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Review article

Advances in organ-on-a-chip technology to examine the impact of air pollutants on epithelial barrier tissues

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ABSTRACT

It is estimated that 99 % of the world population is exposed to air pollution above air quality guidelines and this is responsible for 6.7 million premature deaths annually. Lung and skin are the first organs exposed to air pollution, and this is associated with carcinogenesis, inflammation and atopic disease. Proposed mechanisms of adverse health effects in lung and skin include oxidative stress, inflammation, and loss of epithelial barrier integrity. Most knowledge has been gained using simple 2D or more complex culture models, however these cultures have important limitations, such as a lack of perfusion and stretching and lack of cell-cell crosstalk. Organ-on-chip (OoC) technology may be used to overcome limitations of the *in vitro* models currently used in air pollution research and opens possibilities for studying the pathways underlying adverse health effects of air pollution on immune-mediated diseases of the lung and skin using more physiologically relevant exposure experiments. In this review we discuss currently used *in vitro* models to study the effect of air pollution on epithelial barrier integrity and development of immune-mediated diseases and identify gaps in current knowledge on adverse health effects of air pollution. We then focus on how OoC technology can enhance mechanistic studies of the skin and lung's response to air pollution.

1. Introduction

The skin and lungs, both epithelial barrier tissues, are continuously exposed to environmental stressors, including air pollutants. Air, soil, and water pollution have garnered most attention, as they are believed to contribute to approximately 9 million deaths annually, with most occurring in developing countries (Fuller et al., 2022). Air pollution is a major contributor to these deaths. According to the WHO, ~99 % of the global population is exposed to air pollution levels that exceed air quality guidelines, leading to 6.7 million premature deaths each year (World Health Organisation, 2024). Air pollution is associated with cardiovascular disease (Dominski et al., 2021) and an increased risk of developing various cancers, including but not limited to acute lymphocytic leukemia (ALL), acute myeloid leukemia (AML), cancers of the upper aerodigestive tract as well as breast and ovarian cancer (Fang et al., 2024; Heck et al., 2024; Kentros et al., 2024; Nagel et al., 2024; Turner et al., 2020; Madrigal et al., 2024). It is also linked to skin issues such as aging, atopic dermatitis, acne, skin cancer, psoriasis, eczema, and melasma/hyperpigmentation (Abolhasani et al., 2021; Gu et al., 2024; Jin et al., 2024; Huls et al., 2019; Patella et al., 2020; Mazur et al., 2023). In the lungs, air pollution is associated with a higher incidence of respiratory diseases (Analitis et al., 2006; Turner et al., 2011; Zanobetti et al., 2009), including lung cancer, development and exacerbation of chronic obstructive pulmonary disease (COPD) and asthma and impaired lung function (Tiotiu et al., 2020; Kyung and Jeong, 2020; Garcia et al., 2019; Herbert and Kumar, 2017; Al-Daghri et al., 2013; Agache et al., 2024; Loomis et al., 2018; Hvidtfeldt et al., 2021).

Air pollution can be categorized into ambient and household pollution, with both serving as significant sources of pollutants that can impact health. Volatile and semi-volatile compounds (VOCs),

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particulate matter (PM), and gaseous compounds such as carbon monoxide (CO), ozone (O_3), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) are of particularly large concern to health (Leung, 2015). Airborne PM consists of a wide variety of compounds, and its chemical composition is significantly influenced by both geographical location and time of year (Chen et al., 2023; Zalakeviciute et al., 2020; van Donkelaar et al., 2019). Polycyclic aromatic hydrocarbons (PAHs), which are often bound to PM are a particularly harmful class of VOCs (Saarnio et al., 2008). PAHs are classified as either group 1 carcinogens (sufficient evidence for carcinogenicity), or group 2A/B carcinogens (probable and possible carcinogens) (Baird et al., 2005). PM_{10} (<10 μm) has greater impact on human health than larger particles, as these can readily enter the body. Respiratory disease prevalence increases with 2.07 % for every 10 μg/m^3 increase in PM_{10}, while mortality increases by 0.58 % (Xing et al., 2015).

There are three primary routes through which pollutants can enter the body: ingestion (Wu et al., 2022), inhalation (Wu et al., 2022; Luo et al., 2021) and absorption through the skin and mucous membranes (Luo et al., 2020). Since the skin covers approximately 1.85 m^2 of surface area, it offers ample opportunity for pollutants to enter the body, potentially causing local and systemic effects. Pollutants enter the skin through three routes: the intercellular route, the transcellular route and via the appendages (i.e., sweat glands, hair follicles). After dermal exposure, the intercellular and transcellular routes are considered the primary routes (Jin et al., 2018; Larese Filon et al., 2015). The stratum corneum, the outermost layer of the epidermis, serves as a protective barrier against the external environment. However, substances like benzo-a-pyrene (BaP) and other PAHs can easily penetrate the skin and are subsequently metabolized in the skin (Kao et al., 1985; Ng et al., 1992; Kim et al., 2023).

In the lungs, deposition and uptake of PM is primarily determined by particle size. PM with different diameters will penetrate to varying depths within the respiratory tract (Deng et al., 2019; Morawska and Buonanno, 2021). Coarse particles (10–2.5 μm) are primarily deposited in the upper airways. Fine particles (2.5–0.1 μm) tend to reach the lower respiratory tract. Particles smaller than 0.1 μm behave like gases and can exchange through the alveoli into the systemic circulation (Sturm, 2016; Yacobi et al., 2010; Oberdorster et al., 2002; Heyder et al., 1986). *In silico* modelling of particle deposition in the human lungs, however, suggests that coarse particles (10–2.5 μm) can also penetrate the lower airways (Deng et al., 2019). Finally, the penetration of particles into the respiratory tract also depends on the route of inhalation (nose versus mouth) and age, with children experiencing higher penetration than adults (Brown et al., 2013).

Various animal and tissue models have been used to investigate the pathways involved in stress responses and disease processes in the lungs and skin. Significant knowledge on how the lungs respond to air pollution has been gained using animal studies, while the skin has been studied less extensively. In rodents, exposure to different air pollutants (DEP, NO_2, $PM_{2.5}$+O_3) enhances asthma susceptibility, and results in influx of neutrophils into the airways, as well as other immune cell types including macrophages, monocytes, lymphocytes and eosinophils (Liu et al., 2008; Han et al., 2017; Cassee et al., 2005; Sidwell et al., 2022; Valderrama et al., 2022). The specific outcome, and immune cell effector functions depends on particle size and chemical composition. Induction of oxidative stress has also been reported, however, more research is needed to identify the causal components of air pollution contributing to oxidative stress (Valderrama et al., 2022). PM as well as O3 exposure affected respiratory epithelial barrier integrity in murine models (Smyth et al., 2021), but more research is needed to elucidate the underlying mechanism and how the specific composition of the epithelial layer is affected. Moreover, epithelial-immune cell interactions have not been studied extensively in this field. After inhalation, cardiovascular effects were also observed, with an increase in systemic IL-6 (Watkinson et al., 2001; Lee et al., 2024a; Kodavanti et al., 2000). Although animal studies often show good concordance with human pathophysiology in general, data cannot always be translated to the heterogeneous human population. Moreover, in a number of studies, intratracheal/intranasal installation was used instead of inhalation. This has many advantages, including achievement of an accurate dose, but can affect the pattern of particle deposition and downstream intra- and extrapulmonary inflammatory responses (Todo, 2017).

In AD-like mice, topical application of PM_{10} resulted in increased AD symptoms and skin inflammation (Woo et al., 2020; Costa et al., 2006; Dijkhoff et al., 2020). In murine models, topical application of PM results in decreased expression of epithelial barrier proteins and increased oxidative stress. PM penetrates into the skin, and the level of penetration increases on disrupted skin. For the development of dermo-protective technologies, more mechanistic knowledge needs to be gained regarding effects of air pollution on the skin. Rodents are not an ideal model for studying human skin responses, as there are important differences between human and mouse skin that influence how substances are absorbed through the skin. For example, mouse skin has more appendages and a thinner epidermis (Huh et al., 2010). Porcine skin is more similar to human skin; however, pigs are costly and difficult to handle in experiments.

From an ethical perspective it is desirable to move towards relevant *in vitro* models. Cell and tissue culture models have provided valuable insights into the health impacts of air pollution on the skin and lungs (Further discussed in section 2; Fig. 1). However, traditional cell culture techniques often lack crucial biophysical cues. Furthermore, most air pollution studies focus on the direct effects of air pollutants by using high concentrations (Rynning et al., 2018; Mokrzyński and Szewczyk, 2024; Institute, 2024). *In vitro* $PM_{2.5}$ exposures reported in the literature sometimes exceed 300 μg/ml, which is significantly higher than the annual average exposure levels, such as ~10 μg/m^3 in Western Europe and ~50 μg/m^3 in India (Klein et al., 2013), as most exposure protocols aim to reduce exposure time and increase PM concentration to obtain the same results as chronic exposure with low concentrations. Moreover, most studies expose the cells to PM by adding these directly to the culture medium. These studies have gained valuable insights into the pathways underlying harmful effects of $PM_{2.5}$ exposure. Direct translation of these results to air pollutant exposure in real-world conditions is difficult since the used models often do not accurately mimic how the skin and lungs are exposed to air pollution Furthermore, long-term and repeated exposures beyond 7 days are understudied. Since immortalized cell lines often overgrow during extended cell culture periods, primary cells and induced pluripotent stem cells (iPSCs) could be used to prevent excessive growth. Experiments that utilize physiological pollution levels and repeated exposure would provide deeper insights into pollution-related diseases. However, to more accurately study the effects of long-term air pollutant exposure, more complex models that also incorporate immune cells are required (Ryu et al., 2019).

In this review we discuss currently used *in vitro* models to study the effect of air pollution, and identify gaps in current knowledge on how exposure to air pollution results in the development of immune-mediated diseases of the skin and the lung, as these two epithelial barrier tissues are the first to be exposed to air pollution. Furthermore, inflammation in the skin affects responses in the lung. For example, atopic dermatitis predisposes to the development of asthma, referred to as atopic march (Somanunt et al., 2017; Spergel and Paller, 2003). We focus in more detail on how organ-on-chip (OoC) technology can enhance mechanistic studies of the skin and lung's response to air pollution.

2. Current *in vitro* models for studying effects of air pollutant exposure

2.1. 2-Dimensional (2D) cell culture models

The simplest models used to study the effects of air pollution on the development and/or exacerbation of immune-mediated diseases of

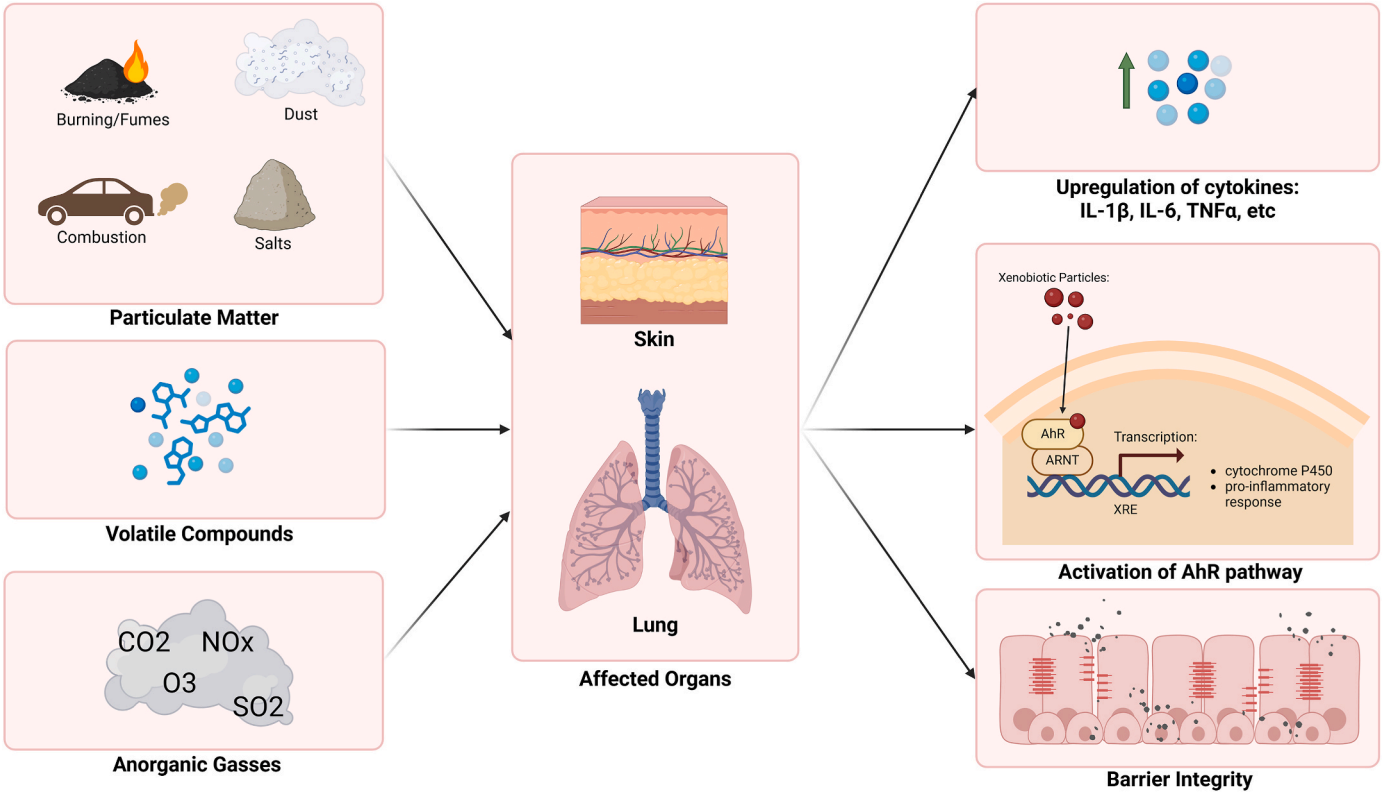


Fig. 1. A schematic representation illustrating the impact of various environmental stressors on the lung and skin tissue. Different environmental stressors, here subdivided into particulate matter, volatile compounds, and inorganic gasses, are depicted. These exposures trigger cellular stress responses, such as the upregulation of cytokines, and a decrease in barrier integrity. Moreover, the Aryl hydrocarbon Receptor pathway is activated by xenobiotic particles like B(a)P, which in turn upregulates other pro-inflammatory responses and transcription of cytochrome P450 for the degradation of these particles. Created in BioRender. Koornneef, S. (2025) https://BioRender.com/o79w301.

epithelial barrier tissues such as the skin and the lung are based on 2D cell cultures using immortalized cell lines or patient derived cells.

Before the development of more advanced co-culture models for lung tissue, lung cell lines were commonly stimulated by adding air pollutants to culture medium, resulting in the production of pro-inflammatory cytokines and oxidative stress (Engels et al., 2024; Grilli et al., 2018; Stringer and Kobzik, 1998). This approach is still used in certain experimental contexts, however, more often lung cells/cultures are exposed to air pollution at the air-liquid interface.

Insights into the cellular pathways affected by $PM_{2.5}$ exposure in the skin have primarily been obtained by exposing keratinocyte monolayers to $PM_{2.5}$, or PAHs in cell culture medium. These experiments demonstrated the induction of oxidative stress, cell death and senescence, as well as decreased barrier integrity and the production of pro-inflammatory cytokines (Piao et al., 2018; Zhen et al., 2019, 2024; Zhu et al., 2022; Herath et al., 2022; Kim et al., 2017, 2021; Choi et al., 2020; Liao et al., 2020; Li et al., 2017; Ortiz et al., 2023).

2D cell cultures using cell lines or primary cells are a well-accepted method and have significantly advanced understanding of toxicity of components of air pollution, and cell-specific responses to exposure to air pollution. These models are highly reproducible, suitable for high throughput and informative in (cyto)toxicity and metabolic effect studies. Furthermore, in 2D cell culture models, elucidating the response of individual cell types is more straightforward. However, cells grown in 2D often display poor differentiation, and altered behavior and morphology compared to the source tissue (Duval et al., 2017) and results do not always translate to *in vivo* exposure in direct comparisons (Sayes et al., 2007).

2.2. Air-liquid interface culture models

By combining keratinocytes with fibroblasts and other cell types found in the skin, such as adipocytes, endothelial cells, nerve cells, melanocytes, immune cells, and appendages, more representative *in vitro* models can be created (Hofmann et al., 2023; Li et al., 1997; Rimal et al., 2024). Models featuring a layer of fibroblasts and a layer of keratinocytes are considered bilayer models, while adding adipocytes creates trilayer models (Randall et al., 2018; Kim et al., 2019; Huber et al., 2016; Vidal et al., 2019; Monfort et al., 2013; Bellas et al., 2012; Sanchez et al., 2022; Trottier et al., 2008). Both bilayer and trilayer models are considered full-thickness models and can be combined with other cell types. They are typically cultured at the air-liquid interface (ALI) on culture inserts. While full-thickness models offer a more realistic tissue architecture and provide an ALI that supports maturation of the epidermis, few studies to date have used these models to investigate the effects of air pollution on the skin. This is likely due to the longer and more complicated culture protocols required.

Similar to the advanced skin models, lung models used for pollution studies often provide an ALI to support the differentiation and maturation of airway epithelial cells (Silva et al., 2023; Gerovac et al., 2014). These models are typically classified as either alveolar or airway models (e.g. bronchiolar) (Eenjes et al., 2021; Lamers et al., 2021; Burgess et al., 2024). Since these cultures mostly consist of epithelial cells, they lack combinations and interaction with other cell types. More advanced models are being proposed that incorporate a combination of mesenchymal, endothelial, epithelial and/or immune cells. Cells used in such models can be patient-derived primary cells or commercial cell lines (Licciardello et al., 2023; Sellgren et al., 2014). Although establishing primary cell cultures is more time-consuming and expensive than using commercial cell lines, models derived from primary cells more closely

replicate *in vivo* conditions and can provide patient-specific insights (Kreimendahl et al., 2019).

Differences in response to components of air pollution were shown between lung cells exposed submerged or at ALI. For example, A549 alveolar cells exposed to SiO_2 or ZnO showed higher IL-8 release when exposed submerged (Lenz et al., 2009; Panas et al., 2014). On the other hand, in the bronchial epithelial cell line 16HBE14o, similar IL-8 responses were found after submerged or ALI exposure, while the ALI dose was 4x lower (Holder et al., 2008). Possible explanations for the observed differences include the differentiation status of the cells, properties of the pollutant themselves in solution or in air, and possible differences in culture conditions. These observations however underline the importance of using relevant *in vitro* models.

2.3. 3D cell culture models

Organoids are self-organized cell clusters that recapitulate a miniaturized organ, exhibiting properties and functions similar to those of the native organ.

Lung organoids are derived from primary lung cells or iPSCs and can be classified into two types: 1) bronchial epithelial organoids and 2) alveolar organoids (Eenjes et al., 2021; Li et al., 2024a). Primary bronchial cells seeded in hydrogel enable robust differentiation towards a mucociliary phenotype, whereas alveolar organoids primarily consist of AT2-like cells. Alveolar organoids can also be guided towards an AT1-like phenotype (Ohnishi et al., 2024). Both organoid models can be based on iPSC-derived progenitor cells, however, obtaining a fully mature AT2/AT1-like phenotype remains challenging (Tiwari and Rana, 2023; Stroulios et al., 2022).

Skin organoids are usually generated using iPSCs and can include skin appendages (Hong et al., 2023; Sun et al., 2021). While existing organoid models do not capture all aspects of the skin, those that include appendages are more physiologically relevant than simpler models.

While organoid models could improve the physiological relevance of air pollution studies, results can potentially be more accurately extrapolated from *in vitro* to *in vivo* situations and allow for cell-cell crosstalk, they have not yet been widely implemented in this field. This is likely due to the lengthy and complex culture protocols, which can take several months. Additionally, iPSC-derived organoids have limited differentiation capabilities due to constraints in the current protocols (Burgess et al., 2024). In contrast, primary cells exhibit strong differentiation capacities (Eenjes et al., 2021). Although obtaining patient material can be challenging, the variation in individual responses to stimuli such as cigarette smoke highlights the importance of including multiple donors in each experiment to adjust for genetic variability and donor-to-donor variation (Katsura et al., 2020). Moreover, for pollution studies involving exposure of the apical side, the organoids would need to be processed prior to use. Protocols to generate apical-out bronchial organoids are available, however these methods often result in reduced numbers of secretory cells (Winkler et al., 2022; Sachs et al., 2019). Nevertheless, once these challenges are addressed, organoids offer a realistic alternative to traditional 2D culture models, more closely resembling native tissue. Furthermore, organoids can serve as a cell source for exposure experiments, as organoids maintain differentiation ability even after being passaged.

3. *In vitro* air pollutant exposure methods

Various methods can be used to expose cell cultures to pollutants, depending on the type of pollutant and the specific culture system (Fig. 2). In *in vitro* experiments, exposures are generally carried out by adding air pollutants to the cell culture medium (Liu et al., 2021; Ke et al., 2018; Zhang et al., 2017). While this has yielded important insights into how pollutants impact individual cell health, it does not accurately replicate *in vivo* exposures. The concentration at the surface of submerged cultures differs from that near the cells. Most particles are hydrophobic, aggregate and tend to sink to the bottom of the well. Consequently, wells with larger volumes or larger culture areas and the same pollutant concentration will have more particles interacting with the cells at the bottom of the dish. In contrast, buoyant nanoparticles may experience the opposite effect, as most particles fail to reach the bottom of the well during submerged exposures (Watson et al., 2016). Therefore, alternative methods for pollution exposure are required.

Aerosolization or exposure via dry particles can help eliminate discrepancies among pollution studies caused by variation in well sizes and volumes, as these techniques enable most particles to directly settle on the culture surface. Commercial systems for aerosolizing air pollutants include the nebulization system Vitrocell Cloud Alpha (formerly known as ALICE (Lenz et al., 2014; Chortarea et al., 2017)) and the CelTox system (Sengupta et al., 2023). Other nebulization systems developed by academic groups and functioning similarly to the Vitrocell system have been reported (Ritter et al., 2001; Aufderheide et al., 2002). These devices utilize a vibrating mesh at the top of an exposure chamber, through which a liquid containing air pollution is vaporized onto the ALI cultures placed in the exposure chamber. While this exposure method closely replicates *in vivo* conditions, it is limited by the particles' ability to dissolve without generating aggregates larger than the pore size of the nebulizer, making it challenging to expose cultures with hydrophobic particulate matter. Alternatively, for exposures involving solid particles, commercial systems such as PreciseInhale® and XposeALI® are available. Solid compounds are introduced into a holding chamber using compressed air and then delivered onto the ALI cell cultures.

Lastly, cell cultures can be exposed to gases by flushing humidified gaseous pollutants into an incubator or culture chamber (Guenette et al., 2022; Horstmann et al., 2021). To protect lab workers from harmful gases, small sized, air-tight exposure chambers can be used. A modified Vitrocell VC 10 Smoking Robot, for example, can be used for gaseous exposure experiments, including combination with cigarette smoke (Breheny et al., 2014). Gaseous exposure methods can be applied to both skin and lung *in vitro* cultures (Ji et al., 2017; Upadhyay et al., 2022a).

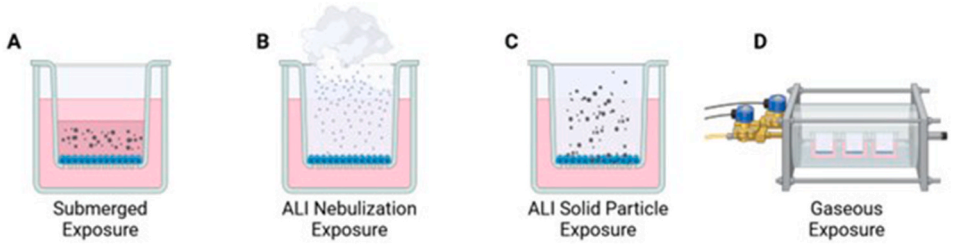


Fig. 2. Schematic overview of different in vitro exposure methods visualized using transwell inserts. A: Submerged exposure: Cultures are exposed to particulate matter by dissolving the environmental stressor in the culture medium. B: Nebulization exposure: Pollutants are aerosolized and delivered to ALI cultures using sedimentation of the droplets. C: Solid particle exposure: Solid particles are brought into a pressurized holding chamber and delivered on top of ALI cultures. D: Gaseous exposure: Cultures are brought into a chamber that holds various gas pollutants. Created in BioRender. Koornneef, S. (2025) https://BioRender.com/x48j138.

These exposure methods could theoretically be integrated in OoC models. In lung-on-chip devices, aerosols are already used to expose the tissues to harmful particles (Todo, 2017; Dwivedi et al., 2018). To our knowledge, these methods have not yet been applied to skin-on-chip devices.

4. Pathobiological pathways/mechanisms underlying adverse effects of air pollution

Pathobiological mechanisms underlying the adverse effects on lung and skin tissues and cells have been studied in *in vivo* rodent models and in *in vitro* systems. Extensive literature is available on effects of *in vivo* exposure on the respiratory tract, whereas effects on the skin have been studied less extensively. Animal studies, controlled exposure in healthy volunteers, and *ex vivo* analysis of human samples suggest that oxidative stress, inflammation and allergic responses, as well as impaired epithelial barrier function, play significant roles in developing immune mediated disease in these barrier tissues. For instance, controlled exposure to $PM_{2.5}$ in both healthy volunteers and rodents led to an accumulation of neutrophils into the lung (Ghio et al., 2000; Li et al., 1999, 2024b; He et al., 2017; Salvi et al., 1999). Exposure to diesel exhaust particles (DEP) resulted in increased levels of eosinophil cationic protein, histamine and IgE in the nasal wash fluid of healthy volunteers (Lee et al., 2024b; Diaz-Sanchez et al., 1996, 2000a; Diaz-Sanchez et al., 2000b). In skin, air pollutants can both exacerbate and result in the development of skin disease. For example, in an atopic dermatitis mouse model, PM_{10} application to the skin resulted in increased severity, with increased expression of proinflammatory genes such as *Il1b* (Watkinson et al., 2001).

4.1. Aryl hydrocarbon receptor signaling

Aryl Hydrocarbon Receptor (AhR) signaling contributes significantly to the body's response to air pollution (Fig. 3). AhR is a ubiquitously expressed cytosolic receptor, with its expression levels varying across and within tissues. AhR is activated by exposure to atmospheric PM, as shown in reporter cell lines, *in vivo* and *in vitro* lung exposure models and *in vitro* skin models (Barhoumi et al., 2020; McDonough et al., 2019; Aryal et al., 2024; Hartung et al., 2025) and its ligands include environmental chemicals, food constituents and endogenous substances. As a result, AhR serves not only as a key regulator of xenobiotic metabolism but also a regulator of various physiologic processes (Abel and Haarmann-Stemmann, 2010). The differences in mechanisms between physiological ligands and xenobiotics are still poorly understood (Stockinger et al., 2024), and investigating these mechanisms in relevant skin and lung models after exposure to air pollution and how this affects surrounding cells would be important in increasing our understanding of the role of AhR signalling in adverse health effects, oxidative stress and inflammation. Exposure to xenobiotic AhR-ligands leads to oxidative stress. For instance, exposure to dioxin or benzo(a)pyrene (BaP) increases the gene and protein expression of AhR, CYP1A1, and CYP1B1 (Ghosh et al., 2018). This is followed by an increase in CYP1A1 and -1B1-mediated generation of reactive oxygen species (ROS), a decline in mitochondrial function, and ultimately, apoptosis in different cell types, including lung cell lines (Zhou et al., 2017; Elbekai et al., 2004; Huang et al., 2021; Bansal et al., 2014). Furthermore, AhR-mediated metabolic activation of PAHs results in the formation of mutagenic DNA and protein adducts (Dipple et al., 1999). The number of DNA adducts correlates with PAH exposure *in vivo*, a relationship already evident in blood samples of newborns (Perera et al., 2005). On the other hand, overexpression of AhR in in lung adenocarcinoma cells resulted in reduced ROS levels and DNA damage following cigarette smoke exposure (Cheng et al., 2012). Additionally, in the skin, UV exposure induces expression of the endogenous AhR ligand FICZ, improving DNA protection (Cheng et al., 2012). Finally, AhR signaling enhances inflammatory responses, as evident in both lung and skin cell lines after

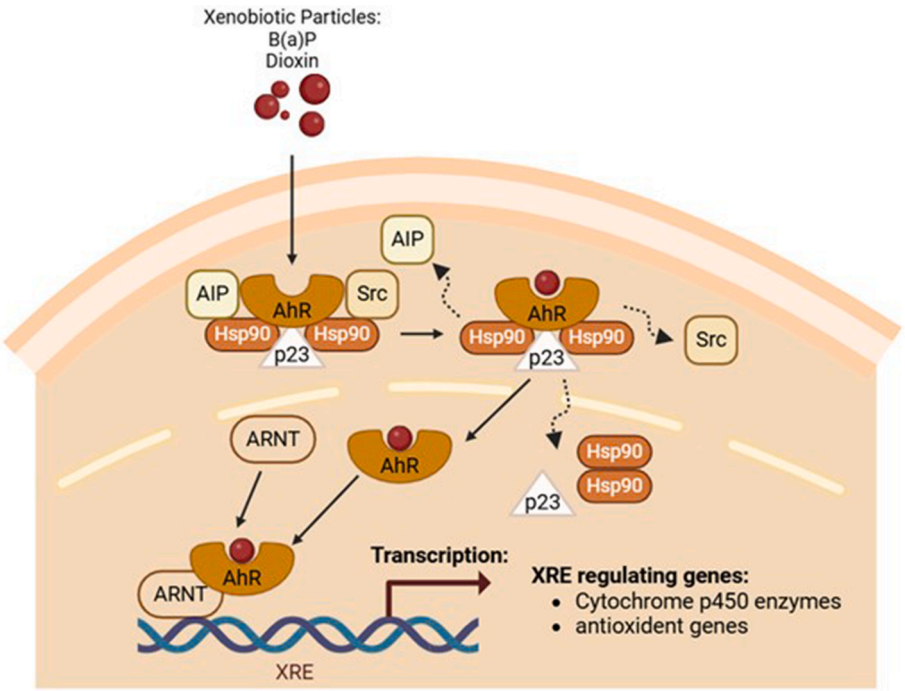


Fig. 3. Aryl hydrocarbon receptor signaling on activation via xenobiotic particles. In its inactive form, AhR complexes with heat shock protein (HSP)90, AhR-interacting protein (AIP), p23 and SRC. After ligand binding, AIP dissociates and AhR translocates to the nucleus, where it binds to ARNT. This complex binds to xenobiotic responsive elements (XREs) in the DNA, regulating gene expression of for example the cytochrome p450 (CYP) enzymes CYP1A1 and CYP1B1 (Abel and Haarmann-Stemmann, 2010; Lag et al., 2020; Rothhammer and Quintana, 2019) and downstream antioxidant genes such as Nrf2, and HMOX1 (Wang et al., 2019). AhR also regulates gene expression of genes that do not have XREs, by interacting with estrogen receptor (Wang et al., 2019), or by controlling the activation of other transcription factors such as NF-κB (Vogel et al., 2014; Ohtake et al., 2003). Created in BioRender. Koornneef, S. (2025) https://BioRender.com/s31m666.

exposure to AhR ligands (Bocheva et al., 2023; Yu et al., 2025).

4.2. Inflammation

In general, exposure of lung and skin models to PM, but also pollutant gases, triggers inflammation (Fitoussi et al., 2022; Zou et al., 2020), with the specific outcome varying, depending on the tissue context.

For example, exposure of bronchial epithelial cells cultured at ALI to $PM_{2.5}$, carbon nanotubes or DEP triggered production of IL-6, IL-8, IL-1β, NF-κB, and TNFα (Lenz et al., 2014; Ji et al., 2017; Zou et al., 2020). This heightened inflammatory response after air pollution exposure may partly be attributed to epigenetic changes, as seen in Treg at the *FOXP3* locus and in bronchial and nasal epithelial cells (Nadeau et al., 2010; Li et al., 2024c; Irizar et al., 2024; Sordillo et al., 2021). However, when bronchial epithelial cells cultured at ALI were exposed to urban aerosols, no differences in DNA methylation were observed in the promotor regions of four selected genes (*IL18*, *AHR*, *CYP1A1* and *CYP1B1*) (Santoro et al., 2024).

In keratinocytes, exposure to $PM_{2.5}$ led to increased gene expression of *IL1A* and *IL1B* (Liao et al., 2020; Kim et al., 2017), as well as elevated protein levels of GM-CSF, TSLP, TNFα, IL-1α, IL-6 and IL-8 (Choi et al., 2020; Li et al., 2017). However, in these experiments, keratinocytes were exposed submerged. *In vitro* culture of alveolar macrophages with PM or BaP results in reduced cell viability and increased levels of TNFα, IL-6, GM-CSF, MIP-1α and IL-1β (Ghio et al., 2000; Jalava et al., 2007; van Eeden et al., 2001; Lecureur et al., 2005; Al et al., 2019; Becker et al., 2003; Mukae et al., 2000).

Especially for skin, as responses can be vastly different between submerged and ALI exposure, future studies should include exposure at the ALI as this is the relevant route of exposure for the skin. Furthermore, while it is important to study inflammatory responses to individual components of air pollution, as ambient air consists of a complex mixture of different pollutants, more research should focus on assessing effects of combined stressors.

4.3. Epithelial barrier function

The epithelium of both the skin and the lung act as a barrier to the outside environment. Exposure to PM adversely affects tissue barrier integrity (Celebi Sozener et al., 2020), where exposure to substances including air pollution results in damage of the epithelium, contributing to inflammation and sensitization (Sun et al., 2024). Epithelial damage to the skin and lung, and the resulting induction of type 2 driven inflammation can ultimately lead to the development of allergic conditions, such as AD, asthma and allergic rhinitis (Reynolds et al., 2023; Celebi Sozener et al., 2022; Zhao et al., 2020).

In the lungs, O_3, DEP and house-dust organic contaminants disturbed the membrane integrity of bronchial epithelial cells *in vitro* (Woo et al., 2020; Reynolds et al., 2023). Further, nasal mucosa downregulated the expression of ZO-1 and vascular endothelial cadherin upon exposure to PM (Marques Dos Santos et al., 2022; Byun et al., 2019). In lung epithelial cells, this resulted in decreased barrier integrity as measured using FITC-dextran transit (Smyth et al., 2020).

In keratinocytes PM exposure decreased expression of structural proteins such as. Filaggrin (Kim et al., 2021) and zonula occludens (ZO)-1 (Choi et al., 2020). Full thickness skin showed a reduction in thickness of the stratum corneum and decreased Filaggrin expression. (Kim et al., 2021; Sun et al., 2024). Additionally, markers for proliferation were decreased upon PM exposure, both in full thickness skin as in 2D models (Dijkhoff et al., 2020).

Again here, effects of pollutants on epithelial barrier integrity of lung and skin models should be assessed at ALI, in addition to effects of exposure to multiple pollutants, and repeated exposures. Furthermore, research should focus on *in vitro* models using cells from patients with respiratory disease (Asthma, COPD for example) or skin disease (atopic dermatitis, psoriasis), to determine how air pollution exposure affects an already perturbed barrier.

4.4. Exposure to combined stressors

Most *in vitro* studies on the health effects of environmental stressors focused on the impact of a single stressor. However, in reality, human tissues are co-exposed to multiple environmental factors simultaneously, which could amplify the harmful effects of air pollutants. Thus although important, the health effects of stressor combinations and the underlying molecular mechanisms remain largely unexplored.

The most studied stressor combination in skin research is photopollution, particularly the combination of PAHs and UV light. UVB light enhances the genotoxicity of PAHs and the resulting phototoxicity is characterized by oxidative stress, mitochondrial damage and impaired DNA-repair (Mokrzyński and Szewczyk, 2024; Larnac et al., 2024). To the best of our knowledge, only one study has explored the combined impact of humidity and air pollution on skin. In this study, semi-dry airflow (45 % relative humidity) exacerbated the negative effects of air pollutants on cellular functionality. However, the impact of humidity on the release of cytokines and chemokines varied depending on the specific pollutant involved (Seurat et al., 2021).

In the lungs, exposure to PM or gases have received most attention. While co-exposure studies of PM with other stressors, such as gaseous compounds, are often overlooked, research shows that exposure to a combination of stressors in the form of DEP in combination with gaseous pollutants has synergistic effects (Upadhyay et al., 2022b).

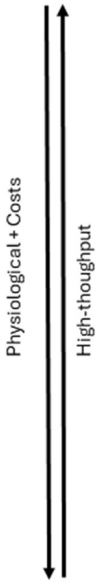
5. Organ-on-chip technology for future research into the effects of pollution

Future research into air-pollution-induced immunological diseases of epithelial barrier tissues should focus on exploring the combined impact of multiple environmental stressors and/or the impact of long term/repeated exposures. Importantly this should be explored in more complex cellular *in vitro* settings than done previously to more accurately mimic the native organ and to facilitate *in vitro-in vivo* extrapolation. More complex models would facilitate the characterization of cell-cell and cell-tissue interactions. This would align with the 3 R principle to reduce, refine and replace animal studies.

As pointed-out before, cell and tissue models can be improved by co-culturing lung or skin cells with other cell types, which facilitates cell-cell interactions and mimics more complex *in vivo* environments. However, traditional cell culture techniques still lack crucial biophysical cues, most notably perfusion and mechanical actuation, limiting their physiological relevance (Table 1). Moreover, the detection of biomarkers is often restricted to end-point measurements, which makes it challenging to study the dynamic kinetics of gene and protein expression over time.

OoC technologies have the potential to drive significant advances in this field. These microfluidic devices maintain cells, tissue explants or organ models under controlled physiological conditions for continuous monitoring. By incorporating features such as perfusion, mechanical stretching and biosensing within the microfluidic system, OoCs can more realistically replicate the native environment of tissues and enable real-time tracking of biomarkers (Fig. 4). Air pollutant exposure technologies can also be incorporated into microfluidic platforms, facilitating long-term/chronic exposure studies and exposure to multiple environmental stressors. While there have been significant advancements in the OoC field, its application in air pollution research remains limited, with few studies using lung and skin models exploring this area (Table 2). This section will discuss the potential of OoC models to investigate the health effects of pollutants on the skin and lungs, as well as the challenges to effectively integrating these technologies into research on air pollution's effects on human health.

Table 1
Summary of the different models used for studying adverse health effects of air pollution.



Model	Advantages	Disadvantages
2D cell culture	• Simple protocol; low cost • Highly reproducible • Useful for (cyto) toxicity studies • More homogeneous response to stimuli • Accepted in regulatory instances	• Cells often display altered morphology and behaviour compared to native tissue, as well as gene and protein expression profiles • Often more sensitive to stimuli • Often poor cell differentiation • Lacks cell-cell crosstalk
ALI-culture	• Mimics exposure to air • Mucociliary differentiation • Moderate cell-cell interactions	• Static condition • Limited cell diversity • Complicated and lengthy culture protocols • Lacks biological cues like flow and stretch
Advanced co-culture models	• Incorporates multiple different cell types • Cell-cell interactions • ECM remodeling studies	• Static conditions • Complex setup • Lacks biological cues like flow and stretch
Organoid cultures	• Moderate Cell-Cell interactions • 3D self-organizing structure • Improved physiological relevance	• Static condition • No waste transport, necrotic centers • Long term growth difficult • Intricate and lengthy culture protocol • Requires expensive factors • Usually outside-in.
Organ-on-Chip	• Tissue-Tissue interactions • Real time monitoring • Microphysiological cues (flow, stretch, ALI)	• Limited tissue interactions • Technically complex • Limited scalability and reproducibility • High costs
Animal models	• Physiologically relevant • systemic response studies • Accepted in regulatory instances	• Moderate prediction of drug responses • Ethical concerns • Requires specialized facilities • Often limited human relevance

6. Organ-on-chip: external cues and sensing

6.1. Perfusion and air flow

Both the skin and the lungs are exposed to air on their apical tissue surfaces and are supplied with blood by the cardiovascular system on their basal side. In the lungs, gas exchange occurs between the alveolar air and blood in the pulmonary capillaries, facilitating the oxygenation of the blood. In both tissues, blood circulation serves as a route for immune cell recruitment, the supply of nutrients and the removal of metabolic waste, but also the dissemination of biomarkers indicative of a disturbance in tissue homeostasis.

Conventional ALI cultures are static models of air and blood, lacking flow cues for the cultured cells. In contrast, various lung-on-chip and skin-on-chip models have been developed to incorporate liquid and/or air flow, separated by a microporous membrane. The liquid component or basolateral side is usually perfused with medium via a pump, while the air compartment or apical side can be either actively or passively ventilated.

In lung-on-chip models, medium flow and airflow cues promote the maturation of blood vessels and the airway epithelium, respectively (Katoh, 2023; Stucki et al., 2015; Meng et al., 2022; Nawroth et al., 2023; Sengupta et al., 2022). The basolateral flow of medium interacts with the endothelial cells and mimics the capillary blood flow. The shear stress generated by this flow modifies endothelial cell permeability and can also influence endothelial cell viability (Smyth et al., 2020; Larnac et al., 2024; Upadhyay et al., 2022b; Zeng et al., 2022). The commercial Emulate lung-on-chip, based on the work published by Huh et al. (Todo, 2017), combines airflow and mechanical stretch. These factors support the maturation of the tissue and aid in the directionality of mucociliary clearance (Seurat et al., 2021). Another alveolar chip model, produced by AlveoliX, demonstrated that a 3D stretchable membrane enhanced the expression of alveolar markers, like AQP5 and SFTPC (Katoh, 2023). Moreover, stress levels decrease over time when stretch or airflow are applied to endothelial or epithelial cultures (Seurat et al., 2021; Katoh, 2023; Meng et al., 2022). However, the barrier integrity of epithelial cells appears to weaken and becomes more sensitive to nanoparticle environmental stressors, such as ZnO and TiO_2, when combined with stretch (Sengupta et al., 2023).

To our knowledge, no studies have directly compared skin epithelial tissue culture with active air flow to static air culture. However, medium flow cues can promote maturation of epithelial tissue in skin models, with higher shear stress levels influencing keratinocyte morphology (Meng et al., 2022; Nawroth et al., 2023; Agarwal et al., 2019). Furthermore, shear stress induced by liquid flow promotes fibroblast migration and organization, with high shear stress leading to cell rounding (Meng et al., 2022; Nawroth et al., 2023). This imposes a limit on the acceptable shear stress levels in microfluidic devices designed for skin culture, while also offering opportunities for improving culture conditions. Perfusion could extend culture duration. However, full-thickness skin models have been cultured in inserts for up to 50 days, whereas skin-on-chip tissues are usually cultured up to 28 days (Zoio et al., 2021a). Therefore, it remains unclear to what extent perfusion improves survival and quality of skin-on-chip models during long term culture, compared to static ALI culture.

In addition to facilitating tissue maturation, more accurately replicating native tissue, flow cues can impact the dynamics of pollutant uptake and downstream responses, likely better replicating *in vivo* uptake dynamics. For example, the uptake of nanoparticles by endothelial cells exposed to flow-induced shear decreases as shear force increases (Charwat et al., 2018). Therefore, fluid mechanics could influence the distribution, bioaccumulation, and clearance of nanoparticles. Moreover, the design of flow channels affects how cells and tissue interact with environmental pollutants. For example, pollutant accumulation in chips with rectangular channels is different compared to the more naturally shaped circular channels (Zhang et al., 2018). Moreover, potentially the improved culture conditions will allow for long-term and/or repeated exposure experiments (Lu and Radisic, 2021) to more accurately mimic real-life situations.

6.2. Mechanical cues

Conventional tissue culture does not include tensile or compressive loads. However, in daily life, both the skin and lungs are subjected to cyclic tensile and compressive forces.

In the lungs, stretching begins in utero, due to internal pressure from internal fluid flow, or later through fetal breathing movements. In mammals, the respiratory muscles periodically contract to alter the volume of the lungs, facilitating the exchange of oxygen and carbon dioxide through changes in pulmonary pressure (Novak et al., 2021). In the healthy lungs, stretching-mediated mechanotransduction promotes pulmonary cell differentiation. For instance, stretching induces the differentiation of alveolar bipotent progenitor cells into AT1 alveolar cells during lung development in a fetal mouse model (Nguyen et al., 2021). In lung-on-chip models, mechanical stretch can be applied to more accurately replicate physiological conditions. Several commercial systems, such as those developed by Emulate and Alveolix, incorporate

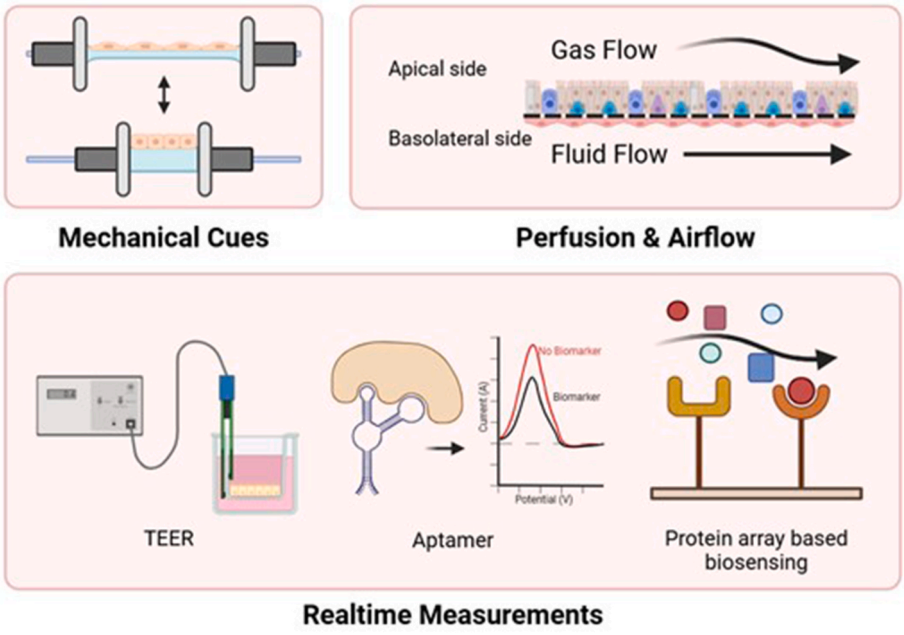


Fig. 4. Various cues and real-time monitoring can be integrated in lung- and skin-on-chip technologies. Mechanical cues: cyclic stretching mimics breathing movements in lung-on-chip systems or mechanical forces in skin-on-chip models. Perfusion and airflow: Controlled media flow mimics blood flow and provides shear stress to endothelial cells. Bi-or unidirectional airflow can be introduced to epithelial cells, enhancing physiological relevance. Realtime Measurements: TEER, aptamer-based sensors, or protein array based biosensing enables continues measurements of dynamic changes within organ-on-chip. Created in BioRender. Koornneef, S. (2025) https://BioRender.com/v11w112.

Table 2
Lung- and skin-on chip air pollution studies to date.

Tissue	Cell type	Type of pollutant	Exposure duration	Exposure method	Ref.	Year
Lung	Cell lines	12 nm Silica nanoparticles	5 h	Thin layer–> submerged	10.1126/science.1188302	2010
Lung	Cell line/primary cells	TiO2, ZnO2 nanoparticles	24 h	Submerged	10.1039/c8tx00156a	2018
Lung	Cell line/primary cells	Polystyrene nanoparticles	24 h	Submerged	10.1016/j.jhazmat.2023.131962	2023
Lung	Cell line/primary cells	TiO2, ZnO2 nanoparticles	48 h	Nebulization	10.3389/fphar.2023.1114739	2023
Lung	Cell line/primary cells	ZnO2 and CCP nanoparticles	48 h	Submerged	10.1021/acs.est.3c03678	2023
Lung	Cell line/primary cells	PM2.5	72 h	Submerged	10.1021/acsbiomaterials.0c00221	2020
Lung	Cell line/primary cells	PM2.5	24 h	Submerged	10.1016/j.ecoenv.2021.112601	2021
Lung	primary cells	PM2.5	96 h	Submerged	10.1007/s13206-022-00068-x	2022
Lung	Primary cells	polystyrene particles	48 h	Nebulization	10.3389/fbioe.2020.00091	2020
Lung	Cell line	PM2.5	24 h	Nebulization	10.1016/j.scitotenv.2020.143200	2021
Lung	Cell line	PM2.5	24 h	Nebulization	10.1016/j.ecoenv.2022.114318	2022
Lung	Cell line	PM2.5	24 h	Nebulization	10.1021/acssensors.3c01744	2022
Lung	Cell line, tissue slices	PM2.5	72 h	Submerged	10.1111/all.16179	2024
Skin	Cell line	PM2.5	24 h	Submerged	10.1016/j.tiv.2017.04.018	2017
Skin	Cell line	Combustion PM	24 h	Submerged	10.1016/j.chemosphere.2018.06.058	2018
Skin	Cell line	UV	0.5 h	N/A (ALI)	10.1039/C6LC00229C	2016
Skin	Cell line	UV	48 h	N/A (ALI)	10.1002/bit.27320	2020

mechanical stretching (Sengupta et al., 2023; Stucki et al., 2015; Nawroth et al., 2023). Studies using these systems have demonstrated that mechanical stretching promotes the maturation of rat AT2 cells (Sanchez-Esteban et al., 2001) and human airway bronchial cells (Nawroth et al., 2023). Mechanical stretching could also influence the response to nanoparticles. For example, the generation of ROS by alveolar cells exposed to silica particles was significantly elevated in the presence of mechanical strain (Todo, 2017). We propose the introduction of mechanical cues will result in more accurate replication of native tissue responses and will facilitate *in vitro-in vivo* extrapolation.

In the skin, stretching affects keratinocyte proliferation, migration and survival (Sanchez-Esteban et al., 2001) and stimulates fibroblast proliferation and collagen production (Lu et al., 2013). Several skin-on-chip studies have incorporated stretching or compression (van Haasterecht et al., 2023; Kim et al., 2022; Lim et al., 2018; Mori et al., 2018; Tokuyama et al., 2015; Wahlsten et al., 2021; Varone et al., 2021; Jeong et al., 2021; Kaiser et al., 2024) to enhance the physiological relevance of the culture conditions. Although the skin is continuously exposed to cycles of stretching and relaxation *in vivo*, and skin-on-chip models incorporating mechanical actuation exist, this factor is typically excluded from skin studies, especially in those investigating the effects of air pollution. To our knowledge, only one study has addressed the combination of pollutant exposure and mechanical tension in a short-term culture study with skin explants cultured under tension (Pambianchi et al., 2023). In this study, static tension modulated ozone-induced antimicrobial peptide (AMP) levels (CAMP, LL-37, hBD2, hBD3), as well as the kinetics of inflammatory molecule expression (COX2, AhR, MMP9 and 4HNE).

6.3. *Real-time monitoring of biomarkers*

The integration of sensors into *in vitro* culture systems can reduce

time spent on sample taking and processing while enabling noninvasive real-time monitoring of cellular biomarkers and culture conditions. While sensors can be integrated into both conventional tissue culture and OoC, sensor miniaturization and design freedom in OoC platforms create flexibility in sensor placement and optimization of the sensing conditions compared to conventional culture. Various optical, physical and electrochemical sensors have been developed for OoC (Shinde et al., 2023). Optical sensors often employ fluorescence, surface plasmon resonance (SPR) or optical coherence tomography. Electrical and electrochemical sensors are usually based on potentiometry/amperometry, field effect transistors or impedance. Physical sensors include pressure gauges, strain gauges and cantilevers. Sensors have also been developed for omics applications and are based on enzymes, antibodies or aptamers with various transducer types.

The most common sensor in studies with barrier tissues, such as the lungs and skin, measures physiological barrier function by assessing the resistance across the barrier. This transendothelial/epithelial electrical resistance (TEER) reflects the integrity of the epithelial barrier and is influenced by factors such as cell junctions, cell layer thickness, and state of confluency [203,204]. TEER is often performed with probes connected to a Voltohmmeter in both conventional culture and OoC but integrated sensors are increasingly common (Nazari et al., 2023a). Several skin-on-chip (Srinivasan et al., 2015; Alexander et al., 2018; Nguyen et al., 2024) and lung-on-chip (Zoio et al., 2021b; Khalid et al., 2020) devices have been reported in the literature with integrated TEER sensors. To measure TEER, the electric circuit must be closed by submerging all electrodes. This is easily achieved in submerged conventional and OoC cultures, however, in ALI cultures, the apical side of the tissue must be periodically flooded for short-duration TEER measurements at specific time-points. Automatic flooding and aspiration of ALI cultures is easier to integrate into OoC platforms than conventional culture plates due to the presence of fluid flow and the flexibility of microfluidic design in OoC. Further, TEER electrode placement is limited to the culture chamber and preferably close to the tissue being studied. Due to the design flexibility of OoC, the space between the sensors and the tissues can be minimized and thin electrodes with optimized geometry can be fabricated using microfabrication techniques (Nazari et al., 2023a). While the existing TEER electrodes in skin-on-chip are opaque, transparent electrodes have been reported in lung-on-chip devices, which facilitates macroscopic and microscopical analysis of the culture (Zoio et al., 2021b; Khalid et al., 2020). Integrating biosensors for other biomarkers, such as proteins and cytokines, could capture fluctuations in markers that are often missed by conventional omics-based and imaging-based end-point analysis at predefined time intervals, hence offering deeper insights into cellular responses and disease mechanisms. By avoiding or simplifying sample taking and processing they also facilitate high throughput applications. Such sensors are prevalent in OoC for diagnostics and therapeutics (Shinde et al., 2023). Various sensors have been integrated in the lung-on-chip for rapid detection of RNAs, and proteins (Ding et al., 2021), however to our knowledge these systems are not used routinely in air pollution research yet. One skin-on-chip study reported an integrated sensor for studying the effects of air pollution on skin (Liu et al., 2021), using an integrated photonic protein array system for studying $PM_{2.5}$-induced cytotoxicity in human keratinocytes, revealing activation of NF-κB and NALP3 signaling pathways and increased production of IL-6 and IL-1β. The latter findings correlated well with results obtained from ELISA, highlighting the system's ability to accurately monitor cellular responses to pollution with proteomics (Zhang et al., 2017).

An important downside of antibody-based biorecognition elements like the photonic protein array system is the accumulation of the biomarkers on the sensor, which reduces its sensitivity. This issue could be resolved by using aptamers, which can regenerate to their unbound state, enabling the measurement of new molecules (Zhao et al., 2011). Aptamers can be designed for various targets, including proteins and nucleic acids (Dunn et al., 2017) with successful applications in OoC (Nguyen et al., 2018).

Additionally, culture conditions on-chip can be monitored by additional sensors. For instance, changes in the pH of the cell culture medium can be optically assessed by measuring the change in phenol red in medium flowing through a separate transparent tube (Khalid et al., 2020). Furthermore, integrated oxygen-sensitive microparticle-based sensor spots allow for continuous optical sensing of oxygen concentration on-chip (Zargartalebi et al., 2024), providing real-time monitoring of oxygen levels within the culture.

More than conventional culture systems, OoC platforms provide the flexibility to combine multiple sensing modalities or to combine sensing with actuation. Despite this flexibility, none of the reported studies with skin- and lung-on-chip models combined TEER measurements with stretching. Since stretching affects tissue maturation and function, including barrier function and uptake of particles, in the lung and skin, integrating TEER sensing with stretching could offer valuable insights into the effects of mechanical load on these tissues and on their response to (air) pollutants. Furthermore, none of the studies referenced above have incorporated TEER alongside other types of biosensors to measure biomarkers involved in stress response of the skin and lungs.

7. Challenges to effectively integrate organ-on-chip technology for pollution research

Scalability and reproducibility remain significant challenges in all organ-on-chip systems, including lung- and skin-on-chip systems (Zirath et al., 2018). Some OoCs, such as the OrganoPlates from Mimetas, are already actively assisting with FDA IND application for drug safety and effectiveness. The systems initially did not support the ALI conditions required for the differentiation of airway and skin cells. Some academic solutions designed plate variations to create ALI for lung cells (Jung et al., 2022). Increased scalability for dynamically co-culturing inserts have also been proposed, such as Simple-flow: multiple linked cell inserts to increase fluidic flow in the basolateral compartment of the cell insert (Leung et al., 2022). However, chips with more advanced mechanics, such as stretch or airflow, often face difficulties in scaling up. As a result, standardization in the field remains limited due to the challenges of achieving scalability and reproducibility. Various organizations, such as ISO, CENELEC, 3RC and ECVAM and nationwide collaboratives such as the Dutch hDMT, are actively collaborating to establish tissue-specific functional parameters. However, for these systems to gain formal acceptance, alignment with established regulatory frameworks is essential. Therefore, EU focus groups and intergovernmental organisation OECD have recently developed a roadmap with guidelines towards developing standards for OoC technology (OECD, 2021). Other considerable research worldwide is contributing to addressing such challenges, paving the way to solid establishment of the technology (Iriondo et al., 2024; Mastrangeli and van den Eijnden-van Raaij, 2021). So far, standardization efforts, like ISO/TC 276/SC 2, have marked 4 standards but are not yet published (ISO). Addressing these regulatory considerations and performance critertia, like reproducibility, is crucial for broader adoption of OoC. Once standards are available, researchers, such as in industry and acedamics, may revise or validate their OoC work to meet the criteria set by the regulatory groups. If these demands are met, industry and regulators, including organizations such as EMA, FDA, and EPA, OoC could fullfill a valuable role in air pollution studies and serve as a replacement model for animal studies.

8. Conclusion

The molecular mechanisms behind air pollution-induced adverse health effects on the lungs and skin remain relatively poorly understood, despite valuable insights gained from simple 2D and more complex 3D culture experiments. However, these culture methods often lack key tissue features such as perfusion, mechanical stretching, and the necessary cell-cell crosstalk between the multiple cell types present in

native lung or skin tissue. We propose to bridge these knowledge gaps in studies on adverse health effects of air pollution by utilizing skin and lung OoC models. OoC platforms can integrate lung or skin cells, tissue models or patient-derived tissues with critical cues such as flow and mechanical stretching, enhancing the (patho)physiological relevance of the models. By incorporating sensors, the culture conditions can be further optimized, and cellular responses can be monitored in real-time, providing a more comprehensive understanding of pollution's impact.

Studies investigating the health effects of air pollution typically expose cells via the culture medium, whereas in reality, both the skin and lungs are exposed to air. PM exposures can be simulated using nebulization systems, whereas gaseous exposures can be replicated using gas chambers. These exposure systems can also be integrated into OoC platforms. By using realistic dosages, combinations of stressors, and mimicking long-term and/or repeated exposures, the field could progress towards more physiological models of air pollution exposure.

CRediT authorship contribution statement

Sem Koornneef: Writing – original draft, Conceptualization. **Fiona J. Horne:** Writing – original draft, Conceptualization. **H. Bing Thio:** Writing – review & editing, Funding acquisition. **Massimo Mastrangeli:** Writing – review & editing, Supervision, Funding acquisition. **Robbert J. Rottier:** Writing – review & editing, Supervision, Funding acquisition. **Willem A. Dik:** Writing – review & editing, Funding acquisition. **Eveline D. de Geus:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

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

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Data availability

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

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