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Review

Olfactory cell cultures to investigate health effects of air pollution exposure: Implications for neurodegeneration

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ABSTRACT

Air pollution is a major, global public health concern. A growing body of evidence shows that exposure to air pollutants may impair the brain. Living in highly polluted areas has been linked to several neurodegenerative diseases, where exposure to complex mixtures of air pollutants in urban environments may have harmful effects on brain function. These harmful effects are thought to originate from elevated inflammation and oxidative stress. The olfactory epithelium is a key entry site of air pollutants into the brain as the particles are deposited in the upper airways and the nasal region. A potential source of patient-derived cells for study of air pollutant effects is the olfactory mucosa, which constitutes a central part of the olfactory epithelium. This review first summarizes the current literature on the available *in vitro* models of the olfactory epithelium. It then describes how alterations of the olfactory mucosa are linked to neurodegeneration and discusses potential therapeutic applications of these cells for neurodegenerative diseases. Finally, it reviews the research performed on the effects of air pollutant exposure in cells of the olfactory epithelium. Patient-derived olfactory epithelial models hold great promise for not only elucidating the molecular and cellular pathophysiology of neurodegenerative disorders, but for providing key understanding about air pollutant particle entry and effects at this key brain entry site.

1. Introduction

1.1. Air pollution is a complex mixture of compounds that critically influences human health

Air pollution is a leading environmental factor for adverse health effects in humans (Cohen et al., 2017; Lim et al., 2012). It has been estimated that 4.2 million people die prematurely due to air pollutants each year (WHO 2008) mostly due to cardiorespiratory reasons (Forouzanfar et al., 2015). However, in addition to those diseases traditionally considered to be caused by exposure to air pollution, emerging evidence points to a connection with other diseases as well. Evidence is mounting for the impact of air pollution exposure on neuroinflammation and several neurodegenerative diseases including Alzheimer's disease (AD) and cognitive impairment (Block et al., 2012; Cacciottolo et al., 2017; Maher et al., 2016; Melinda C. Power et al., 2016). However, the actual exposure levels, types, and routes leading to impaired brain health remain poorly known.

Air pollution particles in the atmosphere originate from different sources and occur in different sizes and chemical compositions. Coarse particles of particulate matter ($PM_{10-2.5}$) (the numbers represent particle size in μm) are usually mechanically generated dusts or soil components (e.g. road dust), whereas fine particles ($PM_{2.5}$) contain more combustion-derived compounds and aged aerosols (Masri et al., 2015; Rönkkö et al., 2018). Ultrafine particles (Dp $<$ 100 nm) are mainly primary exhaust particles or formed via gas to particle conversion. These particles can be released to urban air from a variety of sources, including road traffic, cooking, airports and wildfires (Venecek et al., 2019). Urban air also contains very small ultrafine particles in high concentrations, which may have an impact on human health, which is not yet fully understood (T. Ronkko and Timonen, 2019). Different particle size classes vary in chemical compositions and toxic properties, depending on the emission sources, the seasons and even the time of the day (Jalava et al., 2015; T. J. Ronkko et al., 2018; Sillanpää et al., 2006).

Given the deposition patterns, the greatest exposure in the nasal region occurs for the smallest ultrafine particles and coarse particles. The coarse particles contain a variety of other compounds that are

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carried on the surfaces of solid mineral particles. Some of the metals carried on the particle surfaces are soluble (Jiang et al., 2014) and may thus affect the cells of the olfactory epithelium upon deposition. It is interesting to note that the role of biometals such as aluminum in AD has been widely discussed (Tomljenovic and Shaw, 2011) but the pollutant-carried metals have not yet been linked to the disease. Ultrafine particles may contain large concentrations of combustion derived components, such as elemental and organic carbon compounds as well as small metal particles (Sillanpää et al., 2006).

Typical emission in the ultrafine size range of air pollution is exhaust from both diesel and gasoline powered engines that could be indicated as black carbon concentrations in the atmosphere (Tobias et al., 2018). In densely populated urban areas, traffic is a major source of atmospheric aerosol particles (Pey et al., 2009). It is intriguing to note that living near to traffic may cause dementia and reduced cognition (Chen et al., 2017; M. C. Power et al., 2011), and that even low concentrations of black carbon in the atmosphere can increase the prevalence of stroke (Ljungman et al., 2019). Moreover, there are new findings on the effect of ultrafine particles on brain cancer (Weichenthal et al., 2019) (Epidemiology, accepted), indicating the ability of ultrafine particles to carry carcinogenic compounds to brain. Even though the ultrafine particles from traffic are rather short-lived, it is assumed that these particles may be of the greatest importance when considering the relationship between traffic pollution and adverse health outcomes also with regard to the olfactory system and the brain. It is interesting to note that in comparison to larger particles, the ultrafines have different mechanisms of translocation in cells and organs. They are able to penetrate cells and move through epithelial barriers in airways even without active uptake processes (Geiser et al., 2005; Nemmar et al., 2006). It has also been shown that solid ultrafine carbon particles are able to translocate in the central nervous system (Oberdörster et al., 2004).

1.2. The olfactory mucosa: an entry point of air pollutant particles to the brain

One of the most feasible brain entry routes of pollutant particles is the olfactory mucosa (OM), since both ultrafine particles and coarse particles (Cheng et al., 1991; Garcia et al., 2015; Gonzalez-Maciel et al., 2017; Heyder et al., 1986; Klocke et al., 2017; Oberdörster et al., 2004; Phalen et al., 2010) are deposited in the upper airways and nasal region. The olfactory epithelium consists of the olfactory mucosa (OM), including basal cells, neurons, sustentacular and mucocilliary cells (Salazar et al., 2019) (Fig. 1). Unlike rats and other mammals, human olfactory mucosa is distributed in patches and is interspersed with respiratory mucosa. Newly replenished neuronal cells grow towards and synapse on specialised glomeruli of the olfactory bulb which projects onto primary olfactory regions such as the piriform, entorhinal cortices and amygdala. Within the projection areas the cells then synapse onto various regions of the brain such as the hippocampus, prefrontal cortex and hypothalamus (Borgmann-Winter et al., 2015). Olfactory information therefore has an ability to influence visceral, cognitive and emotional brain functions.

Olfactory ensheathing cells (OECs) located beneath the sensory epithelium within the lamina propria accompany the neuronal axons to the olfactory bulb. The multipotency of the OECs is due to the association of glia with the olfactory nerve (Ohnishi et al., 2015). OEC's are heterogeneous in nature, a quality reflective of their highly plastic nature and ability to be manipulated with varying growth conditions. A second population of stem cells located below the olfactory epithelium are known as ecto-mesenchymal stem cells (EMCs), and possess both mesenchymal and neuronal characteristics. In culture these can generate human olfactory neurospheres (hONS). The hONS hold a capability to be differentiated to neuron- and neuro-glial like cells (Matigian et al., 2010; Roisen et al., 2001; Stewart et al., 2013) without a need for genetic reprogramming. It has been shown that these cultures of olfactory cells also retain the epigenetic and environmental changes occurring over time (Vitale et al., 2017).

The highly plastic olfactory system has several important advantages for research, including easily accessible mucosa with multipotent adult neural stem cells, applications for studying entry of airborne pollutants and infectious agents into the body, and for investigating the relationship between environmental factors and neurodegeneration (Marin et al., 2018; Roisen et al., 2001).

Both animal and post mortem studies have shown translocation of various particles to the brain (Garcia et al., 2015; Gonzalez-Maciel et al., 2017; Klocke et al., 2017; Oberdörster et al., 2004). Specifically, Oberdörster et al. reported the translocation of inhaled ultrafine elemental ^{13}C particles to the brain via the olfactory nerve shortly after a 6-h inhalation exposure to ultrafine particles (Oberdörster et al., 2004). However, the anatomical differences between human and obligatory nose breathers, such as rodents, may bias towards trafficking and signaling pathways which may not be as crucial in human. Subsequently, González-Maciel et al. first reported the deposition of combustion ultrafine particles in neuronal mitochondria in a post mortem study of young and older urbanites (Gonzalez-Maciel et al., 2017). These findings revealed the adverse health effects of ultrafine air pollutants, resulting in growing awareness by environmental agencies. Nonetheless, ultrafine particles are yet to be accounted for in the air quality index (see waqi.info). Although the consensus is that the olfactory mucosa can be responsible for translocation of the pollutants to the brain, whether specific cell types in the tissue are more susceptible to pollutant effects remain controversial. Thus, the identification of susceptible cell types may be a starting point to elucidating cellular mechanistic impact of air pollutant particles.

1.3. Current in vitro models of the olfactory epithelium

Neurodegenerative diseases represent a group of disorders that are characterized by the progressive death of neurons leading to the deteriorated function of the central and/or peripheral nervous system (Katsuno et al., 2013; Kori et al., 2016). Globally, these disorders affect millions of people every year and despite many clinical trials and years of research, cures do not exist (Centeno et al., 2018; Heemels, 2016). This poor translational outcome is partly a consequence of the failure to properly model the complex nature of neurodegenerative disease pathogenesis (Korhonen et al., 2018; McGonigle, 2014). Post-mortem brain tissue provides important information on cellular and molecular changes associated with neurodegeneration, but this tissue is often limited by availability, and appropriate quality due to tissue degeneration (Centeno et al., 2018). Furthermore, it often represents a very late disease state. Utilization of *in vivo* animal disease models allows the study of both physiological and behavioral mechanisms of disease, but due to the inter-species differences, there are a number of limitations to translation of the results to humans (McGonigle, 2014). Patient-derived *in vitro* models can offer a solution for these drawbacks. These models can be used to identify significant patient-specific changes and possible biomarkers for drug discovery (Mackay-Sim, 2013). While embryonic stem cells and induced pluripotent stem cells are frequently utilised to study neurodegenerative diseases *in vitro*, some concerns related to their use include ethical issues, genetic instability and lack of epigenetic markers (Mackay-Sim, 2013). A potential source of patient-derived cells for the study of air pollutant effects is the OM (Murrell et al., 2005). Obtaining cells of the OM for *in vitro* studies is relatively straight-forward. A biopsy or a brush swab of the OM is performed by otorhinolaryngologist and provides a primary source of the cultures of olfactory cells. For the purposes of this review we focus only on cells obtained from the OM via biopsy, brush swab or postmortem from human, rodents and pigs.

In humans the biopsy of the OM is performed under local anesthesia and it generally includes both cells of the olfactory epithelium and the underlying lamina propria. Due to the high regeneration capacity of the

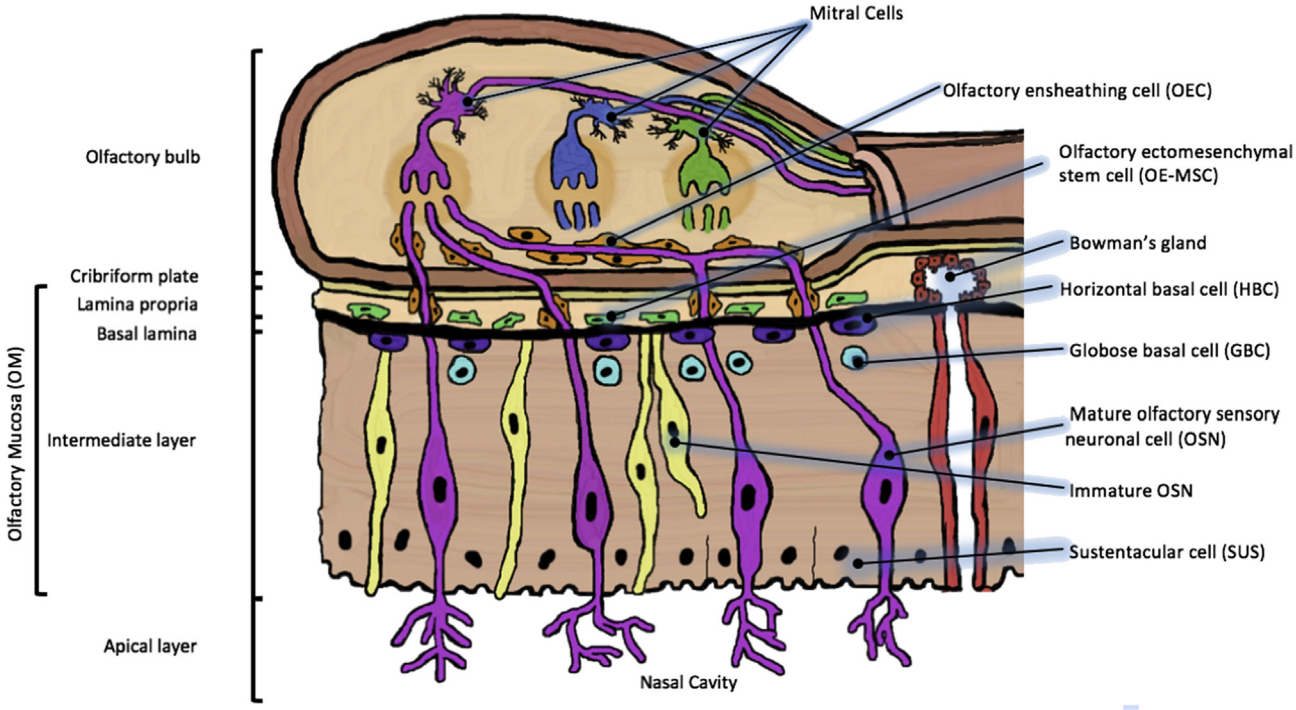

Fig. 1. A depiction of the suggested cytoarchitecture of the olfactory mucosa (OM), which provides a link between the brain and the external environment. Shown is the rodent OM. Human OM is thought to largely recapitulate histologic and molecular characteristics of rodent PM although the precise level of similarity is still to be determined (Borgmann-Winter et al., 2015). Specialised cilia within the nasal cavity are able to trap molecules as they pass the epithelium, this allows the transmission of external information to the receptors in the olfactory bulb and to the brain. Damage to the mucosa has been observed in the biopsies of Alzheimer patients and may occur at any of these structures.

olfactory mucosa, patients have not reported disturbances in their sense of smell after biopsy (Holbrook et al., 2016). If the method for obtaining the biopsies is not well validated, variability in the cell type content and their proportions can be considered as disadvantages of the OM biopsy-derived cell cultures (Benitez-King et al., 2011; Holbrook et al., 2016). A less-invasive method for harvesting the olfactory cells is a brush swab of the OM, where the cells are cultured from the obtained exfoliates (Benitez-King et al., 2011; Jimenez-Vaca et al., 2018). In contrast to the OM biopsy, exfoliation does not reach the cells in the lamina propria (Jimenez-Vaca et al., 2018). However, it has been shown that exfoliates contain neurons in different stages of differentiation and progenitor cells capable of forming neurospheres (Benitez-King et al., 2011; Jimenez-Vaca et al., 2018).

The nomenclature for defining the cell populations derived from biopsies of olfactory mucosa is highly variable. Currently the main *in vitro* models of olfactory cells consist of primary cell cultures of patient-derived olfactory neurosphere-derived cells (ONS), or cultures of olfactory ensheathing cells (OECs). In most of the studies, the cell material is obtained via the biopsy of the OM. However, the subsequent biopsy processing steps, cell culture practices and culture medium compositions vary between studies. In all studies, the primary culture of OM-derived cells is a heterogenous population of multiple cell types. Upon culturing, the OM-derived primary cells form a monolayer, similar to *in vivo* where the OM is also a monolayer (pseudostratified columnar epithelium). Olfactory cell preparations can display multipotency as they may contain olfactory mucosal-derived stem cells (OMCs), which are not derived from haematopoietic stem cells (Murrell et al., 2005).

Original research began more than 20 years ago from cadaveric olfactory epithelium in which the stem cells of the OM were referred to as neuroblasts. In 2010, Matigian et al. proposed neurospheres as a more efficient method to propagate stem cells than past methods Matigian et al. (2010). In the generation of neurospheres, biopsies of olfactory mucosa are gown in primary cultures using serum-free media with supplemented fibroblast growth factor 2 and epidermal growth factor. Upon generation, the neurospheres are dissociated and grown in standard serum-containing medium as adherent cultures. The resulting hONS cells are homogenous neural stem cell populations which also express markers of mesenchymal stem cells.

Another source of stem cells is found in primary cell cultures derived from OM biopsies. We have recently established these cultures from healthy individuals as well as patients with mild cognitive impairment and Alzheimer's disease (unpublished work by our group). These cultures consist of horizontal (HBCs) and globose basal cells (GBCs), which are stem cell-like residents at the basement membrane of the olfactory epithelium and in the lamina propria. These cells are the key contributing factor to the regeneration of the mammalian olfactory mucosa, with documented evidence of their ability to give rise to sensory neurons, sustentacular cells and microvillar cells (Huard et al., 1998; Murrell et al., 2005; Roisen et al., 2001). The physiological and clinical relevance of the basal cells will in the future enable the important characterization of disease-specific pathways and epigenetics.

Unlike induced pluripotent stem cells, cells derived from the OM do not require reprogramming. They can be rapidly generated and can produce homogenous populations after having their differentiation directed by the culture conditions (Stewart et al., 2013). Hence the use of OM cells is highly reproducible and can aid high-throughput screening during drug development. The ecto-mesenchymal stem cells (OE-MSCs also referred to as hONS) have been shown to possess the ability to migrate towards sites of induced neuronal damage, where they stimulated endogenous neurogenesis, differentiated into neurons, enhanced long-term potentiation and restored synaptic plasticity in mice (Nivet et al., 2011). In addition, these cells express neural stem cell markers such as nestin, polysialylated neural cell adhesion molecule, ßIII-tubulin, MAP2 and glial fibrillary acid protein expressed (Tome et al., 2009; DeLorme et al., 2010; Nivet et al., 2011).

The olfactory receptor neurons obtained from the human OM can be cultured as *in vitro* primary cell cultures longer than neurons derived

from many other areas of the brain due to their regenerative nature in the olfactory mucosa (Ghanbari et al., 2004). The cultured human olfactory neurons derived from patients with AD have elevated levels of oxidative stress compared to olfactory neurons derived from their controls (Ghanbari et al., 2004). For permanent olfactory receptor neuron cultures, there are publications describing immortalized cell lines of murine olfactory receptor neurons responding to stimulus by odorants (Barber et al., 2000). The OM-derived stem cells have been shown to hold a capacity for differentiation to dopaminergic neuron-like cells (Alizadeh et al., 2019a,b) and as motor neuron-like cells (Bagher et al., 2018). Evidence for direct reprogramming of adult murine OECs to neuron-like cells with overexpression of one transcription factor, Neurogenin 2, has been recently presented (Sun et al., 2019).

Some studies have used commercially available immortalized cell lines, that do not, however, model the entire OM but rather consist of certain specific cell types. As the OM is in a direct contact with the environment, the cultures of primary olfactory epithelial cells have in the recent years gained an interest in drug delivery and uptake studies. Compared to the tumor cell line RPMI 2650, originally obtained from the nasal septum, primary porcine olfactory mucosal cells have been shown to grow as monolayers, have more cilia and tight junctions and produce more mucus, when cultured at air-liquid interface. This culture may more closely resembles the cytoarchitecture of the human olfactory mucosa (Ladel et al., 2019), thus emphasises the use of primary cells for drug uptake or other exposure studies, including those assessing the effects of air pollution exposure.

1.4. Alterations of the olfactory mucosa are linked to neurodegeneration

Several neurodegenerative disorders are associated with olfactory dysfunction (Doty, 2017). Although biopsies of the OM have exhibited both cellular and molecular alterations, the degree to which the cultures exhibit the pathophysiological features and mechanisms in patient brains remains unknown (Borgmann-Winter et al., 2015). While these cells provide a viable model, results need to be appreciated in the context of the neurobiological characteristics which are specific to the olfactory system. Nonetheless, disease-specific alterations even in multifaceted genetic diseases have been observed in both the gene expression and functions of the olfactory cells such as shorter cell cycles, enhanced proliferation rates, and oxidative stress (McCurdy et al., 2006; Cook et al., 2011).

The ability to produce large stocks and biobank ONS cells has enabled investigation of several patient and control trials, which can harness individual phenotypic differences and elucidate disease-associated variability (Mackay-Sim, 2012). ONS cells derived from patients with neurodevelopmental and neuropsychiatric disorders (Rett's syndrome (Ronnett et al., 2003), Fragile X syndrome (Abrams et al., 1999) schizophrenia, bipolar disorder (Matigian et al., 2010; McCurdy et al., 2006) and neurodegenerative diseases (Parkinson's disease (Matigian et al., 2010), AD (Wolozin et al., 1992)) display disease-specific alterations. Recent studies of ONS cells have shown that neurodevelopment-associated pathways such as the G1/S phase transition are significantly different in cells derived from schizophrenia patients (McCurdy et al., 2006). Furthermore, ONS cells derived from ataxia-telangiectasia (A-T) patients recapitulate the A-T phenotype and have the capacity to differentiate into neurons without the requirement for genetic reprogramming (Stewart et al., 2013). Several non-neural lineages have also been derived from ONS cells when provided with the appropriate tissue-derived signals (Murrell et al., 2005).

OM cells from several mammalian genera (rabbit, sheep, dog, mouse, rat etc.) have been studied and displayed similar key features to the human counterparts (Veron et al., 2018a). Taken together, cells derived from the OM hold great potential for finding new, early biomarkers for neurodegenerative diseases, and understanding cell biology behind psychiatric and neurodevelopmental disorders with autologous cells derived from living doors.

1.5. Therapeutic applications for cells of the olfactory mucosa

In some studies, the OM cells have been used as *in vitro* models of disease, and others have used them for testing therapeutic approaches, for example through transplantation (Chabrat et al., 2019). The hONS are considered suitable candidates for autologous transplantation as they overcome both technical and ethical concerns which are often encountered by other stem cell types. Furthermore, several of the hONS properties meet the prerequisites for clinical trials based on cell therapies, which could be utilised in several stem-cell studies.

Intriguingly, the hONS cells can cross several metabolic barriers and be injected into both the circulation and cerebrospinal fluid. Upon transplantation, differentiated hONS cells may establish functional neuronal networks through integration into their nearby environments. The characteristic of axonal growth assistance coupled with associations with the central nervous system has made specifically the OEC cells promising candidates for both brain and spinal cord repair (Tisay and Key, 1999). However, various limitations have been encountered with the implementation of OECs in research due to the difficulty of distinguishing these cells from the Schwann cells of the trigeminal nerve (Ohnishi et al., 2015). Furthermore, several studies differ along many dimensions such as the source of the OECs (mucosa or olfactory bulb preparations) leading to different outcomes. Other factors include variation in the developmental stages of the cells, differentiation methods, species used, and varying culture conditions.

At present, the state-of-the-art for therapeutic application of OM cells and stem cells lies in regenerative therapy for spinal cord injury, which has been discussed in detail (Assinck et al., 2017; Zadroga et al., 2017). However, alternative application of primary OM cells and stem cells have been demonstrated in the brain and peripheral nervous system, albeit not as well-established as therapy against spinal cord injury. The engraftment of OE-MSCs into rat hippocampus was demonstrated by Veron et al. to treat global cerebral ischemia by inducing neuroblast and glia proliferation (Veron et al., 2018b). The authors observed restoration of cognitive function in ischemic rats within four weeks of engraftment, when compared to the sham. Similarly, transplantation of olfactory stem cells in the substantia nigra of Parkinsonian rats were shown in multiple studies to elicit a therapeutic effect via differentiation into dopaminergic neurons and integration into the tissue microenvironment (Abdel-Rahman et al., 2018; Simorgh et al., 2019). The therapeutic potential of olfactory stem cells in Parkinson's disease was further supported by successful differentiation of hONS into functional dopaminergic neurons by several groups (Chabrat et al., 2019; Zhuo et al., 2017; Alizadeh et al., 2019a,b). In addition, it has been shown that electrophysical and mechanical cues can be manipulated to direct specification of human OE-MSCs into motor neuron-like cells (Bagher et al., 2018, 2019). Collectively, these studies seem to encourage the prospect of generating diverse brain cells for disease modelling and ultimately, autologous cell therapy.

More interestingly, the restorative capacity of olfactory cells has also been explored in rodent models of hyposmia, hearing loss and sciatic nerve repair. The reader may refer to the review on utilizing OM-derived stem cells for hearing regeneration by Young et al. (2018). Contrary to differentiation of OE-MSCs into the cell type of interest, a study by Xu et al. reported that OE-derived neural stem cells implanted into rat cochlea remained viable and protected against the detrimental effects of noise-induced hearing loss by secreting nerve growth factor and neurotrophin-3 (Xu et al., 2016). Salehi et al. showed another example of nerve repair by co-administering OE-MSCs and biomimetic hydrogel into the injury site of an adult rat model of sciatic nerve injury (Salehi et al., 2019). Lastly, the enrichment of primary globose basal cells for restoration of olfactory function in an inducible mouse model of hyposmia promoted the therapeutic application of bone fide olfactory cells, as opposed to prerequisite reprogramming into stem cells

Table 1
List of selected *in vitro* studies on human nasal epithelial cultures including olfactory cells.

Model(s)	Pollutants & exposure conditions	Key findings	Year	Reference
primary HNECs	PM2-0.25, 0–300 μg/ml for 6h	IL-8 ↑ Dose-dependent apoptosis	2013	Pirela et al., (2014)
primary nasal tissue culture model	PM, 20 μg/cm3 for 24h	H2O2 levels ↑ IL-8 ↑ in a dose-dependent fashion.	2014	Cho et al., (2014)
Primary HNECs at ALI for 21d	PM10, 1.4–2.8mg/m3 for 2h PM > 10, 1 μg/cm^2 for 2h	IL-6, IL-8 expression ↑ No significant cytotoxicity	2015	Hawley et al., (2015)
Healthy and allergic rhinitis primary HNECs	DEP, 0–100 μg/ml for 24 h	GM-CSF, IL-8 & RANTES ↑ in allergic rhinitis HNECs ↑ JNK and ↓ NF-κB expression from HNECs with polyps.	2016	Ozturk et al., (2017)
nasal fibroblasts	DEPs, 0–800 μg/mL for 72h	IL-6 and IL-8 at mRNA and protein levels ↑. DEP induced phosphorylation of p38, AKT, and NF-κB.	2016	J. A. Kim et al., (2016)
primary HNECs	DEPs, 0–400 μg/mL for 72h	Activation of p38 MAPK and CREB. *MUC4* expression ↑.	2016	Park et al., (2016)
RPMI-2650	PM2.5, 0–800 μg/mL for 48h	viability↓, intracellular ROS and Nrf2 ↑. superoxide dismutase, catalase and glutathione peroxidase activity ↓. GM-CSF, TNF-α, IL-13 and eotaxin production ↑.	2016	(Hong et al., 2016)
RMPI-2650	$PM_{2.5}$, 0–160 μg/mL for 24h	induced epithelial barrier dysfunction; TEER ↓. ZO-1, occludin and claudin-1↓.	2018	(R. Zhao et al., 2018)
Nasal epithelial cells	PM2.5, 0–120 μg/ml for 24h	autophagic flux ↑. IL-6 and TNF-α secretion ↑.	2019	R. W. Zhao et al., (2019)
primary HNECs, at ALI	DEPs, 0–100 μg/ml for 72h	viability ↓ with increasing doses and exposure. TEER ↓ at ALI day 20 post-exposure. Dextran permeability ↑. MLCK ↑↑, occludin, ZO1, claudin-1, and E-cadherin ↓.	2019	N. Kim et al., (2019)
Cilia, apical side of primary HNECs	PM2.5, 0–12μg/mm2 for 24h	Ciliary beat frequency (CBF) ↑ at the relative lower dosage groups. CBF and ciliary beat pattern (CBP) ↓ at higher dose Basal cell population ↑.	2019	(Jia et al., 2019)
Primary OM cells	PM1-0.2,PM2.5–1,PM10–2.5, 50ug/ml for 24h	Viability ↓, cytotoxicity ↑, intracellular ATP ↓, ROS levels ↑ Mitochondrial respiration ↓, loss of MMP, mtDNA content ↓ Mild inflammatory response	2019	Unpublished work

Abbreviations: ALI; air-liquid interface, DEPs; diesel exhaust particles, HNECs; human nasal epithelial cells, IL; interleukin, LPS; lipopolysaccharide, mtDNA; mitochondrial DNA, OM; olfactory mucosa, PM; particulate matter, ROS; reactive oxygen species, TEER; transepithelial electrical resistance.

prior to engraftment (Kurtenbach et al., 2019).

Despite the therapeutic potential shown in animal models and success in clinical trials for spinal cord injury, it is unlikely that the transplantation methods of OM-derived cells will meet regulatory approval in most jurisdictions. This is due to the large expenses and limited patentable intellectual property which would be attractive to commercial investors (Ohnishi et al., 2015). However, research in this area is imperative with the ever-increasing global age demographic and pharmaceutical company withdrawal from neuroscience due to a lack of translation of successful drug candidates from current models to clinical trials.

1.6. Air pollution effects on olfactory epithelial cells in vitro

To date, the study of air pollutant effects on human cells have relied heavily on primary nasal epithelium and commercial cell lines. Conversely, air pollutant effects on the olfactory mucosal cells are not as well-documented. *In vitro* exposure studies have employed conventional methods to assess cell viability, toxicity, oxidative stress and inflammatory response with dose-dependent exposure to pollutants. Specific key findings of these studies are summarized in Table 1. Air pollutant particle effects in cellular viability and toxicity are conventionally assessed by measuring NADPH-dependent oxidoreductases and lactate dehydrogenase release respectively (Brooks and Olken, 1965; Shelton and Schneider, 1952). We (via unpublished results) and others have shown that exposure to PM led to reduced viability and cytotoxicity in olfactory and primary human nasal epithelial cells (Hong et al., 2016). Apoptosis has also been reported to increase with increasing concentrations of air pollutants (Khatri et al., 2013). Some studies have correlated these observations with oxidative stress. For example, increased hydrogen peroxide levels and impaired superoxide dismutase function have been reported to contribute to oxidative stress (Cho et al., 2014; Hong et al., 2016). One known consequence of oxidative stress is DNA damage, also a trigger for apoptosis, and has been reported in commercial cell lines (T. J. Ronkko et al., 2018). Typical methods to assess DNA content and damage include quantification of mitochondrial DNA copy number and DNA strand breaks using the comet assay (Duez et al., 2003). These methods have been applied to the analysis of human samples in population studies of air pollution (Barth et al., 2017). Mitochondrial dysfunction has been identified to trigger oxidative stress, which is well-studied in aging and heart diseases (Chistiakov et al., 2018; Moro, 2019). Primarily, mitochondrial function can be characterized via measurement of oxygen consumption and glycolysis as well as mitochondrial membrane potential. By far, few studies, including unpublished data from our group, have identified mitochondrial targets of air pollutants (Leclercq et al., 2018). Furthermore, innate inflammatory response and epithelial barrier function are frequently examined in *in vitro* studies in nasal epithelial cell cultures, owing to specific function of this cell type in the tissue. Inflammatory response associated with exposure to air pollutants consists of secreted cytokines such as interleukins (IL) and activated NF-κB, as reported by most of the studies highlighted in Table 1. Some studies have reported that exposure to air pollutants disrupts the epithelial barrier via down-regulation of tight junction proteins (N. Kim et al., 2019; R. Zhao et al., 2018). These studies confirm the harmful health effects of air pollution using physiologically relevant models.

Targets at multiple levels and different signaling pathways have been elucidated and are in agreement, presenting a strong foundation for development of certain therapeutic strategies. However, the diverse composition of air pollutants may complicate identification of the constituents directly affecting viability, inflammatory response or epithelial barrier. Hence, there may be a need to generate cohesive and comprehensive experimental data for environmental agencies worldwide to make informed decisions on air quality control. As mentioned, the different OM cell populations may obscure cell type-specific responses to pollutants, which adds to the challenge of identifying the specific subcellular targets. A few of the studies have also investigated adaptive mechanisms in cells following exposure, which may provide important insight into developing therapeutic interventions. Although treatment strategies have only been briefly explored, the growing body of evidence for mechanistic targets to be identified from such studies will most likely propel future translational research in combating air pollution-induced adverse health effects.

2. Summary and conclusions

In conjunction with the increasing global age demographic, chronic age-related diseases will become more widespread. Consequently, neurodegenerative disorders such as AD become increasingly prevalent. It is only now becoming evident that the environmental conditions, including air pollution exposure levels and types, greatly influences brain health and can be implicated in brain pathology. Due to the pathological complexity of neurodegenerative disorders and limited access to living human brain tissue, generating highly relevant *in vitro* models for research is challenging. The lack of access to living brain tissues has been a major limitation in delineating the neurobiological irregularities present in patient brains and is therefore a critical component of research yet to be developed (Veron et al., 2018a). Though several traditional *in vitro* models may not fully recapitulate the disease phenotypes *in vivo*, human OM-derived cells have been shown to exhibit neurodegenerative disease-specific mechanisms and have been applied to restorative therapy of spinal cord injury in humans, as reviewed (Assinck et al., 2017; Zadroga et al., 2017). In the future the *in vitro* modelling of the OM may take advantage of the knowledge provided by single cell RNA-sequencing of the murine olfactory mucosa and the olfactory receptor neurons (Keller and Margolis, 1975; Saraiva et al., 2015). In addition, attempts for modeling the OM in three dimensions on microfluidic chips (Na et al., 2017) will be highly interesting as they allow highly physiologically relevant modelling similar to that observed in living humans.

Patient-derived OM models hold great promise for not only elucidating the molecular and cellular pathophysiology of neurodegenerative disorders, but for providing key understanding about air pollutant particle entry and effects at this key brain entry site. In such studies the control groups must be selected in a fashion which minimises the effects of confounds such as age, duration of illness and exposure to treatments (Mackay-Sim, 2012; Veron et al., 2018a). Furthermore, the increased use of air-liquid interface exposure systems, which possess several advantages over the traditional submerged cell cultures (Upadhyay and Palmberg, 2018), will in the future continue to provide important, highly physiologically relevant knowledge on the health impacts of air pollution exposure, especially when used with co-cultures of primary human cells.

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Appendix A. Supplementary data

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