**Length Dependent Toxicity of TiO2 Nanofibers: Mitigation via Shortening**

Massimiliano G. Bianchia, Luisa Campagnolob, Manfredi Allegria, Simona Ortellic, Magda Blosic, Martina Chiua, Giuseppe Taurinoa, Valentina Lacconib, Antonio Pietroiustib, Anna L. Costac, Craig A. Polande, Daniel Bairde, Rodger Duffine, Ovidio Bussolatia\*, & Enrico Bergamaschif

**Supplementary Results**

***Effects of TiO2 nanofibers on Raw264.7 macrophages***

Supplementary Figure 1 reports the effects of L- or S-TiO2 NF on Raw264.7 macrophages incubated in the presence of the materials for 24, 48 or 72h.

Viability, as assessed with the resazurin method, was not decreased at any dose of either NF type (A, B, C). Rather, especially at lower times, a marked increase in fluorescence was detected, while MWCNT NM-401, used as a benchmark material, caused the expected, time-dependent loss of viability. At 24h, the effect was more evident with S-TiO2 NF at all the doses, and with the highest doses only at 48 and 72h. To check if fluorescence increase was associated with cytotoxicity, we performed a LDH assay in the extracellular medium and found no increase in cytotoxicity at any time of incubation independently of NF type and dose.

The increase in fluorescence detected in macrophage cultures incubated with L- or S-TiO2 NF cannot be attributed to an effect of the materials on the dye, since this type of interference was preliminarily excluded (see Materials and Methods), or to the insensitivity of the experimental system, since MWCNT caused the expected fluorescence decrease. On the other hand, the fact that no significant LDH release was detected excludes that TiO2 NF exert a sizable acute toxicity on macrophages (at least, in the first 72h of incubation). We can hypothesize that, since resazurin method is based on dye reduction, this reaction is stimulated by the interaction between the dye and the cells in the presence of NF. Interestingly, the fluorescence increase is more pronounced with S-TiO2 NF, although their chemical and surface properties are the same than L-TiO2 NF. It is therefore tempting to attribute this difference to the different size of the NF and to the ability of S-TiO2 NF to be internalized, promoting the metabolic activation of the macrophages.



**Supplementary Figure 1.** Effect of TiO2 nanofibers on macrophage viability. Raw264.7 macrophages were exposed for up to 72h to the indicated doses of long (L-) or short (S-) TiO2 NF. Cell viability (A, B, C) was measured with the resazurin method at 24, 48, or 72h. At the same times, LDH activity (D, E, F) was measured in the extracellular medium as a proxy for cytotoxicity. MWCNT NM-401 were used as a benchmark material in the resazurin assay at 80 μg/cm2. POS represents a positive control for LDH activity provided by the kit. Data are means ± S.D of five independent determinations. \*\* *p* < 0.01 L-TiO2 NF *vs.* S- TiO2 NF (two-way ANOVA).