Monitoring diesel exhaust particles deposition on lung cells *in vitro* by lock-in thermography

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Introduction

Diesel exhaust particles (DEPs) can deposit onto the respiratory epithelial surface upon inhalation exposure. Cellular burden of DEPs is important for dose-response relationships in the cells, however, non-invasive methods to continuously monitor DEPs on cells during exposure are still challenging and not well established.

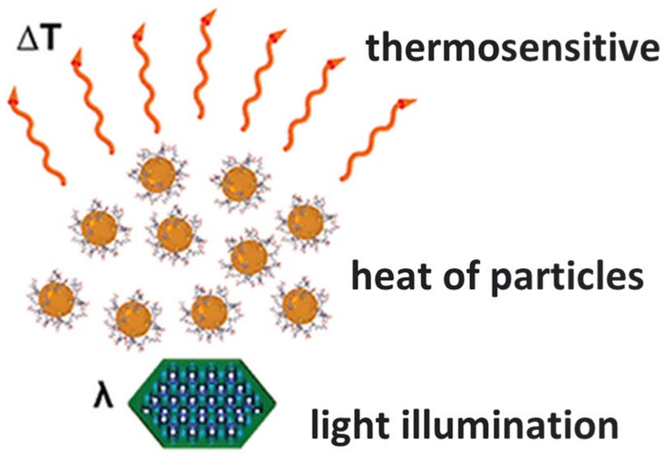

Figure 1. Schematic representation of the lock-in thermography method for DEP detection, adapted from Steinmetz et al. (2021) [1].

Lock-in thermography (LIT) is a thermosensitive detection method that applies light illumination to induce heat of the carbon-based particles (**Figure 1**). An alternative particle detection system, *i.e.,* the miniaturized lock-in thermography (Calorsito mini, NanoLockin GmbH, Switzerland) was explored in this study for DEP detection on cells, combined with the Vitrocell® Cloud Alpha exposure system.

Methods

Lung epithelial A549 cells were exposed to DEPs by nebulization in the Vitrocell® Cloud Alpha exposure system (**Figure 2A**). The deposited DEPs on/in the A549 cells were measured by the Calorsito mini under lights with a wavelength of 525 nm (1 Hz and 60 cycles) in this study (**Figure 2B**) and the generated thermal emission signals from the tested samples were recorded.

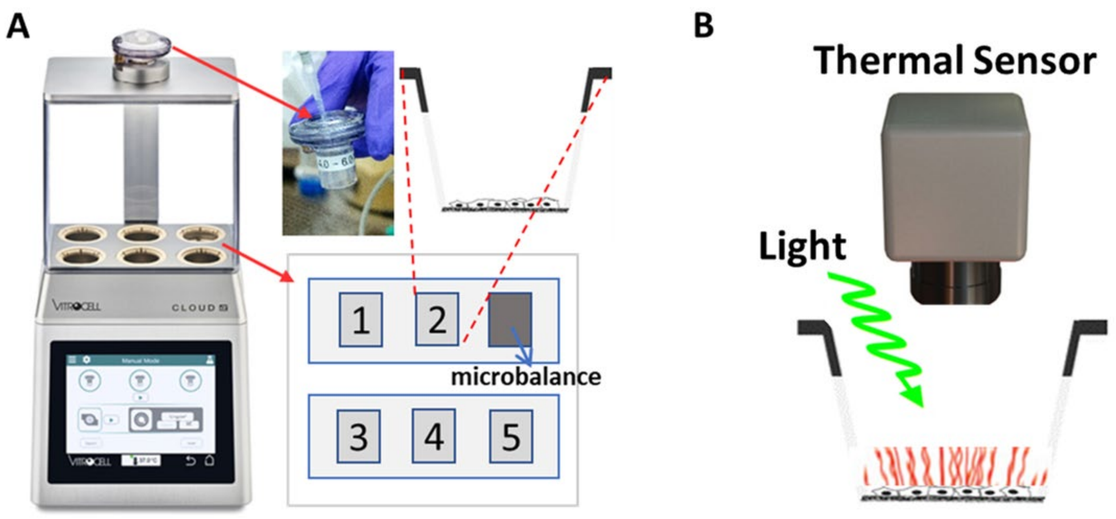

Figure 2. The combination of the Vitrocell® Cloud Alpha exposure system (**A**) and the Calorsito mini (**B**) integrates the cell exposure to DEP aerosols and the detection of the deposited DEPs on/in the cells.

Results

1. Detection of DEPs on cells

DEPs at 240 and 440 ng/cm^2 deposited on the A549 cells were visualized by transmission electron microscopy (TEM, **Figure 3A**) and detected by the Calorsito mini, with the generated thermal images. The homogeneous thermal signal intensity of DEPs on cells can be seen at both concentrations (**Figure 3B**), and the detected thermal signals increased with the DEP levels (**Figure 3C**).

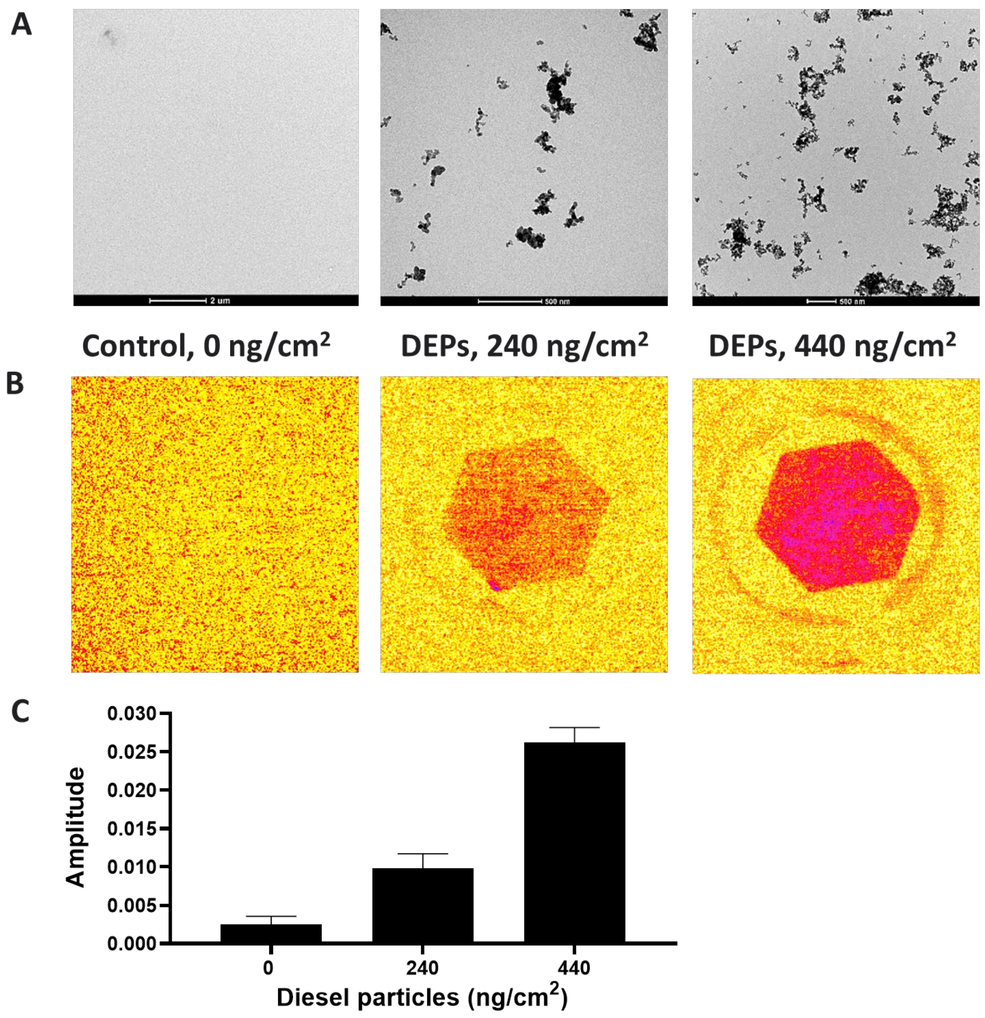

Figure 3. Deposition measured by TEM (**A**), thermal imaging recordings (**B**), and thermal signals (**C**) of DEPs at 240 and 440 ng/cm^2 on the A549 cells.

2. Correlation between thermal signals and DEP levels on cells

DEP solutions with different concentrations were nebulized to obtain the DEP levels from 0 to 450 ng/cm^2 on the A549 cells, which were measured by the Calorsito mini. As shown in **Figure 4**, there is a positive correlation (R^2 = 0.98) between the detected thermal signals and the deposited DEP levels on cells.

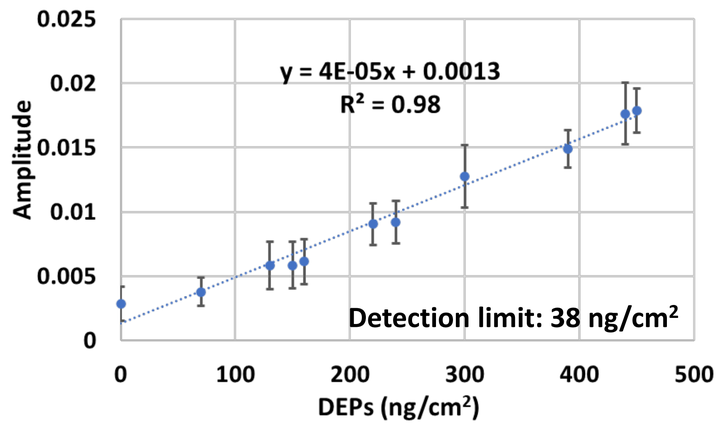

Figure 4. Correlation between the detected thermal signal amplitudes and DEP levels on the A549 cells.

3. Detection of DEPs on/in cells after exposure

A549 cells were exposed to DEPs at 310 ng/cm^2, followed by LIT measurements immediately after exposure and after 24-hour exposure (with 2 times wash). The detected thermal signals of tested samples were comparable before and after 24-hour exposure (**Figure 5A**). The homogeneous signal intensity of DEPs on/in cells can be seen after 24-hour exposure (**Figure 5B**), indicating that the DEPs are retained in the cells.

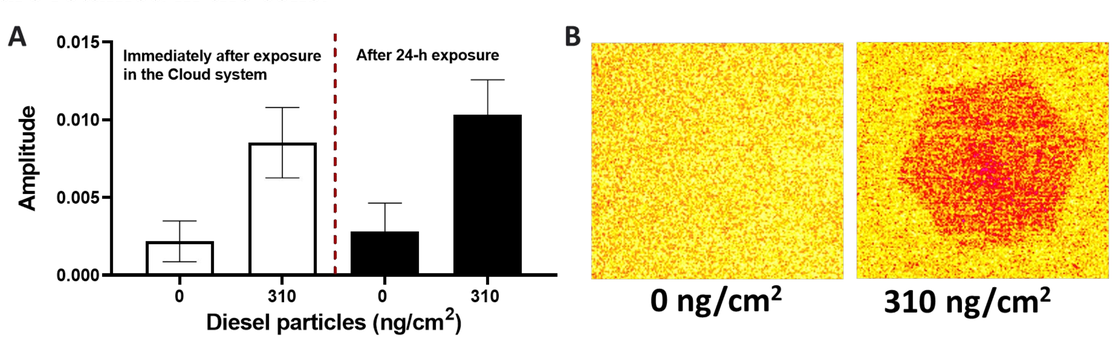

Figure 5. Thermal signals (**A**) of DEPs on/in cells immediately after exposure and after 24-hour exposure, with the thermal imaging recordings (**B**) after 24-hour exposure.

Summary

- The combination of the Vitrocell® Cloud Alpha system and the Calorsito mini integrates the cell exposure to DEPs and the detection of the exposed DEPs on the cells.
- DEP concentrations on cells correlate linearly (R^2 = 0.98) with the generated thermal signals.
- Our results suggest the potential of this combined system for continuously quantifying DEPs on/in the cells during exposure.

References

1. Steinmetz, L. et al., Experimental and theoretical validation of plasmonic nanoparticle heat generation by using lock-in thermography. The Journal of Physical Chemistry C, 2021, 125, (10), 5890-5896.

Acknowledgement

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