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Review

In vitro toxicity testing of cigarette smoke based on the air-liquid interface exposure: A review

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ABSTRACT

Cigarette smoke is a complex aerosol comprising particulate phase and gaseous vapour phase. The air-liquid interface exposure provides a possible technical means to implement whole smoke exposure for the assessment of tobacco products. In this review, the research progress in the *in vitro* toxicity testing of cigarette smoke based on the air-liquid interface exposure is summarized. The contents presented involve mainly cytotoxicity, genotoxicity, oxidative stress, inflammation, systems toxicology, 3D culture and cigarette smoke dosimetry related to cigarette smoke, as well as the assessment of electronic cigarette aerosol. Prospect of the application of the air-liquid interface exposure method in assessing the biological effects of tobacco smoke is discussed.

Contents

1. Introduction

The *in vitro* toxicity testing is very effective for evaluating the relative toxicological effects of cigarette smoke (Andreoli et al., 2003). In recent years, to reduce the hazards of smoking on human health, the tobacco industry and other relevant research institutions all over the world have extensively assessed the harmfulness of tobacco products, and these studies were based on the amount of the harmful chemical components yielded by tobacco products and the toxicological effects of cigarette smoke. The Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) established *In vitro* Toxicology Task Force to work on *in vitro* toxicity testing for tobacco smoke in 2002, and the Task Force recommended the *in vitro* toxicity testing of tobacco products using three aspects: bacterial mutagenicity, mammalian cytogenetics/genotoxicity and the cytotoxicity assay using an appropriate cell line (Bombick et al., 2002). Health Canada recommended three *in vitro* toxicity assays for mainstream cigarette smoke, including the bacterial reverse mutation assay, neutral red uptake cytotoxicity assay and the *in vitro* micronucleus assay (Health Canada, 2004a, 2004b, 2004c). The US Food and Drug Administration (FDA) authorized the Institute of Medicine (IOM) to develop "Scientific Standards for Studies on Modified Risk Tobacco Products" (IOM, 2012), in which the *in vitro* toxicology testing was proposed to be an important part of the evaluation process. A variety of *in vitro* assays have been used to assess the toxicity of

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tobacco and tobacco smoke (Johnson et al., 2009; Manuppello and Sullivan, 2015).

Cigarette smoke is a complex aerosol comprising particulate phase and gaseous vapour phase (GVP) containing more than 5000 compounds (Rodgman and Perfetti, 2013). The main harmful constituents in the particulate phase of cigarette smoke are nicotine, aromatic amines, phenols, tobacco-specific nitrosamines, and polycyclic aromatic hydrocarbons *etc*., and CO, nitrogen oxides and volatile organic compounds in the gas phase, whereas other harmful components including aldehydes and ketones carbonyl compounds, hydrogen cyanide, and ammonia are present in both the gas phase and the particulate phase. Meanwhile, ageing of fresh tobacco smoke occurring within seconds, results in dynamically partition of components between the gas phase and the particulate. Thus, due to the complex characteristics of cigarette smoke, the *in vitro* toxicological effect of cigarette smoke is a multifactorial problem. Previous studies on the *in vitro* toxicity of cigarette smoke have been mainly concentrated on the toxicity assessment of extracts of particulate phase or GVP, respectively, which could not fully reflect the true biological effects of the mixture system of cigarette smoke. In reality, it is difficult to directly expose *in vitro* cultured cells or bacteria to whole cigarette smoke of defined age.

With the development of direct exposure technology, an exposure system to implement the air-liquid interface exposure (Fig. 1) became available, allowing for the simulation of the actual contacting state of the substances to be tested (Aufderheide et al., 2002, 2003; Aufderheide and Mohr, 2004; Aufderheide, 2005; Ritter et al., 2001, 2003). The air-liquid interface exposure provides a possible technical means to implement whole smoke (WS) exposure in experiments. To date, the studies based on experiments with WS exposure have been carried out increasingly. There are a variety of WS exposure systems available (Aufderheide et al., 2011; Ritter et al., 2004; Thorne and Adamson, 2013). For example, the Mimic Smoker-01 (MSB-01) was produced by Burghart (Scian et al., 2009a, 2009b); the self-developed exposure system of WS manufactured by Borgwaldt KC was used by the Reemtsma Cigarettenfabriken of Germany (Wieczorek and Röper, 2008); the self-designed exposure chamber by British American Tobacco (BAT) (Maunders et al., 2007; Phillips et al., 2005) and VITROCELL® system were used by BAT; the CULTEX® system was used in Japan Tobacco Inc., Korea Tobacco and the Ginseng Corporation; and the VITROCELL® system is being used by Japan Tobacco International, Lorillard Tobacco Co., Philip Morris International Inc. and Zhengzhou Tobacco Research Institute of China National Tobacco Corporation, as well as some self-designed smoke exposure systems (Lam et al., 2011; Lu et al., 2007; St-Laurent et al., 2009). In addition, the EpiAirway 3D cell construct and the MucilAir model will be potential models applied to the assessment of *in vitro* toxicity of tobacco smoke aerosol when they are combined with the air-liquid interface exposure (Balharry et al., 2008; Sexton et al., 2008, 2011; Talikka et al., 2014; Zavala et al., 2016).

Recently, the *in vitro* toxicological studies of cigarette smoke based on the air-liquid interface exposure involve mainly cytotoxicity testing, genotoxicity/mutagenicity testing, oxidative stress and inflammation testing, systems toxicology approach, 3D culture model, smoke dosimetry, and electronic cigarette aerosol evaluation. In these studies, cell cultures (Table 1), bacteria (Table 2) and 3D tissue cultures (Table 3) are exposed to tobacco smoke aerosol.

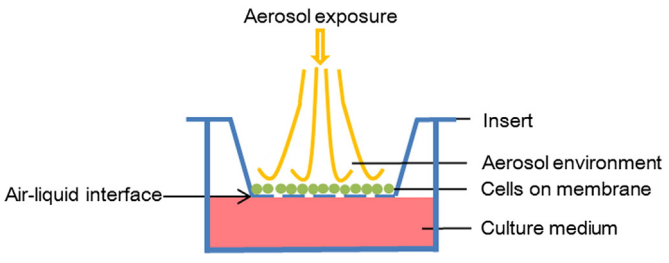

Fig. 1. A schematic of the air-liquid interface exposure. The aerosol is delivered to contact with cells grown on the membrane, and medium below the membrane provides nutrition for cells. Cells are exposed directly to the aerosol at the air-liquid interface.

2. Cytotoxicity testing

For the *in vitro* cytotoxicity testing of cigarette smoke based on the air-liquid interface exposure, the selected cells for the experiments included a variety of immortalized cell lines and *in vivo* isolated primary cells, such as Chinese hamster ovary (CHO) cells (Li et al., 2012a, 2012b, 2013, 2014; Nara et al., 2013), Chinese hamster lung (CHL/IU) cells (Okuwa et al., 2010), mouse fibroblasts BALB/c 3T3 (Thorne et al., 2014, 2015a), human lung adenocarcinoma cells A549 (Fukano et al., 2004, 2006; Li et al., 2015; Majeed et al., 2014; Weber et al., 2013), human bronchial epithelial cells BEAS-2B (Garcia-Canton et al., 2014; Majeed et al., 2014; Weber et al., 2013), human lung epithelial cells NCI-H292 (Adamson et al., 2011; Azzopardi et al., 2015; Phillips et al., 2005), human bronchial epithelial cell line (16HBE14o-) cells (Aufderheide et al., 2011), airway epithelial cell line MM-39 (Beisswenger et al., 2004), normal human primary bronchial epithelial cells (Aufderheide et al., 2015; Beisswenger et al., 2004; Maunders et al., 2007; Scheffler et al., 2015a, 2015b), mouse primary respiratory epithelial cells (Lam et al., 2011), and Sprague Dawley (SD) rat primary bronchial epithelial cells (St-Laurent et al., 2009). The selected cells could be target organ-derived cells in the respiratory tract or non-target organ-derived cells. Under the conditions of the testing, these cells showed a good dose-response effect to smoke toxicity and can be used as the test models for the *in vitro* evaluation of smoke toxicity. Normal human primary cells are preferred to be selected for assessing the biological effects of tobacco smoke due to the status of them is similar to that of *in vivo*. The immortalized cell lines are available conveniently and the passage of cells is easy as well as the consideration of the costs, but their rapid proliferation rates and other genetic abnormalities also can cause different results from normal human primary cells (Johnson et al., 2009). Based on this, selection of the immortalized cell lines is a better choice for the purpose of comparison of the toxicity of different tobacco products.

The methods of the cytotoxicity testing include the neutral red uptake assay, the MTT assay, the lactate dehydrogenase (LDH) release assay and the water soluble tetrazolium salt (WST-1) assay. Different testing methods show different sensitivities to smoke toxicity (Fukano et al., 2004; Li et al., 2015). The air-liquid interface exposure experiment could distinguish cytotoxic differences between different cigarette samples (Li et al., 2012a; Thorne et al., 2015a) and determine the cytotoxicity of gaseous components (Azzopardi et al., 2015; Li et al., 2012b; Thorne et al., 2015a), as well as the impact of different smoking regimes on the toxic effects of cigarette smoke (Azzopardi et al., 2015; Li et al., 2012b).

3. Genotoxicity testing

There are many biological indicators reflecting the genotoxicity of cigarette smoke. Currently, the testing methods based on the air-liquid interface exposure in studies of the *in vitro* genotoxicity of cigarette smoke include the micronucleus assay (Li et al., 2013; Okuwa et al., 2010), the comet assay (Weber et al., 2013) and γH2AX analysis (Garcia-Canton et al., 2014). Using the CULTEX® exposure system, Okuwa et al. (2010) compared the incidence of micronuclei in cells induced by WS and the gaseous components under the smoking conditions of the International Organization for Standardization (ISO) and the Health Canada intensive (HCI). Using the VITROCELL® 24 air-liquid interface exposure system, Weber et al. (2013) evaluated the DNA-damaging effects of cigarette smoke. The results of the comet assay for A549 cells and BEAS-2B cells showed that this system could reproducibly reflect the dose-response relationship between the DNA damage