Nanotubes of imogolite do not activate macrophages and modestly perturb the barrier properties of airway epithelial cells in vitro

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7.1.10 Nanotubes of imogolite do not activate macrophages and modestly perturb the barrier properties of airway epithelial cells *in vitro*

Ovidio Bussolati[1], Bianca Maria Rotoli[1], Pier Paolo Zanello[1], Barbara Bonelli[2], Cristina Zanzottera[2], Edoardo Garrone[2], Ivana Fenoglio[3], Bice Fubini[3], Mara Ghiazza[3], Enrico Bergamaschi[1]

[1]*Dept. Cl. Medicine, Nephrology and Health Sciences & Dept. Experimental Medicine, University of Parma, Italy*
[2]*DISMIC, Politecnico di Torino, Italy and INSTM Unit of Torino Politecnico, Italy*
[3]*Interdepart. Centre "G. Scansetti" and Dept. of Chemistry, University of Torino, Italy*
Email: enrico.bergamaschi@unipr.it

Since airways represent the first barrier for inhaled particles, the effects of nanomaterials on the cells of Lung Blood Barrier (LBB) should be investigated. Previous findings showed that MWCNT impair airway barrier function and are toxic to macrophage lines [1]. Here we investigate the effects of nanotubes of imogolite (INT), a hydrated alumino-silicate with the formula $(OH)_3Al_2O_3SiOH$, previously proposed by some of us as a possible negative control for HARN [2].

INT - i.d. 1 nm, BET 394 $m^2\ g^{-1}$, total and microporous volume of 0.27 and 0.11 $cm^3\ g^{-1}$, respectively - were synthesized via sol-gel procedure and found organized into fibres at FESEM [3]. As *in vitro* models of LBB cells, we used two murine macrophage cell lines (Raw264.7 and MH-S) and the human airway epithelial cells Calu-3. Cell viability was assessed with resazurin. RT-PCR was used to study the expression of NOS2 and ARG1, markers of, respectively, macrophage classical or alternative activations, and concentration of nitrites in the culture medium was measured as an indicator of NO production.. Epithelial barrier integrity was evaluated from the trans-epithelial electrical resistance (TEER). At the same doses, INT caused much smaller effects than MWCNT on macrophage viability, while no significant damage was observed up to 40 $\mu g/cm^2$ of monolayer for exposure times up to 24h. The incubation of macrophages with INT at doses as high as 120 $\mu g/cm^2$ for 72h did not alter either NOS2 or ARG1 expression nor increased NO production. In Calu-3 monolayers exposed to INT (120 $\mu g/cm^2$ for 7d) only modest TEER changes were recorded (< 20%).

As a whole, in spite of their fibrous nature, INT appear not markedly toxic for *in vitro* models of LBB cells and could represent a low-toxicity reference for *in vitro* toxicological studies on HARN, should further tests confirm their inertness.

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