In vitro model adapted to the study of skin ageing induced by air pollution

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HIGHLIGHTS

- Air pollutants exposure and skin ageing.
- 3D-in vitro skin model adapted for evaluating environmental pollutants.
- Air-liquid exposure close to human exposure.

ARTICLE INFO

ABSTRACT

More than a barrier against environmental agents, skin reflects individual health and is a visible sign of ageing with the progressive loss of skin integrity. In order to evaluate the consequences of an environmental complex mixture, with tobacco smoke (TS) as model, on cellular and morphological changes, a 3D skin model was used. Morphologically, tissue integrity was intact after one TS-exposure while the superficial layers were drastically reduced after two TS-exposures. However, TS modified epidermal organisation at the molecular level after just one exposure. A decrease in loricrin protein staining was showed in the epidermis, while production of inflammatory cytokines (IL-8, IL-1α, IL-18) and metalloproteinase (MMP-1, MMP-3) were stimulated. Oxidative stress was also illustrated with an increase in 4-HNE protein staining. Moreover, terminal differentiation, cell–cell junction and anchorage gene expression was down-regulated in our model after one TS-exposure. In conclusion, tobacco smoke impacted the fundamental functions of skin, namely tissue anchorage, cornification and skin desquamation. Oxidative stress resulted in skin ageing. The tissue was even reactive with the inflammatory pathways, after one TS-exposure. The 3D-RHE model is appropriate for evaluating the impact of environmental pollutants on skin ageing.

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1. Introduction

The impact of air pollution is a major public health concern. Eyes, ears, nose and skin are both gateways and barriers to the entry of pollutants. More than a barrier against environmental agents, skin reflects an individual's health and obvious signs, such as dry skin, rashes, eczema and progressive loss of skin integrity, are signs of premature ageing (Farage et al., 2013). Several factors may modify the skin's appearance. Intrinsic factors such as genetic factors controlling telomerase genes may induce programmed cell death, including apoptosis and cell turnover (Libertini, 2014).

Other environmental factors may be responsible for premature skin ageing such as UV, climate conditions, and air pollutants. UV exposure has been shown to have a direct impact on the elastic fibre system and oxidative stress (Gilchrest, 2013; Kammeyer and Luiten, 2015; Sherratt, 2013) and climate conditions, such as changes in environmental humidity, contribute to seasonal exacerbations of skin disorders, such as psoriasis and atopic dermatitis (Singh and Maibach, 2013). Atmospheric pollutants (gases, hydrocarbons, fine particles and ozone) exert deleterious effects on skin, especially for sensitive skins which can overreact to external aggression (Krutmann et al., 2014). Likewise, behavioural factors are additional factors for skin ageing (Cutillas-Marco et al., 2012; Valacchi et al., 2012). Several reviews have agreed that tobacco smoke has a strong impact on dermatology, causing poor wound healing, wrinkling and premature skin ageing, squamous cell carcinoma, psoriasis, hidradenitis suppurativa, and chronic dermatoses (Freiman et al., 2004; Metelitsa and Lauzon, 2010; Ortiz and Grando, 2012).

Several physiological mechanisms, such as impairment of inflammatory cell function and extracellular matrix turnover are the main causes of skin ageing (Thomsen and Sorensen, 2010). Some preclinical models have been used to study skin ageing and associated factors. In vivo models have been developed to study size modifications of skin epithelial cells by confocal imaging (Sokolov et al., 2015), impacts on signalling pathways via the activity of enzymes such as catalase (CAT) glutathione peroxidase (GPX) superoxide dismutase (SOD) and malondialdehyde (MDA) content (Wan and Song, 2015), the expression of human matrix metalloproteinase-1 (MMP-1) induced by dermal collagen fibrils and fibroblast spreading modifications (Xia et al., 2015), and increases in cytoplasmic translocation of high mobility group Box 1 (HMGB1) protein associated with collagen loss after exposure of rats to sidestream tobacco smoke (Chaichalotornkul et al., 2015). The 3Rs principle (Reduce, Refine, Replace) advocates using in vitro studies rather than animals. In vitro models were developed to better understand the role of various physiological pathways involved in skin ageing, such as oxidative stress in primary cultures of human keratinocytes (Ido et al., 2015), cell sizing in primary cultures of human skin epithelial cells (Sokolov et al., 2015), and mitochondrial dysfunction in epidermal stem cells (Velarde et al., 2015). Reconstructed skin 3D models are also of interest for protein expression and interaction in different skin layers (Dos Santos et al., 2015; Pageon et al., 2015; Pennacchi et al., 2015). Data, however, are often generated after exposure to only one pollutant and rarely with complex mixtures combining several pollutants.

In summary, the protective ability of the skin is not limitless and many environmental pollutants can affect its barrier function and play a role in the onset of various skin alterations such as skin ageing. Though genetically programmed, skin ageing can be accelerated by pollutants. Few data are available concerning the impact of environmental pollutants, such as tobacco smoke, on the epidermis and on the expression of proteins involved in inflammation and oxidative stress signalling pathways for example. Exposure to environmental pollutants causes imbalance between oxidants and antioxidants, and this oxidative stress has been implicated in the aggravation of various skin disorders. Tobacco smoke is a pertinent model to mimic an environmental complex mixture since the majority of polycyclic aromatic hydrocarbons and volatile organic compounds present in air pollution can be found in tobacco smoke (Schick et al., 2014).

The purpose of this study was to evaluate the impact of tobacco smoke (TS) on physiological and morphological skin changes, using an in vitro 3D model of skin, a reconstructed human epidermis (RHE).

2. Material and methods

2.1. Chemicals

Culture media (SkinEthic, France), maintenance medium for experimental days and growth medium for non-experimental days, were supplemented with a 1% Penicillin-Streptomycin mixture (penicillin: 100 µg/mL; streptomycin: 100 UI/mL) (Invitrogen, WI, USA). Phosphate-buffered saline (PBS) was provided by Biosolve Chimie (France). Calcium chloride, goat serum, glycine, bovine serum albumin, glycerol, eosin, sodium azide, Eukitt®, sodium cacodylate, glutaraldehyde, hexamethyldisilazane, osmium tetroxide, hydrochloric acid, and propylene oxide were purchased from Sigma-Aldrich (France). Acetic acid and Triton X100, from Merck (Darmstadt, Germany); paraffin (Labonord, France); Ethanol, from VWR International (PA, USA); Xylene and Paraformaldehyde (32%), from Euromedex (France) and Sodium Chloride, from Minisol (France). Mayer Haematoxylin (RAL Diagnostics, France) and Eosin (Merck, Germany).

2.2. In vitro skin model

The Reconstructed Human Epidermis (RHE) model consists of differentiated 3D epidermal tissue derived from human keratinocytes obtained from a young donor (2 years old) (Rosdy and Clauss, 1990; Rosdy et al., 1993). RHE was supplied by SkinEthic™ (Lyon, France) and the maintenance conditions were described by Poumay (Poumay et al., 2004). Experiments were performed using the same batch of cells (same donor, MF22-5). Signed informed consent and ethical approval were obtained by the supplier.

After 11 days of culture in specific medium at the air-liquid interface, RHE tissues were considered morphologically differentiated by SkinEthic™ and deliver for use. Just after reception, at day 11, RHEs were placed in a 24-well plate at 37°C and 5% CO_2 in a humidified incubator with growth medium on basal side. On the eve of the experiment day (16 h before), the growth medium was replaced with maintenance medium. All experiments started one day after RHE delivery.

2.3. Tobacco smoke exposure

Tobacco smoke (TS) was generated by combustion of a French brand cigarette composed of 87% tobacco, 6.5% flavor agents, 6.5% cigarette paper, and producing 7 mg of tar, 0.6 mg of nicotine, 9 mg of carbon monoxide as described by Bardet and others (Bardet et al., 2016). Fresh smoke, composed by both gas and particulate matter, was collected with a syringe at a rate of 25 mL per puff with a total of 10 puffs, and delivered to the apical side of RHE tissue using a static system adapted from the Vitrocell Cloud™ chamber (Vitrocell™ System, Germany). An average of 200 µg/cm² of particulate matter was deposited onto the RHE tissue. Deposition did not vary from one generation to another as shown by repeatability and reproducibility experiments (n = 4, coefficient of variation equal to 10%).

24 h after the last exposure (Air- or TS-exposure), T48 for one exposure or T72 for two exposures, culture media were collected to assess biological activity (cytokine and MMP production). At the end of the experiment (T72), RHE tissues were sacrificed to perform histological analysis and to determine mRNA by RTqPCR. Two controls, air-exposure or incubator condition (without air-exposure) were performed. As no difference was observed between these two controls, only the air control was used in subsequent work. The complete exposure design is summarized in Table 1.