Comparative effects of TiO_2 nanofibers of different aspect ratio on airway barrier cells *in vitro*

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Abstract

Background: The biological effects of TiO_2 nanofibers, a novel nanomaterial increasingly used in a wide variety of fields, are far from being completely elucidated. In this study we have evaluated the toxicity of anatase TiO_2 nanofibers (NF, length, 0.2-30 μm; thickness, 0.2 to 0.6 μm; aspect ratio 1:28), composed by discrete TiO_2 nanoparticles, before and after extensive ball-milling with microspheres of zirconium, which lowered aspect ratio to 1:8. Biological endpoints, including cell viability, inflammatory markers in macrophages, trans-epithelial electric resistance and oxidative stress were benchmarked against TiO_2 nanoparticles and carbon nanotubes.

Results: At 80 μg/cm_2 (72h), TiO_2 NF caused a 45% decrease of viability in A549 epithelial cells but only a 20% reduction in Raw 264.7 macrophages. Ball-milling significantly lowered these effects. Dose- and time-dependent decrease of the Trans-Epithelial Electrical Resistance of CaLu-3 cell monolayers was also detected (40% decrease at 40 μg/cm_2) and mitigated by ball milling (no significant decrease at the same dose). TiO2 NF activated macrophages inducing the expression of inflammatory markers (NO production, induction of *Nos2* and *Ptgs2*, cytokine secretion). All these effects were greatly amplified by ball milling leading, for instance, to a 10-fold enhancement of *Nos2* induction.

Conclusions: This study indicates that TiO_2 NF exert significant toxic effects on airway barrier cells including cytotoxicity, macrophage activation and impaired epithelial barrier integrity. Although the reduction of TiO2 NF aspect ratio mitigates the effects on cell viability and barrier competence, it enhances the pro-inflammogenic activity of the nanomaterial.