Use of cell media nicotine concentration as a marker to predict cells surface deposited nicotine in transwells after fresh smoke/aerosol exposure

CORESTA SSPT CONFERENCE 6-10th October 2019, Hamburg STPOST 37

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1. INTRODUCTION/OBJECTIVES

Exposure of organotypic 3D lung models at the air liquid interface (ALI) to fresh whole smoke/aerosol provides a more human relevant exposure assessment of combustible cigarette smoke and e-cigarette aerosol when compared to submerged cultures.
The aim of this study was to develop a method for the determination of nicotine deposition at a position equivalent to the human organotypic tissue surface in transwells of 24 multiwell plate (MWP). Nicotine serves as a general marker of exposure due to its high transfer rate in smoke/aerosol and its chemical stability. However, due to the absorption of nicotine into the cells, accurate measurement of nicotine deposited on the cell surface is difficult to quantify.

2. MATERIALS AND METHODS

2.1 Smoke / aerosol generation
Fresh smoke and aerosol were generated on the SAEIVS (See Table 1 for aerosol generation regimens).

2.2 Test Samples
Smoke of Reference Cigarette 3R4F
Aerosol of myblu™ Tobacco flavour; 1.6% nicotine

2.3 Cell exposure containers
24 MWP (FALCON #353047) assembled with Inserts (NUNC #40620) and Transwells (COSTAR #3470)

2.5 In vitro exposure system
Imperial Brands' Smoke Aerosol Exposure In Vitro System (SAEIVS; Burghart Tabaktechnik, Wedel, Germany) [1]. The exposure to the undiluted aerosol and diluted smoke was performed in 24 MWP format in inserts / transwells

Table 1: Aerosol was generated for products using the following regimens:

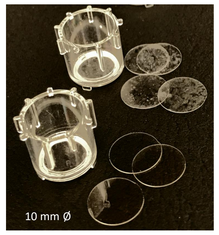
Sample	Smoking Regime	Puff Volume (ml)	Puff Duration (Seconds)	Puff Interval (Seconds)	Vent Blocking	Puff Profile
3R4F Cigarette	Health Canada Intense [2]	55	2	30	Yes	Bell Shaped
myblu™	Coresta Recommended Method 81 [3]	55	3	30	N/A	Square Wave

2.6 Nicotine evaluation
Nicotine was quantified using LC-MS/MS method (IS: Nicotine-d4). Nicotine trapped in cell media and PBS was measured directly without any further sample preparation. Whereas the nicotine trapped on the surface of the glass disc was eluted with 2-propanol before final measurement

2.7 Tissues
MucilAir™ tissues, a fully differentiated 3D airway epithelium, were purchased from Epithelix Sàrl and cultured for more than 7 days before use. Tissues were repeatedly ALI exposed (3 times per week) for 4 weeks to either 30, 60 or 90 puffs of aerosol/smoke and filtered humidified air as the control using Imperial Brands' SAEIVS (2.5). Cigarette smoke was diluted with filtered humidified air 1:17 times whilst myblu™ aerosol was exposed directly to the cells surface undiluted.

3.1. RESULTS

Smoke particulate matter deposition in the inserts cell exposure area (24 MWP)



The surface area of the glass discs used for smoke particulate matter/nicotine deposition experiments was the same as surface area of inserts used routinely for fresh smoke exposure in the SAEIVS.

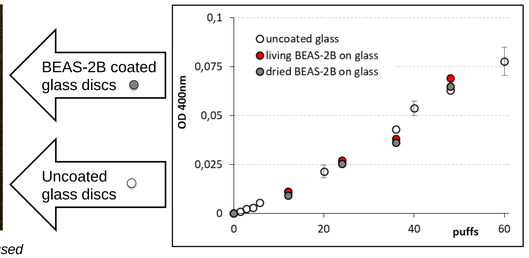
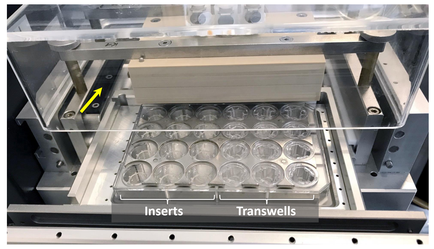


Figure 1: Optical density of glass discs (uncoated, coated with dried and living BEAS-2B cells) exposed to different puffs of 3R4F cigarette smoke



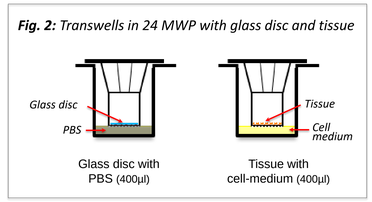
SAEIVS exposure chamber (open) with 24 MWP with inserts (left) and transwells (right). A stainless steel lid covered raw of wells for puff specific exposure (yellow arrow).

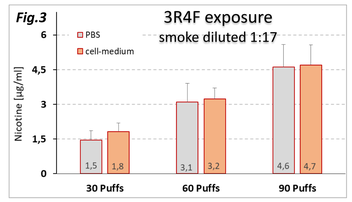
The glass discs were washed with 2-propanol. The amount of deposited particulate matter on glass discs was evaluated by reading the optical density (OD 400nm) in a plate reader. The OD 400nm of cell coated and uncoated glass to the puff number shows a high degree of concordance (See Figure 1). The deposition of smoke particles on to the glass discs did not differ between blank glass and when covered in a layer of cells.

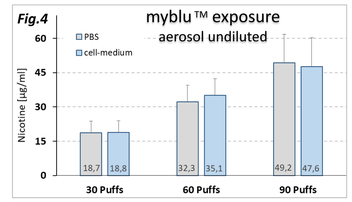
3.2. RESULTS

3.2.1 Nicotine deposition directly after smoke and aerosol exposure in basal medium vs. PBS

During the four weeks of repeated ALI exposure of MucilAir™ tissues to diluted 3R4F smoke and undiluted myblu™ aerosol, the cell-medium were collected for nicotine quantification directly after exposure. Parallel transwells with glass discs and or PBS were exposed (Fig.2).



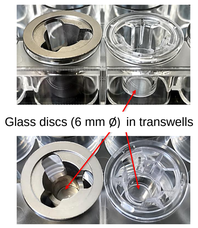


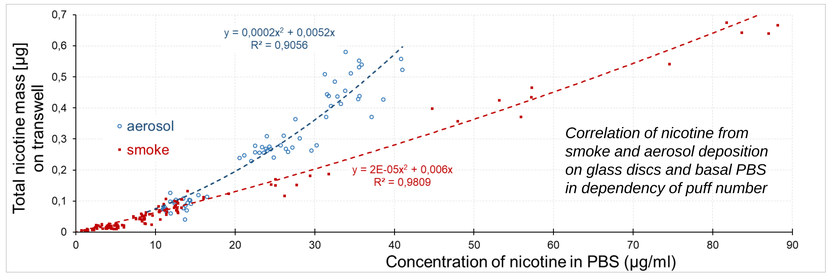


Nicotine concentration of PBS (transwell+glass discs) or wells containing medium (3D tissues) correlated well with increasing puff numbers. No differences in nicotine concentration between PBS and cell medium were found (Fig. 3 and 4).

3.2.2 Correlation of smoke/aerosol to nicotine deposition on transwell exposure surface

Glass discs were placed in stainless steel transwells containing 400µl PBS were exposed to different puff numbers of diluted and undiluted smoke from 3R4F cigarette and aerosol generated by myblu™

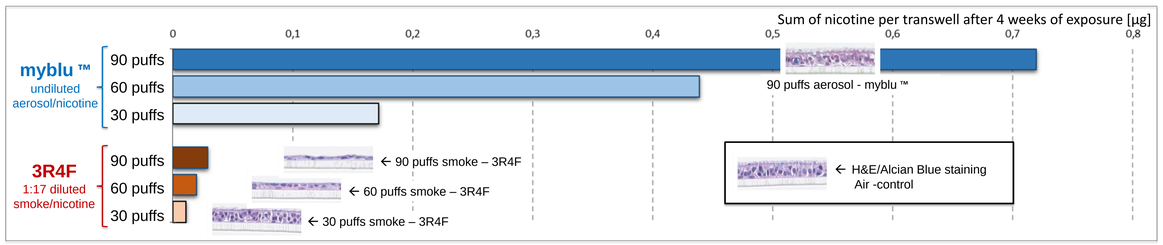




Nicotine deposition on glass discs in the transwells correlated well with the nicotine concentration in PBS, with increasing puff numbers, dilution factors and the surface area of the glass discs. Therefore, the nicotine concentration in exposed basal medium is well correlated with nicotine deposition on the cell surface. Nicotine from the aerosol showed a higher deposition rate on to the glass discs than from smoke (calculated per delivered nicotine in smoke and aerosol).

3.2.3 Nicotine deposition on tissues and histology staining

Sum of nicotine quantities over 4 weeks exposure (µg per tissue) calculated by nicotine measured in cell-medium compared to morphological effects on tissues (H&E/Alcian Blue staining).



Puff based toxicological effects were correlated with nicotine delivery from test articles observed over the exposure time were compared. Tissues were exposed to undiluted aerosol from myblu™ and 1:17 diluted smoke from 3R4F.
The myblu™ -exposed tissue preparations showed little to no histological structural changes, whilst the total nicotine delivery was significantly more than that measured for 3R4F.

4. CONCLUSIONS AND OUTLOOK

- The deposition of smoke particles on to the glass discs do not differ between blank discs and those coated with cells
- The nicotine concentration in exposed basal medium can also be considered as a proxy in relation to nicotine deposition on glass plates
- Although myblu™ delivered up to 25 time more nicotine (by 90 puffs) compared to the 3R4F cigarette smoke, it did not trigger significant toxicological response in histology of the 3D tissue model compared with matched air control
- Furthermore, the data shows that the SAEIVS robot can deliver biologically relevant compounds to the cellular system at concentrations that are physiologically relevant

REFERENCES

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