Validation of the in vitro comet assay in conjunction with an air-liquid interface exposure of cigarette smoke in human lung epithelial cells

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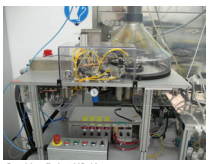
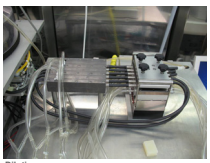
Introduction and Objective

In order to evaluate the toxicological effects of cigarette smoke under quasi-realistic conditions in vitro, we here introduce the use of a novel air-liquid interface exposure system to determine the DNA-damaging activity of cigarette smoke. Using this system, A549 and BEAS-2B cells were exposed to different flows of freshly generated, diluted mainstream smoke from the Reference Cigarette 3R4F and screened for DNA-strand breaks using a standard comet assay protocol.

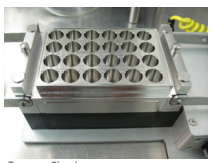
Methods



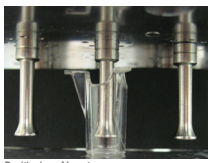
Smoking Robot VC-10



Dilution Chamber



Exposure Chamber



Positioning of Inserts

Dilution with Humidified Synthetic Air (l/min)	No. of Cigarettes per Dilution
0.2	0.045
0.5	0.027
1	0.016
1.5	0.011
2	0.009
2.5	0.007
3	0.006
4	0.005

Whole Smoke Exposure in l/min and number of cigarettes per dilution

Remark: The flow of the synthetic air in this setup was in reciprocal relation to the smoke concentration, i.e., the higher the dilution flow, the lower the concentration of smoke constituents.

Smoke Generation

University of Kentucky Reference Cigarette 3R4F (total particulate matter [TPM] yield ~10 mg/cig.). Cigarettes (10 per batch) were smoked on a VC10 smoking robot in basic conformity with ISO standards (1991).

Exposure of cells

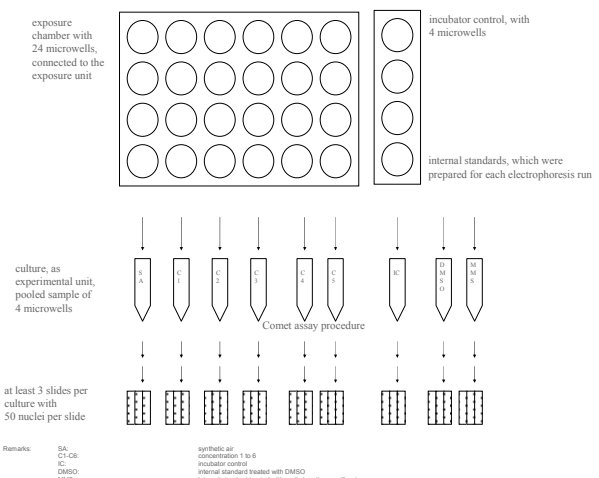
The fresh diluted whole smoke (5 puffs per minute x 35 ml = 175 ml/min) was passed puff-wise through the dilution system and diluted with at least five different permanent flows of humidified synthetic air.

Cell Culture

A549 and BEAS-2B cell lines were kept under standard cell culture conditions.

Experimental Design

Comet Assay Design per VITROCELL®24 Exposure Run: Replicate Cultures (at least 3), providing a measure of variability
(Lovell and Omori, 2008)

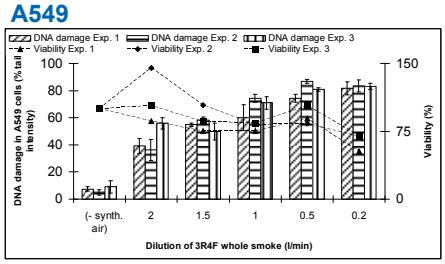


Results

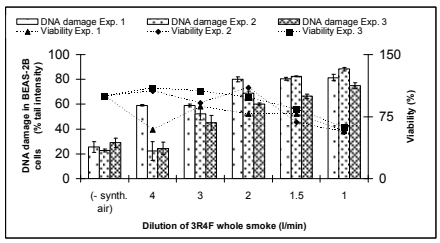
Reproducibility:

- 3 assays on the same day

A549

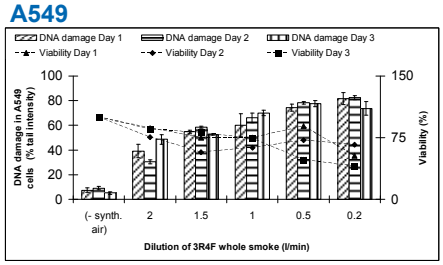


BEAS-2B

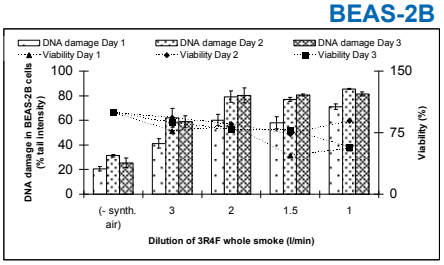


- 3 assays on 3 different days

A549



BEAS-2B



• Assay Variability

A549

	Dilution of whole smoke (L/min)					
	Synthetic air	2	1.5	1	0.5	0.2
General mean	7.14	42.08	54.77	68.32	79.59	80.92
Repeatability						
variance	7.14	77.04	16.82	51.03	22.02	13.90
RSD (%)	**37.44**	**20.86**	**7.49**	**10.46**	**5.90**	**4.61**
Between-day						
variance	0.92	49.211	2.78	0	0	20.20
RSD (%)	**13.44**	**16.67**	**3.05**	**0.00**	**0.00**	**5.55**
Reproducibility						
variance	8.06	126.25	19.60	51.03	22.02	34.10
RSD (%)	**39.78**	**26.70**	**8.08**	**10.46**	**5.90**	**7.22**

BEAS-2B

	Dilution of whole smoke (L/min)				
	Synthetic air	3	2	1.5	1
General mean	25.85	51.94	69.65	72.83	80.22
Repeatability					
variance	18.92	37.25	55.37	43.32	26.01
RSD (%)	**16.83**	**11.75**	**10.68**	**9.04**	**6.36**
Between-day					
variance	15.40	68.89	50.47	88.197	34.86
RSD (%)	**15.18**	**15.98**	**10.20**	**12.90**	**7.36**
Reproducibility					
variance	34.33	106.14	105.84	131.52	60.87
RSD (%)	**22.66**	**19.83**	**14.77**	**15.75**	**9.73**

A clear dose-dependent increase in DNA-damage, expressed as tail intensity, was obtained in all experiments for both cell lines, with A549 cells demonstrating a higher resistance to genotoxic insults than BEAS-2B cells. For some dilutions, mainly with higher concentrations of whole smoke, the viability was lower than 75%. However The DNA damage was accompanied in most cases by a viability of at least 75%, which indicates effects linked to the genotoxicity of the test substance.
Reproducibility and repeatability were acceptable, with a relative standard deviation of approximately 25%. The increases in response to whole smoke from the 3R4F over the synthetic air control were up to 3.9-fold (BEAS-2B) and 17.3-fold (A549), demonstrating a distinct DNA damaging effect of all smoke dilutions applied.

Conclusion

The in vitro comet assay in combination with the VITROCELL 24® air-liquid exposure system is able to detect cigarette-smoke-induced DNA damage in a reproducible and repeatable manner with a higher throughput.

PMI RESEARCH & DEVELOPMENT

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