Air to Liquid Interface (ALI) cell exposure under transient driving of gasoline vehicles

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INTRODUCTION

The problem

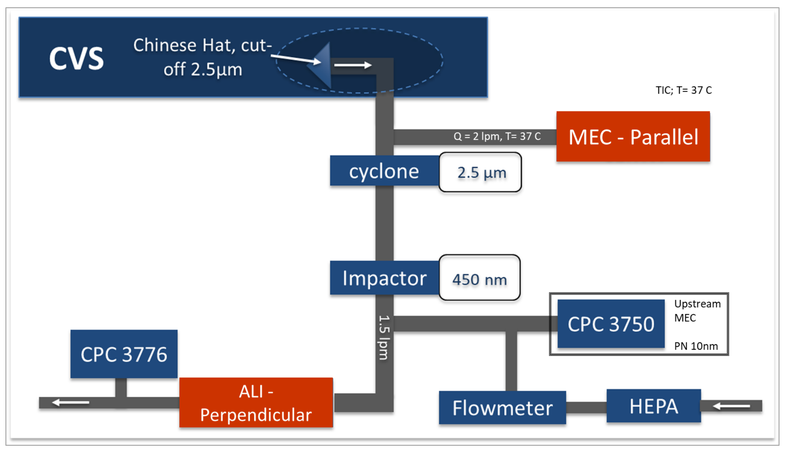
Air pollution, including exhaust emissions, especially in urban areas, has been suggested as one possible cause of the increased risk of respiratory and cardiovascular diseases, including asthma, lung cancer, and stroke. In particular, the ultrafine particles (UFPs) present in emissions are particularly harmful due to their small size, which allows them to penetrate deep into the lungs and even pass into the bloodstream, brain and placenta [1, 2].

The objective

- To investigate the potential health effects associated with different vehicle emission standards and driving cycles.
- To evaluate the impact of two different designs of Air Liquid Interface (ALI) in-vitro cell exposure chambers through cell exposure experiments. The main difference between the exposure chambers is the direction of the exhaust flow (parallel and perpendicular) that also affects the emission doses.

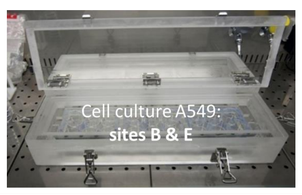
METHODOLOGY

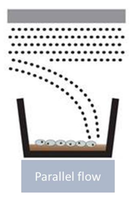
Experimental Setup



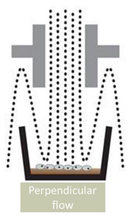
In-vitro Cell* Exposure Systems

(a) Multiculture in-vitro Cell exposure Chamber (MEC) – Parallel flow [4] :
the flow is parallel and particulates are deposited due to diffusion at Air-to-Liquid Interface (ALI)





(b) ALI system – Perpendicular flow [5]**:**
the flow is perpendicular, and particulates are deposited due to diffusion with minor enhancement caused by the stagnation point flow



*A549 human epithelial cells

Emission standards and driving cycles

Vehicles:

- Gasoline Direct Injection (GDI) vehicle equipped with a Gasoline Particle Filter (GPF)
- Gasoline Port Fuel Injection (PFI) **hybrid** vehicle

Driving cycles / dynamics of driving:

- **Mild** one at urban streets ("mRDE" driving cycle, cold start)
- **Dynamic** one, mainly at highway ("Combined" driving cycle, cold start)

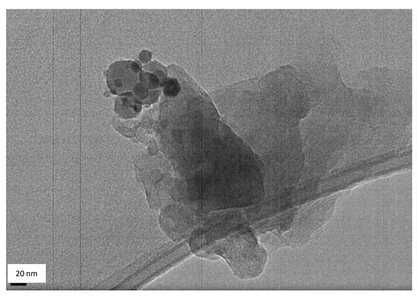
RESULTS

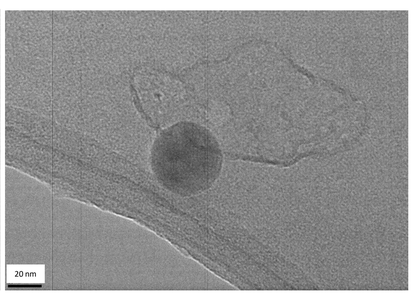
Homogeneity assessment of MEC – parallel flow

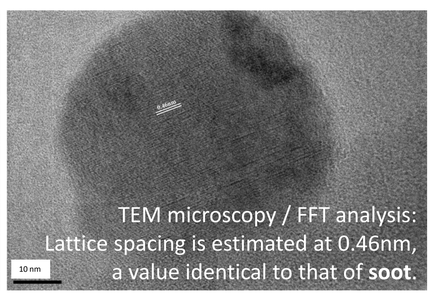
MEC - Parallel has equivalent sites by design, for multiple ALI cell exposures. Between randomly chosen sites B and E, a low, acceptable **overall error of 4%** is observed based on the Alamar Blue cell viability assay, that confirms homogeneity

Experiment No.	Site B	Site E	Average	Std.Dev.	Error (B,E)
	cells/cm^2 (*10^3)				(%)
exp1	281	338	309	40	**13%**
exp2	262	308	285	32	**11%**
exp3	245	250	247	3	**1%**
exp4	354	352	353	1	**0%**
exp5	364	383	374	13	**3%**
exp6	463	465	464	1	**0%**
exp7	427	457	442	21	**5%**
exp8	403	400	402	2	**1%**
exp9	396	404	400	6	**2%**
exp10	346	360	353	10	**3%**
exp11	373	349	361	18	**5%**

TEM Characterization at MEC – parallel flow







Dosimetry

Doses are determined according to the **deposition efficiency of each system** and are **equivalent to human inhalation during realistic daily exposure** [6].

$$\text{Dose} = \frac{C_{TWA} * Q\text{exposure}}{\text{Cell density} * \text{Deposited surface}} * \text{Deposition Efficiency } [=] \text{ particles \#/cell/h}$$

where:

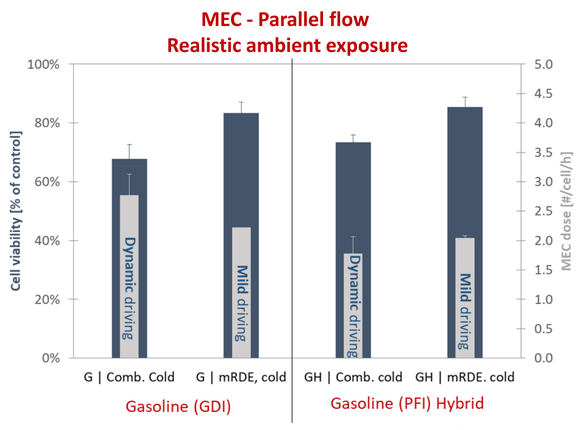
TWA: Time Weighted Average: $C_{TWA} = \frac{\int_{t=1s}^{t=\text{end of cycle}} C_i}{t_{cycle}}$

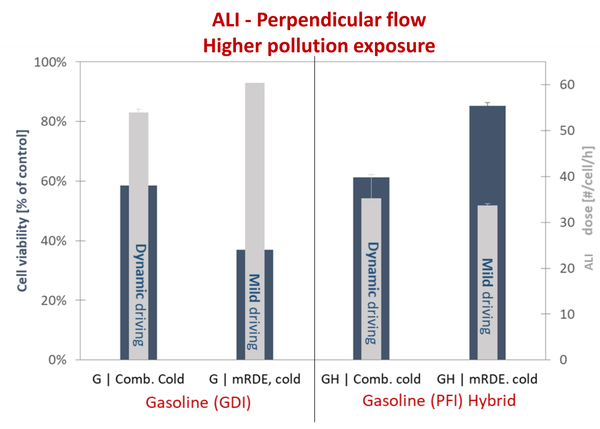
Cell density is calculated from the Alamar Blue assay at control experiments [cells/cm^2]

(a) MEC - parallel dose = 1.6 – 3 #/cell/h	**(b) ALI - perpendicular dose = 27 – 57 #/cell/h**
Equivalent to human inhalation at daily realistic ambient exposure [6]	In the range of equivalent to human inhalation at higher pollution levels [6]

MEC dose ~ 15x ALI dose

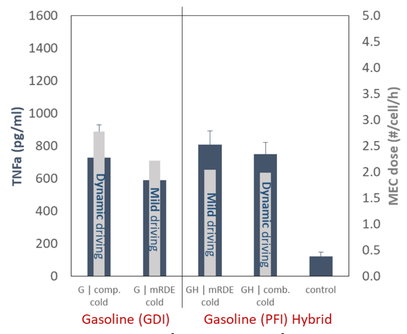
Cell viability at different exposure doses

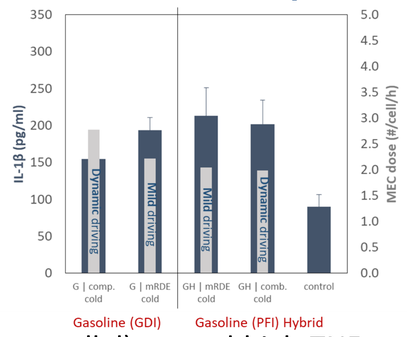




- At realistic ambient exposure (MEC - parallel) dynamic (combined) driving of both GDI and hybrid G-PFI caused higher cell mortality compared to the mild (mRDE) driving.
- At higher pollution exposure (ALI - perpendicular) cell mortality is higher for the GDI vehicle compared to the hybrid G-PFI one. No clear image for the effect of driving dynamics.

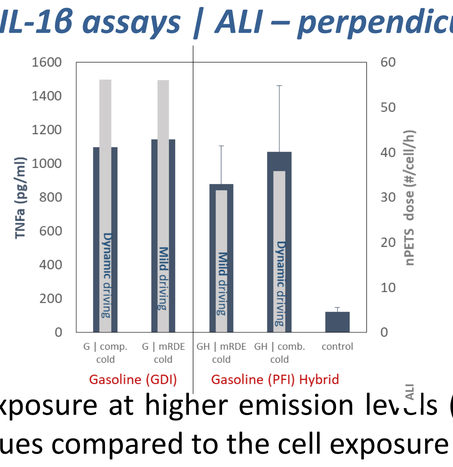
TNFa & IL-1β assays | MEC – parallel flow → Realistic ambient exposure

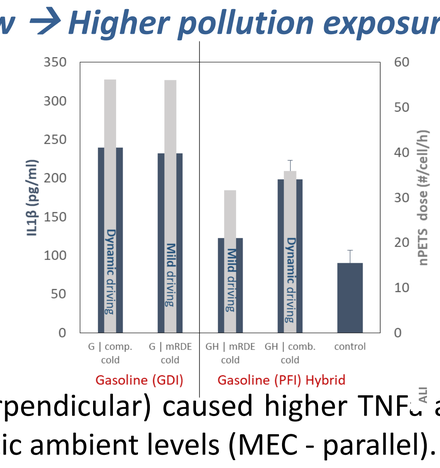




- Cell exposure at realistic ambient exposure (MEC - parallel) caused high TNFa and IL-1β compared to the control.
- At such low doses, no significant effect is observed between the vehicle type (GDI and hybrid G-PFI) and between the driving dynamics (combined and mRDE driving cycle).

TNFa & IL-1β assays | ALI – perpendicular flow → Higher pollution exposure





- Cell exposure at higher emission levels (ALI - perpendicular) caused higher TNFa and IL-1β values compared to the cell exposure at realistic ambient levels (MEC - parallel).
- Both TNFa and IL-1β values are higher for the GDI vehicle compared to the hybrid G-PFI .
- For hybrid gasoline PFI vehicle, dynamic (combined) driving causes higher exposure doses compared to the mild (mRDE) driving.

CONCLUSIONS & FUTURE WORK

- At both exposure doses (parallel & perpendicular flow system), dynamic (combined) driving caused higher cell mortality compared to the mild (mRDE) driving, while the higher biological effect was observed at higher doses emitted by GDI vehicle during dynamic driving.
- At ambient dose (parallel flow system) increase of cytokines and flammability was observed but not significantly affected by the vehicle type and dynamics of driving.
- At higher dose (perpendicular flow system) GDI vehicle caused higher cytokines and flammability compared to the hybrid G-PFI.
- Future investigation of other type of fuel and vehicles (e.g. CNG) is planned with incorporation of Quartz Crystal Microbalance (QCM) to the MEC - parallel system (for real time dose measurement).

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