MUCILAIR™ VERSUS RAW 264.7 CELLS IN NANO-TOXICOLOGY

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OBJECTIVE

With increasing applications of engineered nano-materials (ENM) resulting in increasing exposure, the safety should be addressed along with the design of new materials. Animal testing may not be a desired test method for thousands of new nanomaterials because of the ethical concerns. Therefore, development of predictive *in vitro* toxicological screening can be valuable to rank ENM to determine priority for subsequent *in vivo* testing. In *in vitro* studies, nano-materials are predominantly studied in A549 or RAW 264.7 cell lines. However, using human 3D airway models opens up new possibilities in predictive *in vitro* testing of nanomaterials. These models consist of fully differentiated human epithelial cells and allow relevant exposure via air as they are cultured at an air-liquid interface. We compared the toxicity of SiO_2 and CeO_2 nanoparticles on MucilAir™ (EpiThelix Sarl) to RAW 264.7 macrophages.

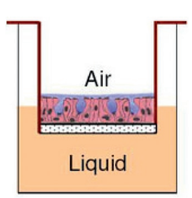


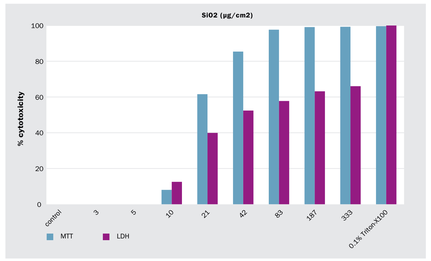
FIGURE 1
Schematic representation of the MucilAir™ model.

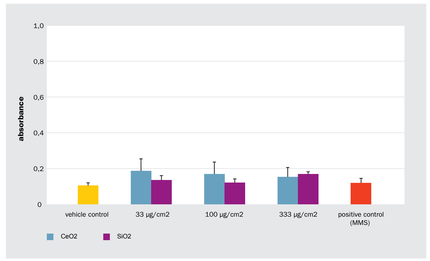
METHODS

MucilAir™ inserts and RAW 264.7 cells were exposed for 24h to the nanoparticles via a droplet on the tissue surface and via the medium, respectively. Cytotoxicity was measured by LDH leakage (both) and TEER (MucilAir™) or MTT conversion (RAW 264.7). Various cytokines were analyzed in culture medium as a measure of inflammation. Oxidative stress and genotoxicity was evaluated by *HO-1* expression and comet assay, respectively.

RESULTS

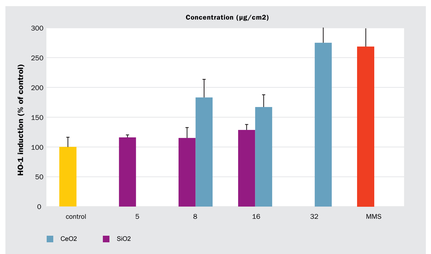
In RAW 264.7 cells, SiO_2 and CeO_2 were cytotoxic at similar concentrations (figure 2b,c). A distinct induction of cytokine release (TNF-α only) was observed with SiO_2 only (figure 3b), whereas HO-1 expression and an increase in % tail DNA was only induced by CeO_2 (figures 4 and 5). In MucilAir™, no clear effects were observed for all endpoints with up to 10-fold higher concentrations (Figures 2a,b; 3-5).





FIGURES 2a and b
LDH leakage and MTT data from RAW 267.4 cells treated for 24h with SiO_2 (fig 2a) and LDH leakage for MucilAir™ inserts treated for 24h with SiO_2, CeO_2 (fig 2b).

Nano-sized SiO_2 and nano-sized CeO_2 give similar results in the LDH leakage and MTT assay (data CeO_2 not shown). IC_{50} values are in the same order (data not shown).
No cytotoxicity was observed in MucilAir™ based on TEER (data not shown) and LDH leakage for both SiO_2 and CeO_2.



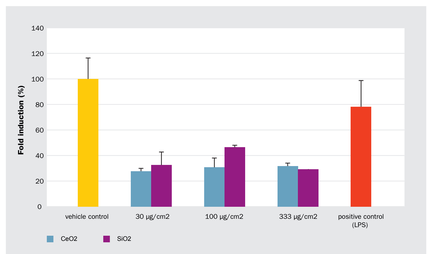
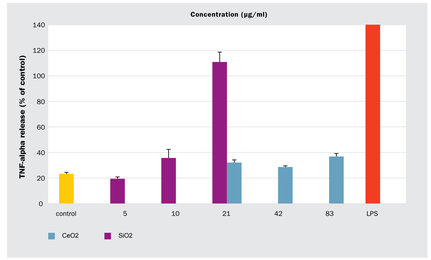


FIGURE 3a and b
HO-1 expression data for RAW 267.4 cells (fig 3a) or MucilAir™ inserts (fig 3b) treated for 24h with SiO_2 or CeO_2.

Although CeO_2 clearly induces HO-1 expression in RAW 267.4 cells, this is not observed in MucilAir™. SiO_2 does not induce HO-1 expression in either RAW 267.4 cells or MucilAir™.



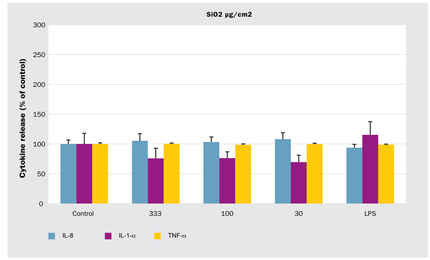
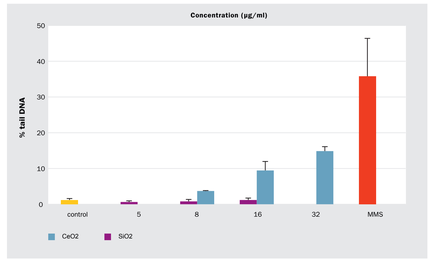
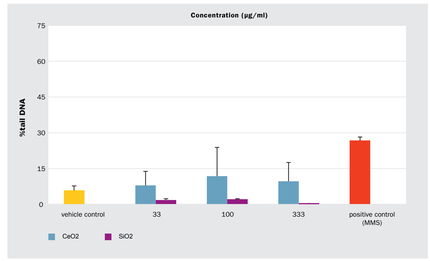


FIGURE 4a and b
TNF-a release for RAW 267.4 cells (fig 4a) or release of cytokines IL-8, IL-1α and TNF-α for MucilAir™ inserts treated for 24h with SiO_2 or CeO_2 (shown for RAW 267.4 cells only).

From a series of 13 cytokines investigated in RAW 267.4 cells, only TNF-α release was increased after exposure to SiO_2. CeO_2 did not increase the release of TNF- α.
In MucilAir™ neither SiO_2 nor CeO_2 demonstrated an increased release of IL-1α, IL-8 and TNF-α.





FIGURES 5a and b
Comet assay for RAW 267.4 cells (fig 5a) or MucilAir™ inserts (fig 5b) treated for 24h with SiO_2 or CeO_2.

CeO_2 induced a clear induction in the % tail DNA in RAW 267.4 cells. In MucilAir™ both damaged and undamaged cells were observed after exposure to CeO_2, indicating that the nanoparticles might have an adverse effect on some cells. However, only a slight induction in % tail DNA was observed which needs further investigation. SiO_2 did not induce an increase in % tail DNA in either RAW 267.4 cells or MucilAir™.

CONCLUSION

MucilAir™ appears less sensitive towards particle induced toxicity. As MucilAir™ enables a more relevant exposure, results from this assay could prove to be more realistic than experiments in cell lines. This will be investigated in further experiments.

FUTURE PLANS

In future, we will optimize experimental conditions and further assess the applicability of 3D airway models, including MucilAir™, by different exposure systems and compare the results with both cell culture and *in vivo* inhalation data. Ultimately, these models may be useful in the first tier(s) of the safety evaluation of engineered nanomaterials.

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