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AKTUELNA TEMA UDC 663.97.05:54-138]:576.7 CURRENT TOPIC

Effects of aerosol from the Tobacco Heating System (THS) on human organotypic cultures of the aerodigestive tract

Efekat aerosola sistema za zagrevanje duvana (*Tobacco Heating System* - THS) na humane ćelijske kulture organa aerodigestivnog trakta

Filippo Zanetti, Anita Iskandar
Philip Morris International, Switzerland

Abstract
Cigarette smoking is responsible for a multitude of health risks and is one of the major modifiable risk factors for many smoking-related diseases. Tobacco Heating System (THS) is an electronic device, which heats tobacco instead of burning it. The THS aerosol has a different chemical composition to cigarette smoke, with its levels of harmful and potentially harmful constituents reduced on average by 90-95%. Advances in tissue engineering have enabled the development of sophisticated, three-dimensional organotypic culture systems that closely resemble human physiology. In several recent studies, buccal, gingival, nasal, bronchial, and small airway human epithelial organotypic cultures were exposed to aerosol from the THS and cigarette smoke. Standard toxicological assays were combined with network-based systems toxicology analyses to identify otherwise undetectable cellular-level effects. The results showed that the THS aerosol has a minimal biological impact on cells in comparison to cigarette smoke.

Key words: Tobacco Heating System (THS), organotypic in vitro cultures, smoking, systems toxicology

Apstrakt
Dim cigarete odgovoran je za mnoštvo zdravstvenih rizika i jedan je od glavnih faktora za nastanak mnogih bolesti povezanih sa pušenjem a na koji se može uticati. Aerosol koji proizvodi sistem za zagrevanje duvana (*Tobacco Heating System* – THS), koji zagreva duvan umesto da ga sagoreva, ima drugačiji hemijski sastav od dima cigarete, sa nivoima štetnih i potencijalno štetnih sastojaka smanjenim u proseku za 90- 95%. Napredak u inženjerstvu tkiva omogućio je razvoj sofisticiranih, trodimenzionalnih organotipskih sistema kultura koji blisko podsećaju na ljudsku fiziologiju. U nekoliko nedavno sprovedenih studija, organotipske kulture epitela bukalne, gingivalne, nazalne, bronhijalne i epitela malih disajnih puteva bile su izložene aerosolu THS-a i dimu cigareta. Standardni toksikološki testovi kombinovani su sa sistemom koji je zasnovan na mreži toksikoloških analiza kako bi se otkrili inače neotkriveni efekti na ćelijskom nivou. Rezultati su pokazali da je THS aerosol imao minimalan biološki uticaj na ćelije u poređenju sa dimom cigareta.

Ključne reči: sistem za zagrevanje duvana (THS), organotipske in vitro kulture, pušenje, toksikološki sistemi

Introduction

Toxicological evaluations designed for mechanistic investigation (e.g., cellular and molecular assessment) are often conducted using animal models to assess the effects of a given toxic compound on the whole organism. However, because of interspecies differences, the results obtained from such studies are limited and cannot fully reflect the pathophysiology of human diseases. In vitro cellular models using human cells can help overcome interspecies differences, and advances in tissue engineering have enabled the development of sophisticated, three-dimensional organotypic culture systems that closely resemble human physiology. In addition, in line with 21st Century Toxicology, these models support the internationally recognized "3Rs" of animal research: replace, reduce, and refine the use of animals in research.

Tobacco harm reduction

Cigarette smoke is the leading modifiable risk factor for many human diseases. Complete smoking cessation is the best approach to reduce the risks of smoking-related diseases. However, while the prevalence of cigarette smoking has been steadily declining

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Corresponding author / Autor za korespondenciju: **Filippo Zanetti**
Philip Morris International, Philip Morris Products S.A., Quai Jeanrenaud 5, CH-2000 Neuchâtel, Switzerland | E-mail: Filippo.Zanetti@pmi.com

over the years, millions of individuals across the globe continue to smoke. Smoking cessation has proven difficult for many smokers, who might benefit from using alternative products that have the potential to reduce the harm caused by cigarette smoke. For smokers who would otherwise continue smoking cigarettes, Philip Morris International's (PMI) goal is to offer reduced-risk products (RRP)* that have the potential to reduce the risk of smokers developing smoking-related diseases relative to continued smoking.

The objectives of the five studies outlined here were to assess the effects of the Tobacco Heating System (THS) aerosol on human epithelial buccal, bronchial, nasal, gingival, and small airway cells by using three-dimensional, organotypic culture systems (Fig. 1) (1-6).

Study design

All five studies used comparable methodologies, and the data from the buccal, bronchial, and nasal studies were evaluated by a meta-analysis. Cells from human donors were grown in specially developed inserts at the air–liquid interface, which represents the physiology of epithelial cells in human biology. The cells were exposed to THS aerosol, cigarette smoke, or filtered air for 28 minutes (repeated on 3 consecutive days in the gingival study).

The nicotine concentrations were matched as closely as possible between THS aerosol and cigarette smoke (Table 1). However, these concentrations varied across the studies because of the morphological differences between the tissue types (e.g., the thickness of the buccal cultures was approximately five times that of the bronchial and nasal cultures). In the gingival study, endpoints were assessed 4 and 24 hours after exposure. In all other studies, the endpoints were assessed 4, 24, 48, and 72 hours after exposure.

Results

Cytotoxicity and histological analysis

The effects of exposure were first assessed by measuring the release of adenylate kinase as a marker of cytotoxicity. This was complemented by histological analysis of exposed tissue cultures. Cigarette smoke exposure resulted in similar cytotoxicity profiles in the bronchial, nasal, and small airway cultures, with cytotoxicity increasing significantly with the dose

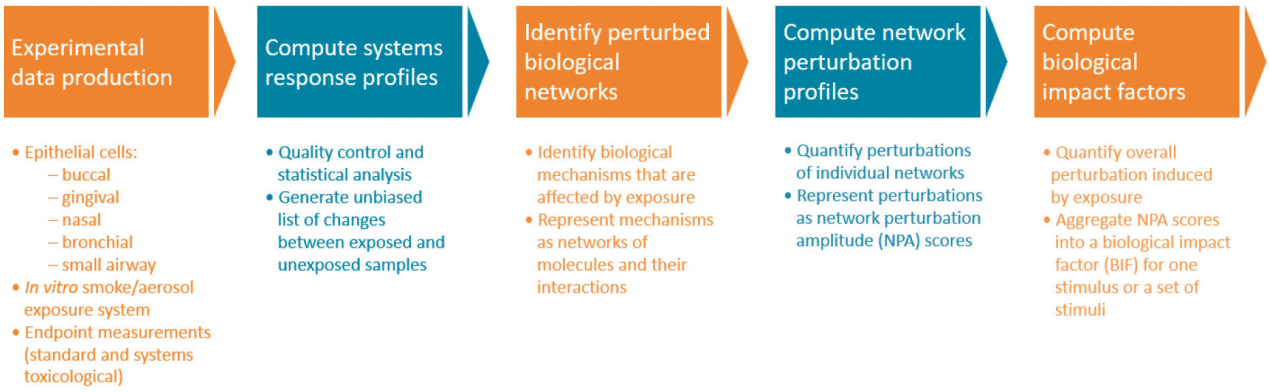

Figure 1. Network-based systems toxicology approach

Table 1. Experimental repetitions, dosing, and endpoints

Tissue	Experimental repetitions	Nicotine concentrations in smoke and aerosol (mg/L)·							
		Cigarette smoke				Tobacco Heating System			
Buccal	4	-	0.32	0.51	-	-	0.31	0.46	1.09
Nasal	4	0.15	0.25	-	-	0.15	0.27	0.44	-
Bronchial	6	0.13	0.25	-	-	0.14	0.25	0.42	-
Small airway	3	0.14	0.26	-	-	0.14	0.30	0.45	-
Gingival	3	-	-	49.4	84.6	-	-	54.6	100.4

Selected endpoints: cytotoxicity; histological changes in the cultures; molecular changes determined by multiomics and network-based mRNA profiling

· **In the bronchial, nasal, buccal, and small airway studies**, nicotine concentrations were determined in the aerosol (expressed in mg nicotine/mL smoke or mg nicotine/L aerosol). **In the gingival study**, nicotine concentration was determined as the concentration deposited in phosphate-buffered saline (PBS) placed in the exposure chamber (expressed in mg nicotine/L PBS).

* Reduced-Risk Products ("RRP") is the term we use to refer to products that present, are likely to present, or have the potential to present less risk of harm to smokers who switch to these products versus continued smoking. We have a range of RRPs in various stages of development, scientific assessment, and commercialization. Because our RRPs do not burn tobacco, they produce far lower quantities of harmful and potentially harmful compounds than found in cigarette smoke.

and duration of exposure. In contrast, THS aerosol exposure resulted in only minimal cytotoxicity in the bronchial, nasal, and small airway cultures at all doses and post-exposure time points. Histological analyses revealed a number of features commonly associated with cytotoxicity in buccal cultures exposed to CS but only minor changes in buccal cultures exposed to THS aerosol. At 24 hours after exposure, major cytotoxicity was observed in gingival cells exposed to cigarette smoke but not in those exposed to THS aerosol. Histological analyses revealed severe damage to buccal and gingival cells after exposure to cigarette smoke, while only minor changes were observed after exposure to THS aerosols at comparable nicotine concentrations.

Multiomics and network-based analyses

In complement to the standard toxicological measurements performed in these studies, causal network enrichment analyses of transcriptomic and metabolomic data were performed to identify otherwise undetectable molecular changes in the five tissue types. In contrast to cigarette smoke, THS aerosol was found to have a significantly reduced impact on key biological networks associated with processes relevant to smoking-related diseases (Figs. 2, 3, 4, and 5).

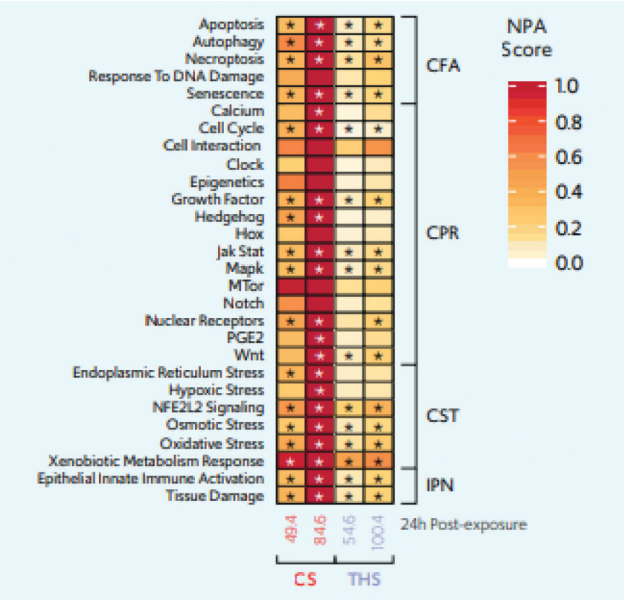

Figure 3. Gingival cultures: Effects of THS and CS exposure on key biological networks related to smoking-related disease processes. Network perturbation amplitude (NPA) scores. CFA: cell fate; CPR: cell proliferation; CST: cell stress; IPN: inflammatory process networks; CS: cigarette smoke; THS: Tobacco Heating System; *: statistically significant.

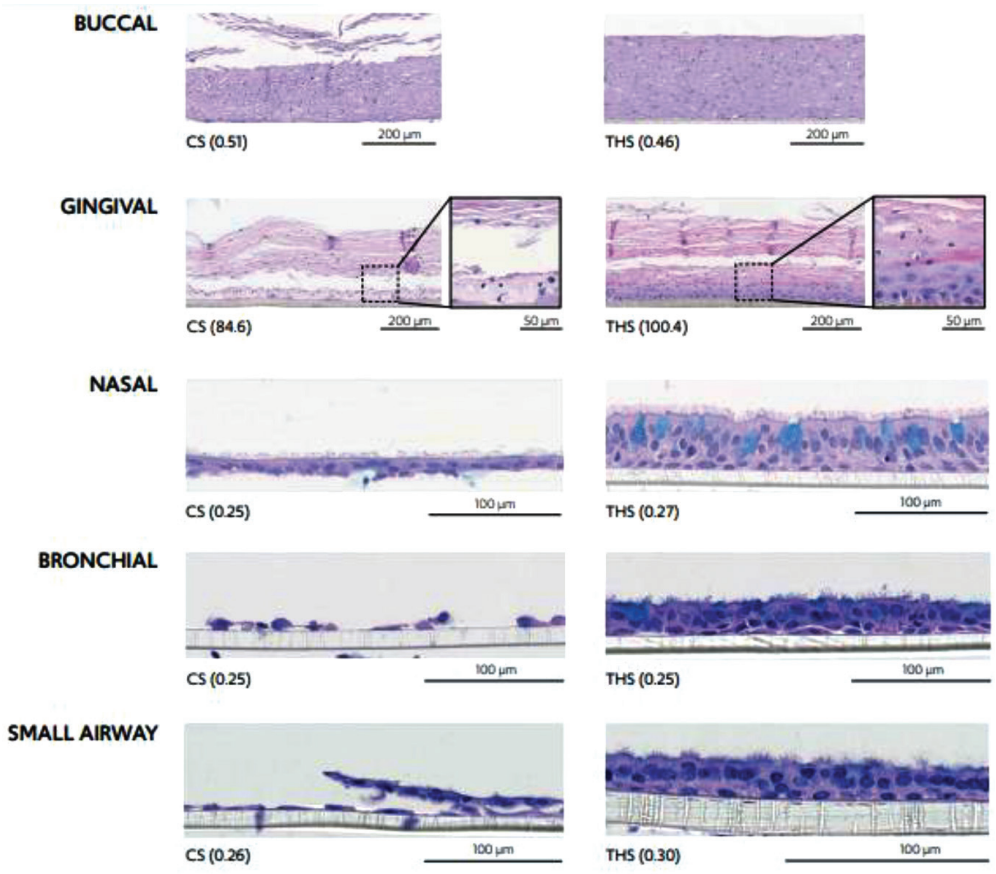

Figure 2. Selected histological findings. Representative images of hematoxylin–eosin and Alcian blue-stained sections observed 72 hours post-exposure in case of buccal, bronchial, nasal, and small airway cultures and 24 hours post-exposure in case of gingival cultures. CS: cigarette smoke; THS: Tobacco Heating System.

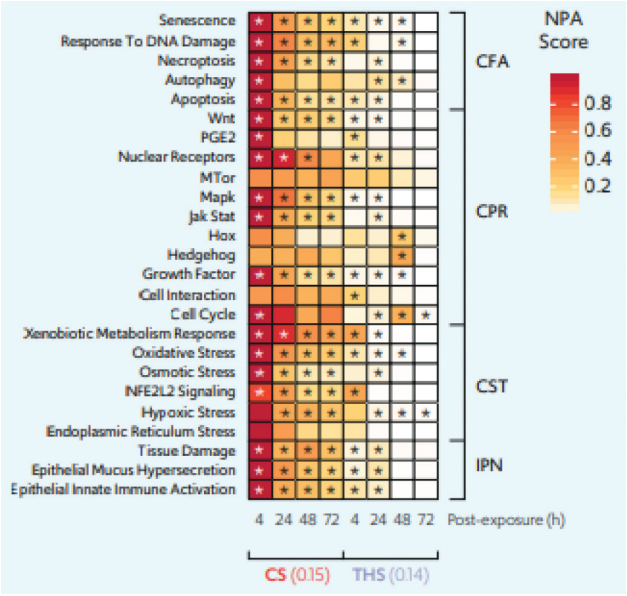

Figure 4. Small airway cultures: Effects of THS and CS exposure on key biological networks related to smoking-related disease processes. Network perturbation amplitude (NPA) scores. CFA: cell fate; CPR: cell proliferation; CST: cell stress; IPN: inflammatory process networks; CS: cigarette smoke; THS: Tobacco Heating System; *: statistically significant.

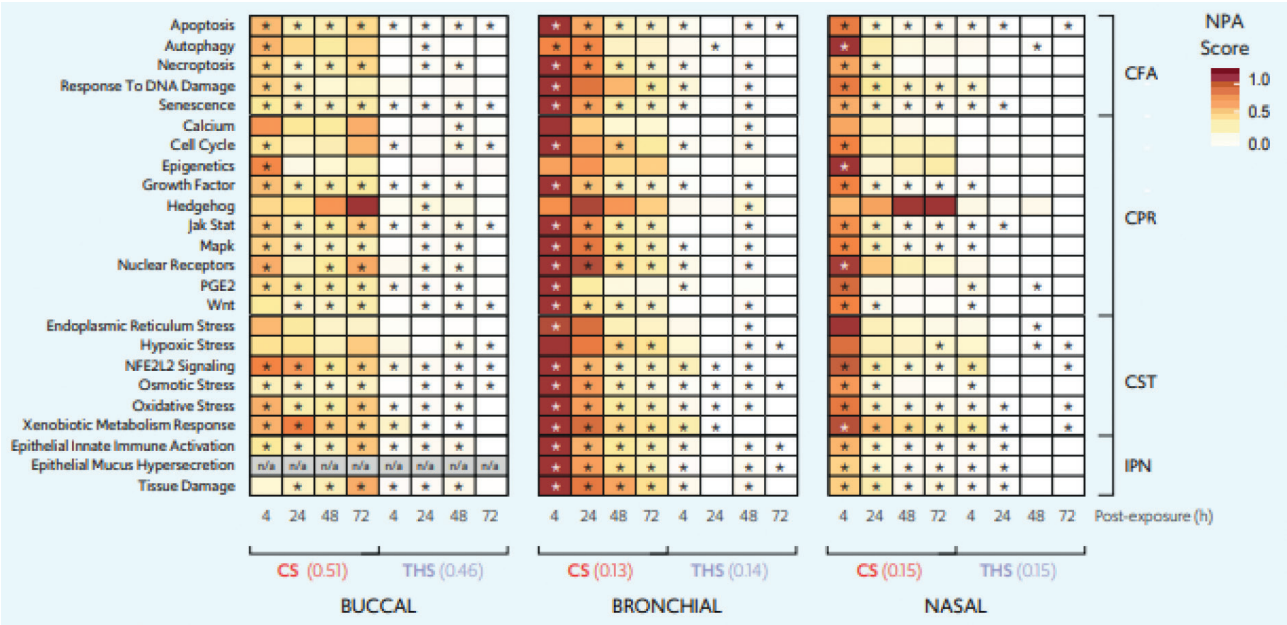

Figure 5. Buccal, bronchial, and nasal cultures: Effects of THS and CS exposure on key biological networks related to smoking-related disease processes. Network perturbation amplitude (NPA) scores. CFA: cell fate; CPR: cell proliferation; CST: cell stress; IPN: inflammatory process networks; CS: cigarette smoke; THS: Tobacco Heating System; *: statistically significant.

Conclusion

The five studies briefly outlined here—in addition to the meta-analysis of the buccal, bronchial, and nasal studies—all indicate minimal biological impact in human epithelial buccal, gingival, nasal, bronchial, and small airway cells exposed to THS aerosol in comparison with the findings in cells exposed to cigarette smoke. These studies further demonstrate the applicability and robustness of a systems toxicology approach for in vitro inhalation toxicity assessment.

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