with reduced staining indicating a reduced cell cycle. Furthermore, senescence can also be measured *ex vivo* and *in vivo* by immunohistochemistry of aforementioned senescence markers (Fig. 5).

5.6. Apoptosis/necroptosis

Wildfire smoke extract exposed to airway epithelial cells in ALI culture induced a remarkable level of blockage of autophagy evidenced by increased levels of microtubule-associated protein 1A/1B-light chain 3 (LC3-II) coupled with increased sequestosome levels. Exposure also induced apoptosis by facilitating the cleavage of (Poly-(ADP)-Ribose Polymerase (PARP), and reduce protein abundance of tight junction proteins zonula occludens-1 and claudin-1 (Roscioli et al., 2018). Necroptosis signalling mediated by receptor interacting protein kinases (RIPKs) and mixed lineage kinase domain-like pseudokinase (MLKL) promote airway inflammation, remodelling, and emphysema (Lu et al., 2021). BFS extract exposure to THP-1 macrophages for 24 h increased the levels of active caspase-1 detected by immunofluorescence or confocal microscopy, decrease protein levels of anti-apoptotic Bcl-2 and increased PARP cleavage detected by immunoblot (Hamon et al., 2018). Therefore, assessment of the potential of BFS to induce apoptosis or necroptosis using these techniques or by specific gene knockout using CRISPR/Cas9 *in vitro* would elucidate their roles in pathogenesis.

5.7. Transepithelial ion transport

An integral component of mucociliary clearance is the maintenance of airway surface liquid (ASL) volume and composition, ensuring effective function (Randell et al., 2006). Transepithelial ion transport plays a crucial role in this, particularly through sodium and chloride transporters that create an osmotic gradient, attracting water for mucus hydration. The best characterised transporter is the cystic fibrosis transmembrane conductance regulator (CFTR), which serves as a cyclic (c)AMP-regulated channel for chloride and bicarbonate efflux (Saint-Criq and Gray, 2017). Other proteins including, epithelial sodium (ENaC), potassium (KCs), Ca^{2+}-activated Cl^- (CaCC's), SLC26A9, and $Na^+/K^+/2Cl^-$ (NKCC) channels, have roles in ion transport in the airway epithelium.

Air pollution and chronic respiratory diseases are associated with acquired dysfunction in epithelial ion transport, though the underlying mechanisms remain unknown. Loss-of-function genetic variants in the CFTR gene, responsible for cystic fibrosis, result in dysregulation of ASL, increasing mucus viscosity, recurrent infections, inflammation and respiratory failure. CFTR dysfunction occurs in asthma, COPD (Dahl and Nordestgaard, 2009) and following exposure to air pollutants of tobacco smoke, ozone, heavy metals, and diesel particulates (Rasmussen et al., 2014). Proposed mechanisms for acquired CFTR dysfunction include CFTR internalization, altered channel gating, and downregulated production. However, the underlying cellular and molecular pathways driving this dysfunction remain unclear.

Several *in vitro* methods are used to assess CFTR function as an anion channel, such as evaluation of anion flux using iodide selective electrodes, radio-isotope tracers or fluorescent probes, electrophysiological methods that measure ionic current using Ussing chambers, patch-clamping and forskolin-induced swelling (FIS) assays on organoids (Ramalho et al., 2022). *Ex vivo* CFTR assessment uses intestinal current measurement (ICM) (Mall et al., 2004), and *in vivo* uses sweat chloride concentration (LeGryset al., 2007), ß-adrenergic sweat test (Quinton et al., 2012), and nasal potential difference (Knowles et al., 1995).

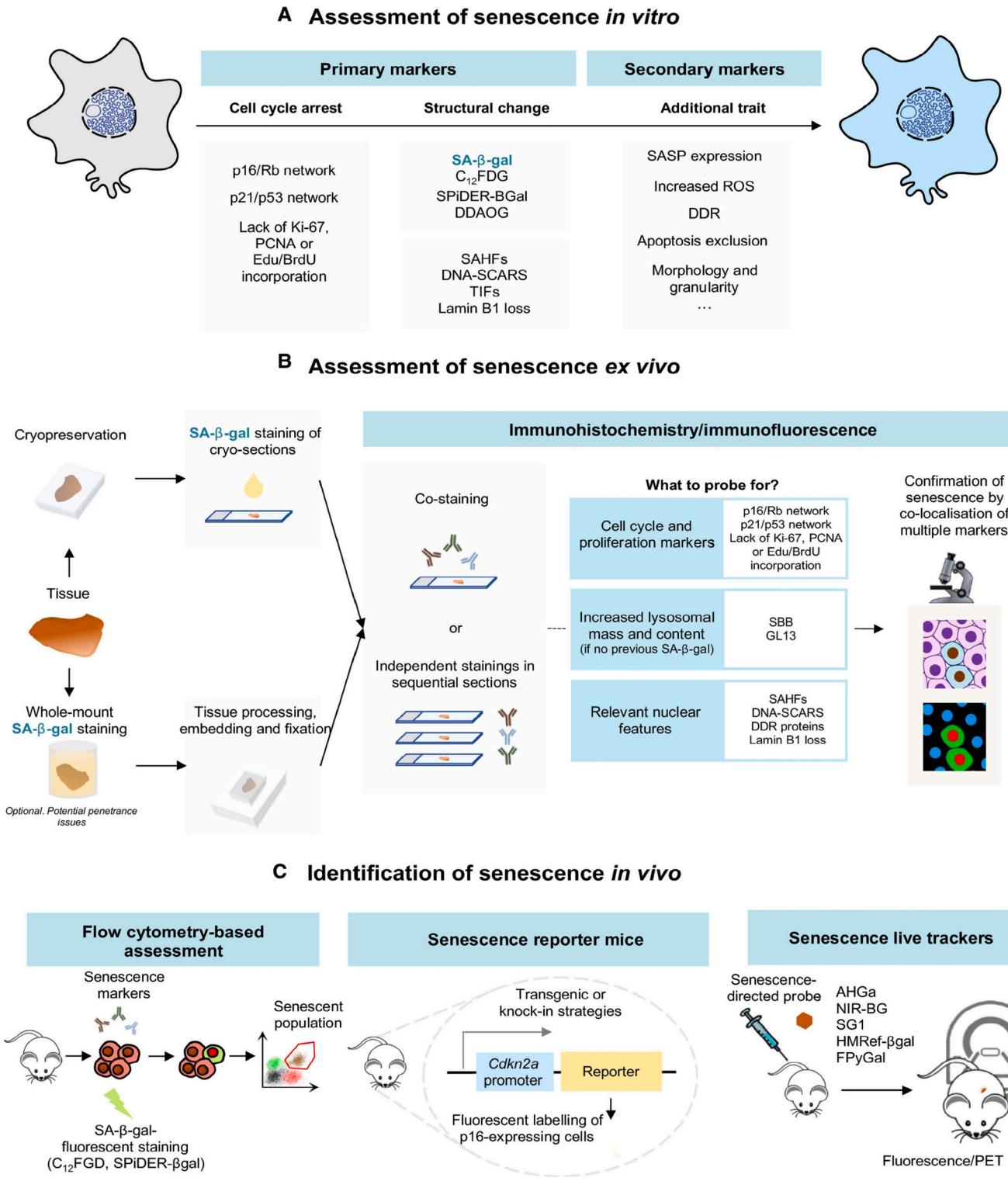
6. Effects of BFS exposure *in vivo*

Establishing *in vivo* models for use across multiple groups studying the same pollutant is commonplace. Here we discuss and make recommendations based on established models from studies using BFS, however, there are few studies that have done this. Several studies undertaken by the Last group were the first and only ones to investigate the effects of BFS PM in an animal model (Wegesser et al., 2010; Williams et al., 2013). BALB/c mice were administered wildfire smoke PM collected from filters during the 2008 California wildfires *via* intratracheal instillation. The outcomes of this study highlighted that wildfire smoke PM was much more toxic to the lung on an equal weight basis than was PM collected from normal ambient air in the same area. Lung damage was observed as high count of neutrophils and level of protein in lung lavage and by histological analysis that showed increased inflammatory cell influx and edema in the lung (Wegesser et al., 2009).

Another study in Calgary, Canada, examined the effects of bushfire pollution in thoroughbred polo horses following a month of exposure ($PM_{2.5}$ daily mean exposure of 35.51 μg/m^3). Exposure of horses (n = 12) to high levels of respirable PM led to smoke-induced mild asthma that was not ameliorated by dexamethasone compared to saline treatment. Aerobic enhancement was attributed solely to improved air quality, with no significant improvements attributed to dexamethasone or salbutamol (Bond et al., 2020). BAL cell counts during and after pollution exposure revealed that the proportion of macrophages increased, lymphocytes and eosinophils decrease, whereas neutrophils and mast cells were maintained (Bond et al., 2020). They suggest that because none of the horses had a history of asthma prior to the exposures that the clinical signs of mild asthma were associated with chronic smoke exposure and recommended an improvement of air quality to improve the respiratory health of horses in the future.

6.1. Inflammation

Measures of inflammation in the lungs assess many parameters as a single metric cannot fully encapsulate the complexity of inflammatory processes *in vivo*. Cell counts in BAL and lung tissue are useful global



(caption on next page)

Fig. 5. Assessment of senescence *in-vitro*, *ex-vivo* and *in-vivo*. (A) Schematic for the investigation of cellular senescence using *in vitro* techniques. Cells of interest are stimulated (example with bushfire smoke PM) to senesce at various time course. Then, the hallmarks of senescence are examined, first confirming cell cycle arrest and the halt of proliferation using markers [Ki-67, proliferating cell nuclear antigen (PCNA)], some senescent cells also grow in size compared to health non-senescent cells. This is the defined by additional characteristics, such as increased SA-β-Gal, changes in organelle structures (Lamin B1 downregulation) and/or markers of epigenetic changes [senescence associated heterochromatin focis (SAHFs)]. Then markers of specific types of senescence such as DNA damage response-related markers, increased reactive oxygen species (ROS) levels or senescence associated secretory phenotype (SASP) expression to define the senescent phenotype. (B) Workflow to assess cellular senescence in tissues *ex vivo*. Tissues are processed by different methods depending on the desired immuno-detection read-out. If histochemical analysis is required, samples are processed, preserved and fixed in a way that is incompatible with subsequent SA-β-Gal staining. For this reason, samples can be whole-mount stained for such SA-β-Gal activity first before processing. Alternatively, if the tissue is cryo-preserved and cryo-sectioned, fresh sections can be stained for SA-β-Gal activity, overcoming the drawbacks associated with whole-mount staining. For subsequent multiparametric staining, probing for a minimum of two additional markers is recommended (if prior SA-β-gal staining was performed) or three markers (if no SA-β-gal staining) that represent cell cycle arrest and absence of proliferation, and increased lysosomal compartment and other relevant nuclear or structural features associated with cellular senescence. Such stainings should be performed in combination on the same tissue section or as independent stainings in sequential sections, so that cellular senescence can be verified with high levels of confidence by the co-localization of multiple parameters in the same cell. (C) Approaches currently used to identify senescence in living animals using flow cytometry-based approaches, senescence-reporter mice or administration of probes that detect cellular senescence *in vivo*. The figure is reproduced with permission from E. Gonzalez-Gualda et al. (2021).

indicators of inflammatory responses (Pinkerton et al., 2022). Detailed characterisation by flow cytometry or cytometry by time of flight (CyTOF) are powerful but caution should be taken when applying changes in whole lung tissue to specific localisations and *vice versa*. Furthermore, cell number alone does not convey activity, with environmental exposures and chronic diseases associated with impaired cellular functions such as phagocytosis and antigen presentation (Li et al., 2022). Changes in inflammatory cytokines and chemokines, whether measured by gene expression or protein levels, are commonly used as indicators of inflammation but their precise impacts are mediated by cellular responses and localisation of effects (de Souza Xavier Costa et al., 2020). With state-of-the-art technologies such as spatial transcriptomics and proteomics, the ability to identify molecular changes driving pathology and cellular responses will be vastly improved and better characterize inflammatory responses in the lungs (Bressan et al., 2023).

6.2. Histopathological and airway remodelling and changes in vivo

Histopathological assessment of lung tissue is a major advantage of mouse models, permitting broad characterisation of structural changes underpinning disease that cannot be achieved in humans without excising the lungs. Assessment of alveolar diameter and destrictive index characterises emphysematous damage, and airway nuclei number and epithelial thickening can be performed with most tissue stains (Liu et al., 2022). More refined measures of airway remodelling require specific staining, such as quantification of collagen deposition using sirus red-fast green or Masson's trichrome, and of mucus secreting cells using periodic acid-Schiff. Additionally, molecular measures of remodelling such as quantification of hydroxyproline or soluble collagen or characterisation of markers of epithelial-mesenchymal transition are important indicators, although they lack spatial selectivity of other methods.

6.3. Lung function

Impaired lung function is a primary outcome in chronic lung diseases, and therefore its assessment in animal models is essential for the translatability of findings to humans. Underlying pathological processes such as inflammation and changes in lung histopathology are not those reported by patients or routinely recorded clinically, whereas respiratory symptoms (*e.g.* breathlessness) and clinical measures of lung function are essential for accurate diagnoses and assessment of patient health.

A variety of techniques have been developed for assessing mouse models. Forced oscillation techniques (FOT) assess airway impedance after emitting different frequencies of oscillatory pressures during respiration (Bhattarai et al., 2020), requiring insertion of a cannula into the trachea and therefore is only measureable as a terminal endpoint. However, this technique permits characterisation of a greater variety of respiratory system mechanical properties including lung and airways resistance, elastance, energy dissipation and lung capacities, and has a greater precision relevant to patients (Mori et al., 2022). Furthermore, FOT can be combined with methacholine challenge to assess airways hyperresponsiveness. For these reasons, FOT is the most commonly reported measure of lung function in mice. Additionally, direct measures of diffusing lung capacity are increasingly implemented in animal studies through instillation and recovery of carbon monoxide to the lungs, which is directly related to diffusing capacity measurements in people. Gomez et al., used a single breath hold maneuver to assess the effect of landscape file smoke PM_{10} airway exposure on gas exchange (diffusing factor of the lung for carbon monoxide), and airways hyperresponsiveness to methacholine using the flexiVent FX1 system (SCIREQ, Montreal, Quebec, Canada). Authors observed that landscape fire smoke airway exposure impairs respiratory function and worsens experimental asthma in mice (Gomez et al., 2024). Other lung function parameters that can be measured in mice and are relevant to human lung function includes lung volume, dynamic compliance, static lung compliance, inspiratory capacity, force vital capacity, total lung capacity (Liu et al., 2022).

6.4. The limitations of interspecies differences in traditional animal models

While small animal like mice and rats are extensively used *in vivo* model to elucidate the impact of pollutant exposure, they possess several limitations of interspecies differences when comorped with human physiology. For example, a) mice/rats are obligate nose breathers and humans are not, b) mice/rats lack extensive respiratory bronchioles like human do, c) there is huge difference in airway surface area–to–body mass ratio, d) mice/rats has thinner epithelial layers, fewer goblet cells, e) particle filtering in the nasal cavity is much higher in mice/rats, f) $PM_{2.5}$ and ultrafine particles deposit differently, g) central *vs* peripheral lung injury patterns do not match humans (Stucki et al., 2024). Anatomically, one of the major differences in human *versus* rats lungs is that human left (two lobes) and right (three lobes) lungs are similar in shape whereas the rat left (one lobe) and right (four lobes) lungs are significantly different. A very detailed summary of key differences in the macroscopic anatomy, microscopic anatomy, and physiology of the respiratory tracts of rats and humans with clear implications for toxicity testing was explained in recent publication by Stucki et al. (2024).

Thus, it is important to select an animal model depending on the question the researcher wants to answer. For instance, if a researcher wants to study healthy humans, a good starting point will be the C57/BL6 background considering this strain shares genetic similarity (Rivera and Tessarollo, 2008). Another consideration to choose animal model is immune system divergence. Researcher should look into different toll like receptor expression patterns (human *versus* mice/rats), stronger neutrophilic bias in mice, divergent inflammasome activation

thresholds, different Th1/Th2/Th17 balance, poor modeling of chronic airway inflammation, the fact that IL-8 (CXCL8), a key human neutrophil chemoattractant, does not exist in mice and its functional substitutes (KC) is not identical (Watanabe et al., 2004). Mice have higher baseline antioxidant enzyme activity. As such mice can tolerate oxidative burdens that damage human epithelium and thus protective pathways may be overestimated in humans (Hamilton et al., 2012). While mouse models provide mechanistic insight, fundamental interspecies differences in airway anatomy, immune signalling, and toxicokinetics limit their predictive value for human bushfire smoke–induced respiratory disease.

7. Use of CRISPR/gene editing techniques

Gene editing techniques are important for studying biological mechanisms, pathophysiology of disease and identifying causative genes and signalling pathways. Identifying causality, however, is time-consuming. To address this, multiplex screening methods of large numbers of genetic perturbations in parallel to select cells with a desired phenotype enables rapid identification of causative genes.

The simplicity of the CRISPR-Cas9 system allows the multiplex of thousands of knock-outs to achieve efficient and accurate gene knockout screening, at the genome scale (Nishiga et al., 2022). CRISPR screens enable unbiased, high-throughput studies of gene function in cells and animals. These genome-wide screens use pooled libraries of single guide RNAs (sgRNAs) to systematically target and perturb individual genes, allowing researchers to investigate their roles across diverse biological processes. Here, CRISPR libraries are introduced into pools of cells using lentiviral plasmids expressing different gRNAs, such that each cell receives a different gRNA that perturbs the target gene (Joung et al., 2017). Perturbed cells are grown and subjected to a particular biological challenge (BFS, infection, metabolite) or drug. Perturbation-induced effects are assessed by high-throughput sequencing of the guide RNAs. The frequency or distribution of gRNAs identifies those that are enriched or depleted with the challenge and therefore those that are causative or repressive of the phenotype of interest. Results rank depleted gRNAs that identify genes whose disruption renders the cell susceptible to challenge, while their enrichment identifies the genes that disruption of which brings selective advantage in response to the challenge.

Selecting the most appropriate model system for screening is critical. Depending on the research question, this may include immortalized cell lines, primary cells, or more complex platforms such as organoids or whole organisms. In parallel, the choice of genetic perturbation method is equally important, particularly when the goal is to induce targeted mutations in the genome. With the advent of CRISPR technologies, genetic screens have expanded beyond simple knockout approaches to include more sophisticated methods such as gene inhibition (CRISPRi), activation (CRISPRa), base editing, and prime editing.

In simple gene knockout (CRISPRko), Cas9 nucleases directed by gRNAs introduce double-stranded breaks (DSBs). These breaks activate endogenous DNA repair pathways, which often results in insertions or deletions that disrupts the gene's reading frame. Beyond traditional gene knockout, a dead Cas9 (dCas9) fused with a regulator (like a repressor or activator) can be guided by sgRNA to specific DNA sites to control gene expression without introducing DSBs. This allows modulation of gene expression without altering the DNA sequence. When dCas9 is coupled with transcriptional repressors or activators, it enables targeted gene silencing or activation, referred to as CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa), respectively. In addition, CRISPR technology has expanded to enable precise modulation of the epigenome allowing control of gene expression without altering the DNA sequence. By fusing dCas9 with epigenetic effector proteins, targeted modification of histone marks such as methylation of lysine residues on histones H3 and H4 (including H3K4, H3K9, H3K27, H3K79, and H4K20), acetylation of H3K27, as well as DNA methylation can be achieved. Additionally, CRISPR base editors fused with dCas9 can be coupled with proteins that chemically modify DNA bases in the genome, allowing the introduction of genetic alterations without inducing DSBs (Komor et al., 2017). Building on this, CRISPR prime editing utilized a dCas9 fused to reverse transcriptase along with a prime editing guide RNA (pegRNA) that directs the insertions, deletion, or substitution of DNA sequences, providing a versatile and efficient tool for precise genome editing without DSBs.

Common readouts in CRISPR screens include cell survival and growth. After the introduction of sgRNA libraries, cells can be cultured with or without BFS extract and those that survive are collected. By comparing sgRNA distribution in surviving and unexposed cells, the sgRNAs that promote cellular survival or increase toxicity can be identified. Additionally, fluorescence signals marked by fluorescent reporters (*e.g.*, antibodies), small molecules monitoring cellular activities, genetically-encoded reporters, or small absorbed particles are used to separate cells based on signal intensity by fluorescence-activated cell sorting (FACS). Recently, CRISPR screens were combined with high-resolution single-cell RNA-sequencing (termed Perturb-seq) that enables the efficient exploration of cellular phenotype by assessing both specific gene function and regulation at a cellular level (Replogle et al., 2020).

gRNAs can also be modified by introducing guide barcode expression cassettes to capture the single cells (Choo et al., 2021). Furthermore, single-cell CRISPR screening coupled with epigenomic profiling such as Perturb-ATAC, enables simultaneous detection of CRISPR gRNAs and genome-wide chromatin accessibility in individual cells using transposase-accessible chromatin with sequencing (ATAC-seq) (Pierce et al., 2021).

Other screening techniques that incorporate high-resolution microscopy-based approaches are termed image-based screening. They use high-content imaging of individual cells as read-outs in CRISPR screens. They substantially expand the phenotypic profiling by enabling the probing of complex cellular phenotypes such as cell morphology or subcellular organizations of cells labeled with gRNAs (Feldman et al., 2019). Imaging-based pooled library screen approaches enable genotyping by sequencing physically isolated cells with specific phenotypes (Wang et al., 2019). Such high-definition CRISPR screens offer exciting new avenues for large-scale mechanistic studies. The detail pictorial representation of how CRISPR/gene editing technology can be utilized to define the effect of BFS is shown in Fig. 6.

8. Translational pathway: from experimental models to patients

The *in vitro*, *ex vivo*, *in vivo* research on bushfire smoke particulate matter inhalation study should be transformed into clinical applications and studied to improve public health strategies. This will help to formulate more effective preventive measures or treatment methods based on the experimental results and promote these measures in actual medical practice. Each methodology can contribute to the translational pathway.

From *in vitro* studies, we can identify the toxic PM fractions ($PM_{2.5}$, ultrafine particles, PAHs, heavy metals) and cellular pathways affected such as oxidative stress (ROS, NRF2, glutathione depletion), inflammatory signalling (NF-κB, NLRP3 inflammasome), epithelial barrier dysfunction, macrophage polarization and impaired phagocytosis, epigenetic and microRNA changes. This could help in clinical translation through biomarker discovery, example: circulating cytokines (IL-6, IL-8, TNF-α), oxidative stress markers (8-isoprostane, MDA) and microRNAs or exosomal markers detectable in blood/sputum of firefighters, first line responder, public, at-risk population (neonates, infant, pregnant, elderly, pre-existing disease). Based on pathological outcome such as oxidative stress and inflammation, we can propose drug repurposing of antioxidants (*N*-acetylcysteine, sulforaphane) and corticosteroid responsiveness testing.

Similarly with *ex vivo* studies, we can conduct human relevance validation. Human airway tissues, BALF cells, lung explants can validate

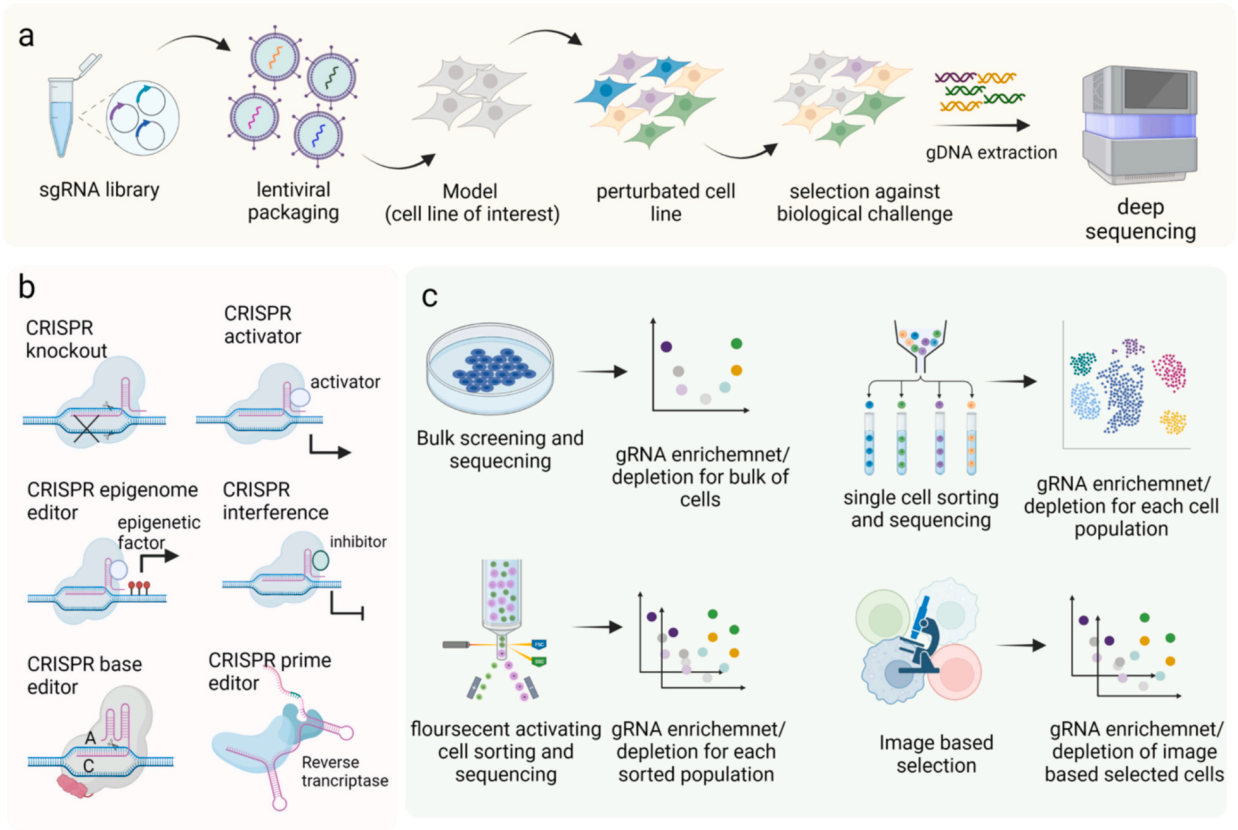


Fig. 6. CRISPR/gene editing technology to define the effect of bushfire smoke in high-definition. (A) Simplified workflow of pooled CRISPR library screening, from sgRNA library delivery and generation of perturbed cells to biological selection and deep sequencing–based identification of enriched or depleted sgRNAs. (B) Illustrative representation of major CRISPR modalities, including knockout (CRISPR–Cas9), activation (CRISPRa), interference/inhibition (CRISPRi), epigenome editing, base editing, and prime editing. (C) Readout strategies for CRISPR screens, including bulk sequencing, single-cell sequencing, FACS-based enrichment, and image-based selection to determine sgRNA abundance and phenotype–genotype relationships.

animal/*in vitro* findings in human-relevant systems. These human samples from health *versus* pre-existing disease (asthma, COPD) and various age group (neonates, infant, adult, elderly) can identify susceptibility differences. This will help stratification of high-risk populations (lung disease, cardiovascular disease), indigenous communities, outdoor workers and personalized the exposure response profiles followed by formulation of targeted preventive guidelines and policies.

Likewise, from *in vivo* studies in small animals which mimic the human physiology to greater extent (considering few inter-species variations between human and animals), we could generate the proof of safety (adverse impact of bushfire PM exposure) and efficacy of potential therapeutics to control the damage due bushfire smoke inhalation. The study of acute *vs* chronic exposure effects of bushfire smoke PM in mice can be translated to human in terms of equivalent concentration and mice-to-human days calculations. This will provide dose–response relationships and help defining the safe exposure thresholds, timing of therapeutic intervention (pre-exposure *vs* post-exposure), and validation of therapeutic windows.

Based on pre-clinical experimental evidence, clinical trial of potential pharmacological prevention could be conducted such as pre-exposure antioxidant therapy in high-risk patients, temporary step-up of inhaled corticosteroids during smoke events, prophylactic bronchodilators for vulnerable populations. On pathological and clinical side, rapid blood/sputum biomarker panels for smoke exposure injury and early lung injury detection before symptom onset could be a potential diagnostic tool.

9. Conclusions

The increasing frequency of forest fires has garnered global attention from researchers, particularly due to the complications they pose for healthy individuals and those with pre-existing health conditions. The impact of bushfires on humans is highly variable, influenced by factors such as global vegetation, atmospheric conditions, and the recent surge in air pollution. This led to an increase in the number of research and review articles published, but what is lacking predominantly in BFS research is guidelines or standards on how BFS research has to be conducted. Therefore, there is an urgent need for global consortia to make guidelines on data collection, data sharing, and ethical guidelines, using a multidisciplinary approach.

Here, we comprehensively explore the array of experimental techniques used to study BFS-induced pathogenesis. From *in vitro* cellular models to sophisticated *ex vivo* and *in vivo* methods, potential use of CRISPR/gene editing technology, each approach contributes to our understanding of the complex interactions between BFS particles and biological systems.

Each experimental models has its own physiological relevance, throughput, cost, advantage and disadvantage (Table 1) but researchers should wisely choose a model that closely mimic human physiology, for example ALI culture are more realistic than 2D submerged culture. Similarly, 3D culture techniques like organoids/spheroids with multiple cell types or microfluidics techniques where cells/tissue are under controlled fluid flow mimicking mechanical forces (blood flow pressure) and nutrient gradients may provide characteristics feature observed inside human body as compared to 2D culture system. Using microfluidics system coupled with organ on a chip can not only be used to

Table 1
Comparison of various experimental models to elucidate the impact of bushfire smoke.

Model	Physiological relevance	Throughput	Cost	Advantages	Disadvantages
2D cell culture	Low due to cells being in solution	Very high	Low	Fast, simple and easy to scale	Doesn't replicate human lungs. Mostly single type of cells.
ALI (air-liquid interface)	Medium-high Suitable to study epithelium	Medium	Medium	Mimics respiratory cilia. Better for PM exposure. Forms tight junctions.	Long setup and maintenance (21+ days). Variability. Mostly suitable for epithelial cells.
Organoids	High (self-organizing 3D)	Low-medium	High	Patient-specific genetics. Complex interactions	Variable size/shape, Patient/sample specific variability. Difficult access to lumen.
PCLS (lung slices)	Very high (native matrix)	Low	Medium-high	Retains full tissue complexity Native immune cells Better visualisation	Short lifespan (<1 week) Hard to source tissue No circulation Technically challenging
LOC (lung-on-chip)	High (adds mechanics/flow)	Low	Very high	Dynamic environment Minimal reagents Multi-organ linking	Technically challenging Materials absorb drugs Bubble issues
Animal models	Highest (systemic complexity)	Low	Medium-high	Gold standard for toxicity Systemic effects. Pharmacokinetics	Ethical concerns Species differences High maintenance

study the impact of bushfire PM but also the *in-vitro*-to-*in-vivo* translation of pharmacokinetic and pharmacodynamics parameters (quantitative prediction) of drugs use to slow down the negative impact of bushfire inhalation (Herland et al., 2020). *In vivo* model using mice, rats, and rabbits *etc.* are considered superior to *in vitro* model as these small animals have many physiological similarities with human suggesting any pathological or immunological changes due to bushfire smoke exposure in small animals are highly translatable to human. By synthesizing together the findings from these diverse methodologies provides valuable insights into the mechanisms underlying pathogenic mechanisms. Moving forward, a number of developing technologies present apparent prospects for future advances. For instance, combining CRISPR-based gene modifications with single-cell sequencing (scRNA-seq and scATAC-seq) could help in the identification of contributing regulators of inflammation, oxidative stress responses, and epithelial repair pathways triggered by BFS. Single-cell CRISPR screenings could identify individual genes, regulatory elements, or cell states that determine susceptibility or resilience to hazardous smoke components, circumventing present limitations caused by cellular heterogeneity and the difficulty to infer causality solely from observational data. Ultimately, integration of advanced experimental techniques with epidemiological and clinical studies will enable the elucidation of the full spectrum of health consequences of BFS inhalation. Such interdisciplinary efforts are essential for informing effective public health interventions and mitigating the adverse effects of BFS on human health.

CRediT authorship contribution statement

Keshav R. Paudel: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Karl J. Hegarty:** Writing – original draft. **Jesus Shrestha:** Writing – review & editing, Software, Methodology. **Kurtis F. Budden:** Writing – review & editing, Visualization. **Nikhil T. Awatade:** Writing – review & editing, Validation. **Tayyaba Sadaf:** Writing – original draft, Software. **Vamshikrishna Malyla:** Writing – original draft, Software. **Shafagh Waters:** Writing – review & editing. **Paris C. Papagianis:** Writing – review & editing. **Jane E. Bourke:** Writing – review & editing. **Peter A. Wark:** Writing – review & editing, Supervision, Funding acquisition. **Philip M. Hansbro:** Writing – review & editing, Validation, Supervision, Funding acquisition, Conceptualization.

Funding

We acknowledge the funding support of MRFF2020 bushfire grant (1201338) and Healthy Environments And Lives (HEAL) National Research Network both to PMH, the latter receives funding from the National Health and Medical Research Council Special Initiative in Human Health and Environmental Change (#2008937). We also acknowledge funding support from Maridulu Budyari Gumal, The Sydney Partnership for Health, Education, Research and Enterprise 2021 seed grant and Thoracic Society of Australia and New Zealand (TSANZ) Maurice Blackburn grant-in-aid for occupational disease to KRP and PMH. KRP, TS, and PMH would like to acknowledge the Australian Respiratory Council funding 2025 for bushfire project.

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.

Data availability

No data was used for the research described in the article.

References

Alasmari, F., Crotty Alexander, L.E., Drummond, C.A., Sari, Y., 2018. A computerized exposure system for animal models to optimize nicotine delivery into the brain through inhalation of electronic cigarette vapors or cigarette smoke. Saudi Pharm. J. 26, 622–628.
Alewel, D.I., Henriquez, A.R., Schladweiler, M.C., Grindstaff, R., Fisher, A.A., Snow, S.J., et al., 2023. Intratracheal instillation of respirable particulate matter elicits neuroendocrine activation. Inhal. Toxicol. 35, 59–75.
Allouche, Y., Marchetti, S., Bengalli, R., Motta, G., Pagliarulo, L., Cazier, F., et al., 2025. Comparison of submerged and air liquid interface exposure: limitations and differences in the toxicological effects evaluated in bronchial epithelial cells. Environ. Res. 279, 121856.
Alsafadi, H.N., Uhl, F.E., Pineda, R.H., Bailey, K.E., Rojas, M., Wagner, D.E., et al., 2020. Applications and approaches for three-dimensional precision-cut lung slices. disease modeling and drug discovery. Am. J. Respir. Cell Mol. Biol. 62, 681–691.
Artzy-Schnirman, A., Zidan, H., Elias-Kirma, S., Ben-Porat, L., Tenenbaum-Katan, J., Carius, P., et al., 2019. Capturing the onset of bacterial pulmonary infection in Acini-On-Chips. Adv. Biosyst. 3, e1900026.
Aydin, M., Naumova, E.A., Bellm, A., Behrendt, A.K., Giachero, F., Bahlmann, N., et al., 2021. From submerged cultures to 3D cell culture models: evolution of nasal epithelial cells in asthma research and virus infection. Viruses 13.
Bannuscher, A., Schmid, O., Drasler, B., Rohrbasser, A., Braakhuis, H.M., Meldrum, K., et al., 2022. An inter-laboratory effort to harmonize the cell-delivered in vitro dose of aerosolized materials. NanoImpact 28, 100439.
Bergeron, C., Tulic, M.K., Hamid, Q., 2007. Tools used to measure airway remodelling in research. Eur. Respir. J. 29, 596–604.
Bhattarai, P., Myers, S., Chia, C., Weber, H.C., Young, S., Williams, A.D., et al., 2020. Clinical application of forced oscillation technique (FOT) in early detection of airway changes in smokers. J. Clin. Med. 9.

Bond, S.L., Greco-Otto, P., MacLeod, J., Galezowski, A., Bayly, W., Leguillette, R., 2020. Efficacy of dexamethasone, salbutamol, and reduced respirable particulate concentration on aerobic capacity in horses with smoke-induced mild asthma. J. Vet. Intern. Med. 34, 979–985.
Borchers Arriagada, N., Palmer, A.J., Bowman, D.M., Morgan, G.G., Jalaludin, B.B., Johnston, F.H., 2020. Unprecedented smoke-related health burden associated with the 2019-20 bushfires in eastern Australia. Med. J. Aust. 213, 282–283.
Bovard, D., Sandoz, A., Luettich, K., Frentzel, S., Iskandar, A., Marescotti, D., et al., 2018. A lung/liver-on-a-chip platform for acute and chronic toxicity studies. Lab Chip 18, 3814–3829.
Bressan, D., Battistoni, G., Hannon, G.J., 2023. The dawn of spatial omics. Science 381, eabq4964.
Bui, D., Davis, S., Flynn, A., Bell, R., Dharmage, S., 2021. Impact of recent catastrophic bushfires on people with asthma in Australia: health, social and financial burdens. Respirology 26, 296–297.
Camargo, L.D.N., Righetti, R.F., de Almeida, F.M., Dos Santos, T.M., Fukuzaki, S., Martins, N.A.B., et al., 2023. Modulating asthma-COPD overlap responses with IL-17 inhibition. Front. Immunol. 14, 1271342.
Choo, X.Y., Lim, Y.M., Katwadi, K., Yap, L., Tryggvason, K., Sun, A.X., et al., 2021. Evaluating capture sequence performance for single-cell CRISPR activation experiments. ACS Synth. Biol. 10, 640–645.
Clarke, P.J., Lawes, M.J., Murphy, B.P., Russell-Smith, J., Nano, C.E., Bradstock, R., et al., 2015. A synthesis of postfire recovery traits of woody plants in Australian ecosystems. Sci. Total Environ. 534, 31–42.
Courtoy, G.E., Leclercq, I., Froidure, A., Schiano, G., Morelle, J., Devuyst, O., et al., 2020. Digital image analysis of picrosirius red staining: a robust method for multi-organ fibrosis quantification and characterization. Biomolecules 10.
Crue, T., Lee, G.Y., Peng, J.Y., Schaunaman, N., Agraval, H., Day, B.J., et al., 2023. Single cell RNA-sequencing of human precision-cut lung slices: a novel approach to study the effect of vaping and viral infection on lung health. Innate Immun. 29, 61–70.
Cui, Y., Liu, K.W.K., Ip, M.S.M., Liang, Y., Mak, J.C.W., 2020. Protective effect of selegiline on cigarette smoke-induced oxidative stress and inflammation in rat lungs in vivo. Ann. Transl. Med. 8, 1418.
Cui, F., Sun, Y., Xie, J., Li, D., Wu, M., Song, L., et al., 2023. Air pollutants, genetic susceptibility and risk of incident idiopathic pulmonary fibrosis. Eur. Respir. J. 61.
Dahl, M., Nordestgaard, B.G., 2009. Markers of early disease and prognosis in COPD. Int. J. Chron. Obstruct. Pulmon. Dis. 4, 157–167.
de Souza Xavier Costa, N., Ribeiro Junior, G., Dos Santos Alemany, A.A., Belotti, L., Schalch, A.S., Cavalcante, M.F., et al., 2020. Air pollution impairs recovery and tissue remodeling in a murine model of acute lung injury. Sci. Rep. 10, 15314.
Dharwal, V., Paudel, K.R., Hansbro, P.M., 2020. Impact of bushfire smoke on respiratory health. Med. J. Aust. 213 (284–284), e1.
Ding, Y., Weindl, P., Lenz, A.G., Mayer, P., Krebs, T., Schmid, O., 2020. Quartz crystal microbalances (QCM) are suitable for real-time dosimetry in nanotoxicological studies using VITROCELL(R)Cloud cell exposure systems. Part. Fibre Toxicol. 17, 44.
Feldman, D., Singh, A., Schmid-Burgk, J.L., Carlson, R.J., Mezger, A., Garrity, A.J., et al., 2019. Optical pooled screens in human cells. Cell 179, 787–799. e17.
Forkink, M., Smeitink, J.A., Brock, R., Willems, P.H., Koopman, W.J., 2010. Detection and manipulation of mitochondrial reactive oxygen species in mammalian cells. Biochim. Biophys. Acta 1797, 1034–1044.
Francis, I., Shrestha, J., Paudel, K.R., Hansbro, P.M., Warkiani, M.E., Saha, S.C., 2022. Recent advances in lung-on-a-chip models. Drug Discov. Today 27, 2593–2602.
Gomez, H.M., Haw, T.J., Ilic, D., Robinson, P., Donovan, C., Croft, A.J., et al., 2024. Landscape fire smoke airway exposure impairs respiratory and cardiac function and worsens experimental asthma. J. Allergy Clin. Immunol. 154, 209–221.e6.
Gonzalez-Gualda, E., Baker, A.G., Fruk, L., Munoz-Espin, D., 2021. A guide to assessing cellular senescence in vitro and in vivo. FEBS J. 288, 56–80.
Graf, J., Trautmann-Rodriguez, M., Sabnis, S., Kloxin, A.M., Fromen, C.A., 2023. On the path to predicting immune responses in the lung: modeling the pulmonary innate immune system at the air-liquid interface (ALI). Eur. J. Pharm. Sci. 191, 106596.
Haghi, M., Traini, D., Young, P., 2014. In vitro cell integrated impactor deposition methodology for the study of aerodynamically relevant size fractions from commercial pressurised metered dose inhalers. Pharm. Res. 31, 1779–1787.
Hamilton, R.T., Walsh, M.E., Van Remmen, H., 2012. Mouse models of oxidative stress indicate a role for modulating healthy aging. J. Clin. Exp. Pathol. Suppl 4.
Hamon, R., Tran, H.B., Roscioli, E., Ween, M., Jersmann, H., Hodge, S., 2018. Bushfire smoke is pro-inflammatory and suppresses macrophage phagocytic function. Sci. Rep. 8, 13424.
Herbert, J., Kelty, J.S., Laskin, J.D., Laskin, D.L., Gow, A.J., 2023. Menthol flavoring in e-cigarette condensate causes pulmonary dysfunction and cytotoxicity in precision cut lung slices. Am. J. Phys. Lung Cell. Mol. Phys. 324, L345–L357.
Herland, A., Maoz, B.M., Das, D., Somayaji, M.R., Prantil-Baun, R., Novak, R., et al., 2020. Quantitative prediction of human pharmacokinetic responses to drugs via fluidically coupled vascularized organ chips. Nat. Biomed. Eng. 4, 421–436.
Howard, Z.L., Carlson, S.J., Baldwin, Z., Johnston, F., Durrheim, D.N., Dalton, C.B., 2020. High Community Burden of Smoke-related Symptoms in the Hunter and New England Regions During the 2019–2020 Australian Bushfires.
Huh, D.D., 2015. A human breathing lung-on-a-chip. Ann. Am. Thorac. Soc. 12 (Suppl. 1), S42–S44.
Jalava, P.I., Salonen, R.O., Hälinen, A.I., Penttinen, P., Pennanen, A.S., Sillanpää, M., et al., 2006. In vitro inflammatory and cytotoxic effects of size-segregated particulate samples collected during long-range transport of wildfire smoke to Helsinki. Toxicol. Appl. Pharmacol. 215, 341–353.
Jha, S.K., De Rubis, G., Devkota, S.R., Zhang, Y., Adhikari, R., Jha, L.A., et al., 2024. Cellular senescence in lung cancer: molecular mechanisms and therapeutic interventions. Ageing Res. Rev. 97, 102315.
Joung, J., Engreitz, J.M., Konermann, S., Abudayyeh, O.O., Verdine, V.K., Aguet, F., et al., 2017. Genome-scale activation screen identifies a lncRNA locus regulating a gene neighbourhood. Nature 548, 343–346.
Kawanaka, Y., Matsumoto, E., Sakamoto, K., Yun, S.J., 2011. Estimation of the contribution of ultrafine particles to lung deposition of particle-bound mutagens in the atmosphere. Sci. Total Environ. 409, 1033–1038.
Kesimer, M., Kirkham, S., Pickles, R.J., Henderson, A.G., Alexis, N.E., Demaria, G., et al., 2009. Tracheobronchial air-liquid interface cell culture: a model for innate mucosal defense of the upper airways? Am. J. Phys. Lung Cell. Mol. Phys. 296, L92–L100.
Knowles, M.R., Paradiso, A.M., Boucher, R.C., 1995. In vivo nasal potential difference: techniques and protocols for assessing efficacy of gene transfer in cystic fibrosis. Hum. Gene Ther. 6, 445–455.
Komor, A.C., Zhao, K.T., Packer, M.S., Gaudelli, N.M., Waterbury, A.L., Koblan, L.W., et al., 2017. Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C: G-to-T: a base editors with higher efficiency and product purity. Sci. Adv. 3, eaao4774.
Kong, J., Wen, S., Cao, W., Yue, P., Xu, X., Zhang, Y., et al., 2021. Lung organoids, useful tools for investigating epithelial repair after lung injury. Stem Cell Res. Ther. 12, 95.
Lang, N.J., Gote-Schniering, J., Porras-Gonzalez, D., Yang, L., De Sadeleer, L.J., Jentzsch, R.C., et al., 2023. *Ex vivo* tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis. Sci. Transl. Med. 15, eadh0908.
Lechasseur, A., Altmejd, S., Turgeon, N., Buonanno, G., Morawska, L., Brunet, D., et al., 2019. Variations in coil temperature/power and e-liquid constituents change size and lung deposition of particles emitted by an electronic cigarette. Physiol. Rep. 7, e14093.
LeGrys, V.A., Yankaskas, J.R., Quittell, L.M., Marshall, B.C., Mogayzel Jr., P.J., Cystic Fibrosis F, 2007. Diagnostic sweat testing: the Cystic Fibrosis Foundation guidelines. J. Pediatr. 151, 85–89.
Lehmann, M., Krishnan, R., Sucre, J., Kulkarni, H.S., Pineda, R.H., Anderson, C., et al., 2024. Precision cut lung slices: emerging tools for preclinical and translational lung research. An official American Thoracic Society Workshop Report. Am. J. Respir. Cell Mol. Biol. 72, 16–31.
Li, C.H., Tsai, M.L., Chiou, H.C., Lin, Y.C., Liao, W.T., Hung, C.H., 2022. Role of macrophages in air pollution exposure related asthma. Int. J. Mol. Sci. 23.
Linderholm, A.L., Franzi, L.M., Bein, K.J., Pinkerton, K.E., Last, J.A., 2015. A quantitative comparison of intranasal and intratracheal administration of coarse PM in the mouse. Integr. Pharmacol. Toxicol. Genotoxicol. 1, 5–10.
Liu, J.C., Peng, R.D., 2019. The impact of wildfire smoke on compositions of fine particulate matter by ecoregion in the Western US. J. Expo. Sci. Environ. Epidemiol. 29, 765–776.
Liu, G., Jarnicki, A.G., Paudel, K.R., Lu, W., Wadhwa, R., Philp, A.M., et al., 2022. Adverse roles of mast cell chymase-1 in COPD. Eur. Respir. J. 60.
Lu, Z., Van Eeckhoutte, H.P., Liu, G., Nair, P.M., Jones, B., Gillis, C.M., et al., 2021. Necroptosis signaling promotes inflammation, airway remodeling, and emphysema in chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 204, 667–681.
Lucas, C., Hennessy, K., Mills, G., Bathols, J., 2007. Bushfire Weather in Southeast Australia: Recent Trends and Projected Climate Change Impacts.
MacIntyre, C.R., Nguyen, P.Y., Trent, M., Seale, H., Chughtai, A.A., Shah, S., et al., 2021. Adverse health effects in people with and without preexisting respiratory conditions during bushfire smoke exposure in the 2019/2020 Australian summer. Am. J. Respir. Crit. Care Med. 204, 368–371.
Mall, M., Hirtz, S., Gonska, T., Kunzelmann, K., 2004. Assessment of CFTR function in rectal biopsies for the diagnosis of cystic fibrosis. J. Cyst. Fibros. 3 (Suppl. 2), 165–169.
Moheimani, F., Shahdab, N., Cummings, S., Hansbro, P.M., Ward, C., 2022. Key role of dysregulated airway epithelium in response to respiratory viral infections in asthma. ERJ Open Res. 8.
Mondonedo, J.R., Bartolak-Suki, E., Bou Jawde, S., Nelson, K., Cao, K., Sonnenberg, A., et al., 2020. A high-throughput system for cyclic stretching of precision-cut lung slices during acute cigarette smoke extract exposure. Front. Physiol. 11, 566.
Mori, V., Vitorasso, R.L., Takeuchi, V.A., Lima, W.T., Oliveira, M.A., Moriya, H.T., 2022. Signal processing to remove spurious contributions to the assessment of respiratory mechanics. Exp. Lung Res. 48, 1–11.
Mori, A., Ito, S., Sekine, T., 2024. A revision of the multiple-path particle dosimetry model focusing on tobacco product aerosol dynamics. Int. J. Numer. Method Biomed. Eng. 40, e3796.
Nishiga, M., Liu, C., Qi, L.S., Wu, J.C., 2022. The use of new CRISPR tools in cardiovascular research and medicine. Nat. Rev. Cardiol. 19, 505–521.
Ortiz-Munoz, G., Looney, M.R., 2015. Non-invasive intratracheal instillation in mice. Bio-protocol 5.
Ortiz-Quintero, B., Martinez-Espinosa, I., Perez-Padilla, R., 2022. Mechanisms of lung damage and development of COPD due to household biomass-smoke exposure: inflammation, oxidative stress, microRNAs, and gene polymorphisms. Cells 12.
Papagianis, P.C., Mason, K., Studley, W., Ratnayaka-Gamage, N., Winship, A., Paudel, K., et al., 2025. Investigating the harmful effects of bushfire smoke exposure in precision cut lung slices. Am. J. Respir. Crit. Care Med. 211, A6821.
Patton, J.S., Byron, P.R., 2007. Inhaling medicines: delivering drugs to the body through the lungs. Nat. Rev. Drug Discov. 6, 67–74.
Paudel, K.R., Wadhwa, R., Mehta, M., Chellappan, D.K., Hansbro, P.M., Dua, K., 2020. Rutin loaded liquid crystalline nanoparticles inhibit lipopolysaccharide induced oxidative stress and apoptosis in bronchial epithelial cells in vitro. Toxicol. in Vitro 68, 104961.
Paudel, K.R., Panth, N., Manandhar, B., Singh, S.K., Gupta, G., Wich, P.R., et al., 2022. Attenuation of cigarette-smoke-induced oxidative stress, senescence, and

inflammation by berberine-loaded liquid crystalline nanoparticles: in vitro study in 16HBE and RAW264. 7 cells. Antioxidants 11, 873.
Paudel, K.R., Clarence, D.D., Panth, N., Manandhar, B., De Rubis, G., Devkota, H.P., et al., 2024. Zerumbone liquid crystalline nanoparticles protect against oxidative stress, inflammation and senescence induced by cigarette smoke extract in vitro. Naunyn Schmiedeberg's Arch. Pharmacol. 397, 2465–2483.
Peters, A., Hume, S., Raidal, S., Crawley, L., Gowland, D., 2021. Mortality associated with bushfire smoke inhalation in a captive population of the smoky mouse (Pseudomys fumeus), a threatened Australian rodent. J. Wildl. Dis. 57, 199–204.
Pezzulo, A.A., Starner, T.D., Scheetz, T.E., Traver, G.L., Tilley, A.E., Harvey, B.-G., et al., 2011. The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of in vivo airway epithelia. Am. J. Phys. Lung Cell. Mol. Phys. 300, L25–L31.
Pierce, S.E., Granja, J.M., Greenleaf, W.J., 2021. High-throughput single-cell chromatin accessibility CRISPR screens enable unbiased identification of regulatory networks in cancer. Nat. Commun. 12, 2969.
Pinkerton, J.W., Kim, R.Y., Brown, A.C., Rae, B.E., Donovan, C., Mayall, J.R., et al., 2022. Relationship between type 2 cytokine and inflammasome responses in obesity-associated asthma. J. Allergy Clin. Immunol. 149, 1270–1280.
Quinton, P., Molyneux, L., Ip, W., Dupuis, A., Avolio, J., Tullis, E., et al., 2012. beta-adrenergic sweat secretion as a diagnostic test for cystic fibrosis. Am. J. Respir. Crit. Care Med. 186, 732–739.
Ramalho, A.S., Boon, M., Proesmans, M., Vermeulen, F., Carlon, M.S., Boeck, K., 2022. Assays of CFTR function in vitro, ex vivo and in vivo. Int. J. Mol. Sci. 23.
Randell, S.H., Boucher, R.C., University of North Carolina Virtual Lung G, 2006. Effective mucus clearance is essential for respiratory health. Am. J. Respir. Cell Mol. Biol. 35, 20–28.
Rasmussen, F., Taylor, D.R., Flannery, E.M., Cowan, J.O., Greene, J.M., Herbison, G.P., et al., 2002. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator FEV1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am. J. Respir. Crit. Care Med. 165, 1480–1488.
Rasmussen, J.E., Sheridan, J.T., Polk, W., Davies, C.M., Tarran, R., 2014. Cigarette smoke-induced Ca2+ release leads to cystic fibrosis transmembrane conductance regulator (CFTR) dysfunction. J. Biol. Chem. 289, 7671–7681.
Replogle, J.M., Norman, T.M., Xu, A., Hussmann, J.A., Chen, J., Cogan, J.Z., et al., 2020. Combinatorial single-cell CRISPR screens by direct guide RNA capture and targeted sequencing. Nat. Biotechnol. 38, 954–961.
Rivera, J., Tessarollo, L., 2008. Genetic background and the dilemma of translating mouse studies to humans. Immunity 28, 1–4.
Roscioli, E., Hamon, R., Lester, S.E., Jersmann, H.P.A., Reynolds, P.N., Hodge, S., 2018. Airway epithelial cells exposed to wildfire smoke extract exhibit dysregulated autophagy and barrier dysfunction consistent with COPD. Respir. Res. 19, 234.
Rothen-Rutishauser, B., Gibb, M., He, R., Petri-Fink, A., Sayes, C.M., 2023. Human lung cell models to study aerosol delivery–considerations for model design and development. Eur. J. Pharm. Sci. 180, 106337.
Saint-Criq, V., Gray, M.A., 2017. Role of CFTR in epithelial physiology. Cell. Mol. Life Sci. 74, 93–115.
Scieszka, D., Bolt, A.M., McCormick, M.A., Brigman, J.L., Campen, M.J., 2023. Aging, longevity, and the role of environmental stressors: a focus on wildfire smoke and air quality. Front. Toxicol. 5, 1267667.
Seo, Y., Qiu, L., Magnen, M., Conrad, C., Moussavi-Harami, S.F., Looney, M.R., et al., 2023. Optimizing anesthesia and delivery approaches for dosing into lungs of mice. Am. J. Phys. Lung Cell. Mol. Phys. 325, L262–L269.
Shrestha, J., Paudel, K.R., Nazari, H., Dharwal, V., Bazaz, S.R., Johansen, M.D., et al., 2023. Advanced models for respiratory disease and drug studies. Med. Res. Rev. 43, 1470–1503.
Si, L., Bai, H., Rodas, M., Cao, W., Oh, C.Y., Jiang, A., et al., 2021. A human-airway-on-a-chip for the rapid identification of candidate antiviral therapeutics and prophylactics. Nat. Biomed. Eng. 5, 815–829.
Stegmayr, J., Wagner, D.E., 2021. The dawn of the omics era in human precision-cut lung slices. Eur. Respir. J. 58.
Steiner, S., Majeed, S., Kratzer, G., Vuillaume, G., Hoeng, J., Frentzel, S., 2017. Characterization of the Vitrocell® 24/48 aerosol exposure system for its use in exposures to liquid aerosols. Toxicol. in Vitro 42, 263–272.
Stucki, A.O., Sauer, U.G., Allen, D.G., Kleinstreuer, N.C., Perron, M.M., Yozzo, K.L., et al., 2024. Differences in the anatomy and physiology of the human and rat respiratory tracts and impact on toxicological assessments. Regul. Toxicol. Pharmacol. 150, 105648.
Studley, W.R., Lamanna, E., Martin, K.A., Nold-Petry, C.A., Royce, S.G., Woodman, O.L., et al., 2024. The small-molecule formyl peptide receptor biased agonist, compound 17b, is a vasodilator and anti-inflammatory in mouse precision-cut lung slices. Br. J. Pharmacol. 181, 2287–2301.
Thai, P., Loukoianov, A., Wachi, S., Wu, R., 2008. Regulation of airway mucin gene expression. Annu. Rev. Physiol. 70, 405–429.
van der Vaart, J., Lamers, M.M., Haagmans, B.L., Clevers, H., 2021. Advancing lung organoids for COVID-19 research. Dis. Model. Mech. 14, dmm049060.
Vardoulakis, S., Jalaludin, B.B., Morgan, G.G., Hanigan, I.C., Johnston, F.H., 2020. Bushfire smoke: urgent need for a national health protection strategy. Med. J. Aust. 212 (349–353), e1.
Wang, C., Lu, T., Emanuel, G., Babcock, H.P., Zhuang, X., 2019. Imaging-based pooled CRISPR screening reveals regulators of lncRNA localization. Proc. Natl. Acad. Sci. 116, 10842–10851.
Watanabe, H., Numata, K., Ito, T., Takagi, K., Matsukawa, A., 2004. Innate immune response in Th1- and Th2-dominant mouse strains. Shock 22, 460–466.
Wegesser, T.C., Pinkerton, K.E., Last, J.A., 2009. California wildfires of 2008: coarse and fine particulate matter toxicity. Environ. Health Perspect. 117, 893–897.
Wegesser, T.C., Franzi, L.M., Mitloehner, F.M., Eiguren-Fernandez, A., Last, J.A., 2010. Lung antioxidant and cytokine responses to coarse and fine particulate matter from the great California wildfires of 2008. Inhal. Toxicol. 22, 561–570.
Williams, K.M., Franzi, L.M., Last, J.A., 2013. Cell-specific oxidative stress and cytotoxicity after wildfire coarse particulate matter instillation into mouse lung. Toxicol. Appl. Pharmacol. 266, 48–55.
Zavala, 2020. New Approach Methods to Evaluate Health Risks of Air Pollutants: Critical Design Considerations for In Vitro Exposure Testing.
Zhu, Y., Sun, L., Wang, Y., Cai, L., Zhang, Z., Shang, Y., et al., 2022. A biomimetic human Lung-on-a-Chip with colorful display of microphysiological breath. Adv. Mater. 34, 2108972.