Endothelial responses of the alveolar barrier *in vitro* in a realistic dose-controlled exposure to diesel exhaust particulate matter

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Exposure to fine and ultra-fine environmental particles is still a problem of concern in many industrialized parts of the world and the intensified use of nanotechnology may further increase exposure to small particles.

The response triggered by air pollutants is not limited to local effects of the respiratory system but is often systemic, resulting in endothelial dysfunction or atherosclerotic malady. The link between air pollution and cardiovascular disease is now accepted by the scientific community, but the underlying mechanisms responsible for the pro-atherogenic potential still need to be unravelled in detail.

A complex tetraculture system was developed (Figure 1) and consists of the alveolar type-II cell line (A549), differentiated macrophage-like cells (THP-1), mast cells (HMC-1) and endothelial cells (EA.hy 926), seeded in a 3D-orientation on a microporous membrane to mimic the cell response of the alveolar surface *in vitro* in conjunction with native aerosol exposure (Vitrocell™ chamber).

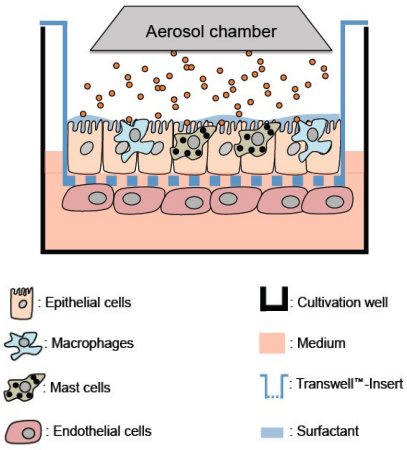

Figure 1. Representative organization and composition of the tetraculture system exposed to diesel exhaust particulate matter. Klein *et al* (2013).

After exposure the expression of different anti-oxidant target genes and inflammatory genes such as *HMOX1*, *NQO1*, *ICAM1* or *VCAM1* as well as the nuclear translocation of Nrf2 was evaluated.

In addition, the potential of diesel particles to induce the upregulation of *CYP1A1* mRNA in the endothelium was analysed.

Diesel particle exposure led not to an upregulation of the anti-oxidant or inflammatory target genes, but to clear nuclear translocation of Nrf2 (Figure 2).

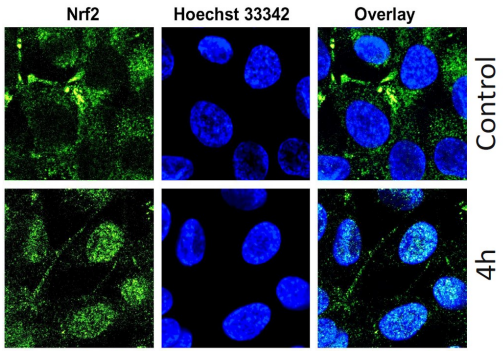

Figure 2. Nrf2 translocation in endothelial cells observed after 4 hours of exposure.

The endothelial cells responded to the treatment also with the upregulation of *CYP1A1* mRNA and nuclear translocation of AhR.

Overall, exposure triggered a response in the endothelial cells after indirect exposure of the tetraculture system to low doses of particles, underlining the sensitivity of ALI exposure systems.

The use of the tetraculture together with the native aerosol exposure equipment may finally lead to a more realistic judgment regarding the hazard of new compounds and/or new nano-scaled materials in the future.

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