A new in vitro testing module for the cytotoxic evaluation of e-cigarette vapor

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Introduction

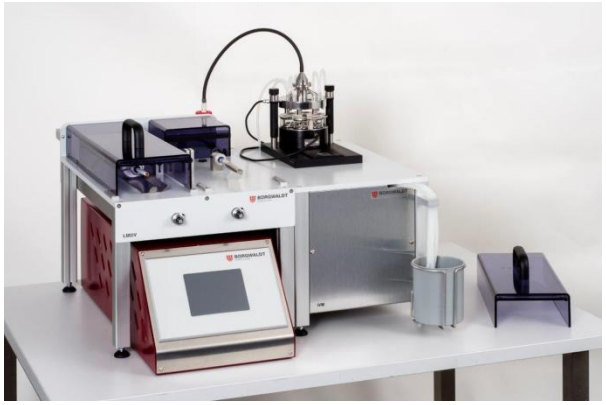
The e-cigarette market has recently been booming, and e-cigarettes are often described as "reduced-risk" nicotine products or alternatives to combustible cigarettes. However, no regulations for e-cigarettes are currently into force, so that the quality and safety of e-liquids is not necessarily guaranteed. There are two major ways to analyze e-cigarette vapor: chemically and biologically. Whereas chemical evaluations are mostly restricted to known toxic components, biological analysis give information about effects triggered in the human body after inhalation. For the generation of relevant data, a system is needed which is able to produce stable data. Therefore, the smoking machine has to produce e-liquid vapor of reproducible quality, which can then be used to expose human bronchial epithelial cells directly at the air-liquid interface. A new flexible compact version of smoking machine and exposition module set-up is introduced. The suitability of the system is demonstrated by presenting dose-response curves for normal human bronchial epithelial cells after direct cigarette smoke and e-cigarette vapor exposure at the air-liquid interface.

Materials and Methods

Cell Cultivation
Normal human bronchial epithelial (NHBE) cells were isolated from a healthy tissue sample derived from of a 75-year old patient with a non-small cell lung cancer (NSCLC) after lobectomy (Bielefeld Evangelical Hospital, Bielefeld, Germany). The received cells were named NHBE48 (10). In accordance with the Declaration of Helsinki, the subjects gave his informed consent to the research use of the removed lung tissue samples. After the first passage, NHBE cells were cultivated in collagen IV coated culture flasks using AEGM Medium (Promocell, Heidelberg, Germany). After reaching 80-90 % confluence, the cells were seeded on collagen IV coated cell culture inserts (seeding density: 2.1 x 10^5/ cm^2). The cells were cultivated under submerged conditions and supplied with AEGM medium for 1 day before the apical medium was removed and the cells were transferred to the exposure module. The exposure experiments were performed with cells of passages 2-4.

E-Liquids and cigarettes
The tested refill e-liquid was purchased from Johnsons Creek (Hartland, WI. USA), flavor Tennessee Cured, with nicotine concentrations of 0.0 %.
For cigarette smoke exposure, K3R4F research cigarettes (University of Kentucky, Lexington, KY, USA) with a standard cellulose acetate filter tip were used.

Exposure system
Size reduced syringe-controller combination capable to generate either smoke from combustable cigarettes or e-cigarettes vapour, depending on the adaption to the syringe. Generated smoke is blown out of the syringe into a dilutor with a total volume of 1.000 [ml], dilution ratio depends on generated puff volume (5 [ml] to 150 [ml]). Constant working vacuum pumps lead diluted smoke or vapour into RFS compact pre chamber and from where it is equally distributed onto exposure chambers .
Pre-calibrated mass flow controllers ensure a constant flow into each exposure chamber.
Nutrient solution for the NHBE cells is filled into each exposure chamber with a pipette. A peristaltic pump pumps the nutrient solution up to the required level into a stand by container.



Exposure
For e-cigarette vapor experiments, a Reevo Mini-S (In-Smoke, Winnenden, Germany) was used, equipped with a battery of 3.3 V/ 900 mAh and a vaporizer with a resistance of 2.2 Ohm. 200 puffs were taken with a puff volume of 35 mL, a puff duration of 2 s and a blow-out time of 7 s. The smoking robot was operated in asynchron mode. For a better distribution, the e-liquid vapor was diluted with synthetic air (1 L/min) before sucked into the CULTEX® RFS compact via a vacuum pump with a flow rate of 5 mL/min/ insert. For mainstream smoke exposure, 10 K3R4F cigarettes were smoked by the smoking robot using the same parameters as described for the e-cigarette. The clean air exposure (clean air control) was performed for 30 min with the flow rates described above.
The flow rates were controlled by mass flow controllers (IQ+ Flow and EL-Flow Select, Bronckhorst, Ruurlo, Netherlands). The exhaust air was directed back to the fume hood.

Analysis
The analyses were done 24 h after the exposure allowing the cells to respond to the exposure. The cell viability was measured using the CellTiter-Blue® Assay (Promega, Madison, WI, USA).

Results

Figure 1 shows the cell viability of primary NHBE cells after the exposure to e-cigarette vapor in dependence to the number of puffs taken (normalized to clean air control). The cell viability decreases linear with increasing number of puffs. Compared to mainstream smoke exposed cells, the cell viability is between 6 (400 puffs) and 10-times (50 puffs) higher. Figure 2 confirms the linear relationship between cell viabilty and number of puffs taken.

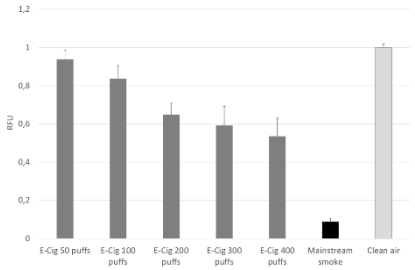


Figure 1: Puff-adjusted values for cell viability of NHBE cells after exposure, normalized to clean air control. The results are given as mean of three independent experiments with three samples each + standard deviation.

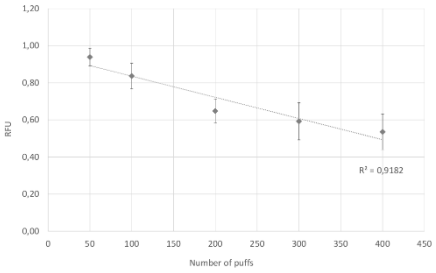


Figure 2: Puff-adjusted values for cell viability of NHBE cells after exposure to e-liquid vapor, normalized to clean air control. The results are given as mean of three independent experiments with three samples each + standard deviation.

Conclusions

The here presented data illustrate the usability of the exposure unit for the *in vitro* analysis of e-liquid vapor on primary NHBE cells. The low standard deviation and the linearity of the dose-response curve confirm the robustness of the system and prove the ability to generate reproducible data. In summary, the in vitro testing module represents a platform for the generation of stable data to evaluate the toxicological potential of e-cigarette vapor.

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