Cellular toxicity of TiO_2-based nanofilaments

A. Magrez et al

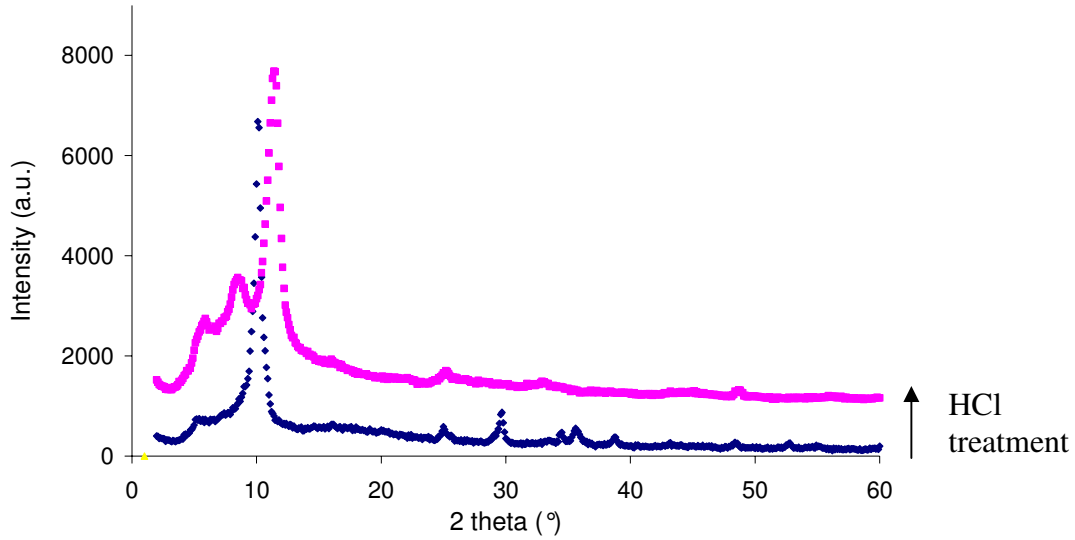
Supplemental table 1: Chemical characterization of TiO_2-based nanomaterials

The chemical compositions have been determined by inductively coupled plasma optical emission spectrometry (ICP-OES).

	%w Na	*%w Ti*	*Formulae*
Na_xTiO_{2+d} NWs	15.6(5)	46.5(5)	$Na_{0.70(6)}TiO_{2+0.35(6)}$
H_yTiO_{2+d} NWs	0.31(3)	59.0(3)	$H_{0.66(5)}Na_{0.01(5)}TiO_{2+0.34(6)}$
H_yTiO_{2+d} NTs	0.45(5)	58.9(5)	$H_{0.65(6)}Na_{0.02(6)}TiO_{2+0.34(6)}$

Supplemental Figure 2: Structural characterization of TiO_2-based nanomaterials by X-ray Powder Diffraction.

The produced nanofilaments (both nanotubes and nanowires) with a composition of Na_xTiO_{2+d} crystallize in the form of a $Na_2Ti_3O_7$ structure (a layered TiO_2-based material). A decrease of the lattice constant, i.e. a shift of the peaks towards larger 2 theta values is attributed to the substitution of Na^+ ions (blue curve) by H^+ (pink curve) yielding $H_2Ti_3O_7$ (H_yTiO_{2+d}).



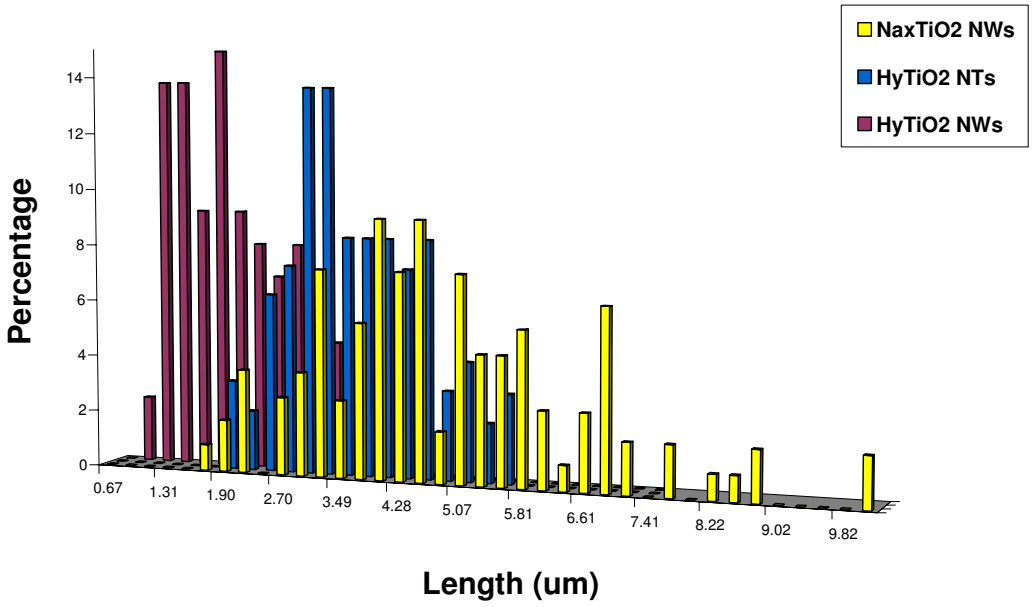
Supplemental Figure 3: Morphological characterisation of various TiO_2-based nanofilaments

The mean length (A), length distribution (B), diameter distribution of nanowires (C1) and nanotubes (C2) and specific surface area (D) were established from SEM and TEM micrographs.

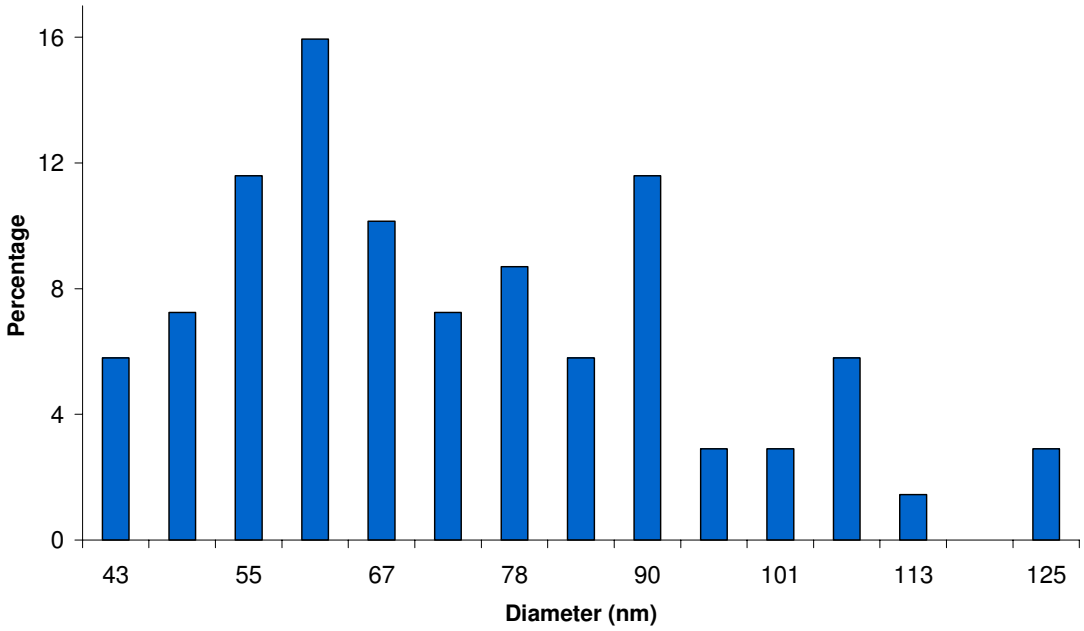
A

	Mean Length (μm)
Na_xTiO_{2+d} NWs	4.9
H_yTiO_{2+d} NWs	2.1
H_yTiO_{2+d} NTs	3.7

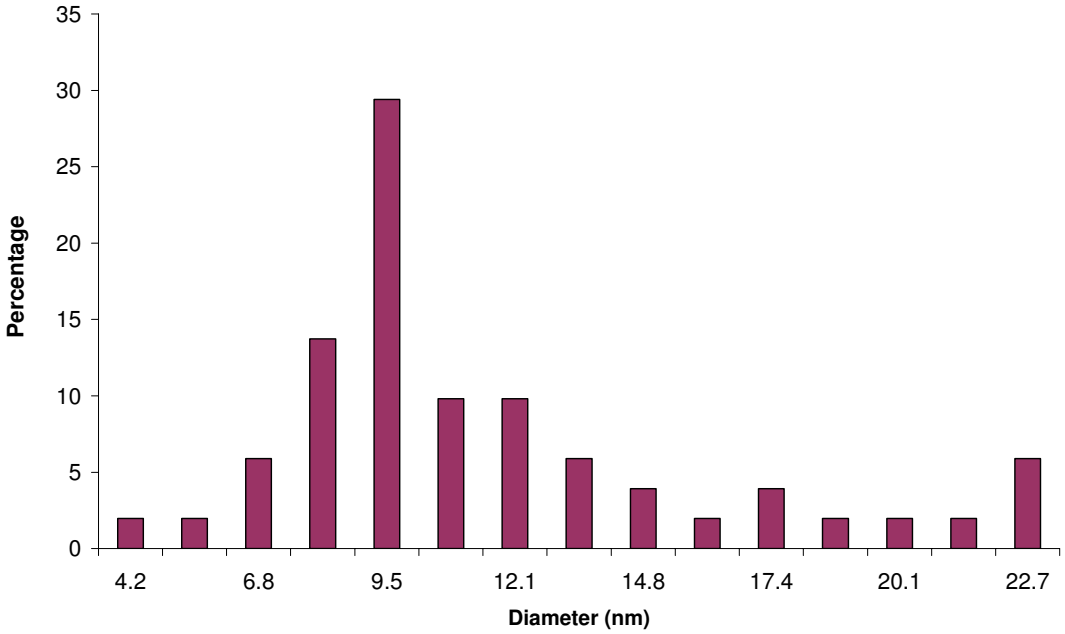
B



C1 Diameter of TiO_2 based nanowires (mean value = 75.9 nm)



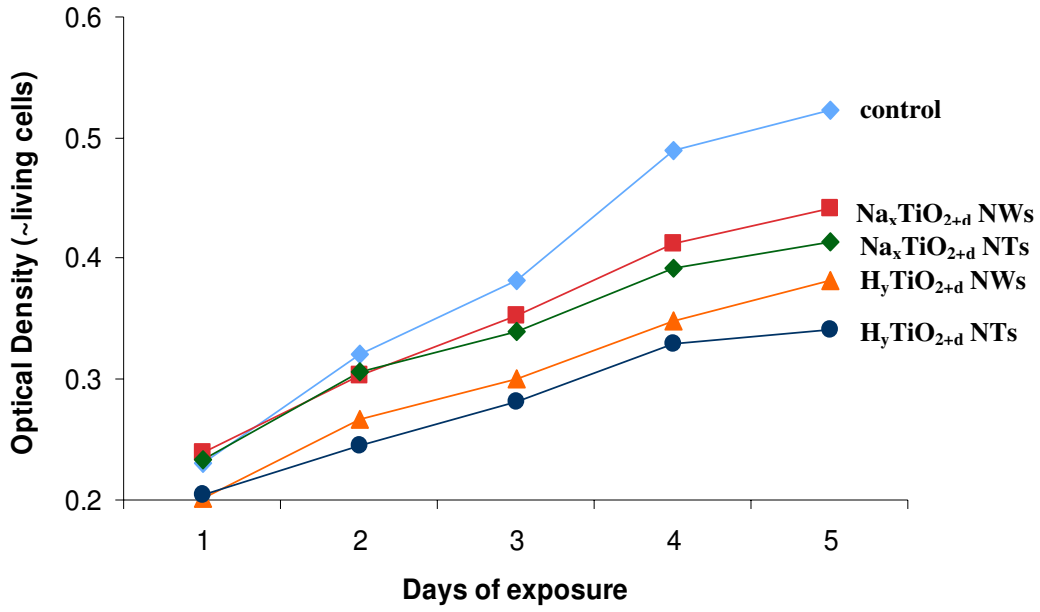
C2 Diameter of TiO_2 based nanotubes (mean value = 12.2 nm)



D Specific Surface Area (SSA) was measured by BET from dried powders.

	SSA (m^2/g)
Na_xTiO_{2+d} NWs	45
H_yTiO_{2+d} NWs	64
H_yTiO_{2+d} NTs	55

Supplemental Figure 4: Growth curve of H596 cells in the presence of TiO_2-based nanotubes and nanowires in the Na^+ and H^+ forms (2 µg/ml). For these experiments, Tween-80 (Sigma-Aldrich, Buchs, Switzerland) was used as the dispersing agent. A comparison with the experiments obtained in gelatin-containing medium (Fig. 2a) revealed the results to be essentially identical, i.e. the toxicity order was as follows: $Na_xTiO_{2+\delta}$ NWs < $H_yTiO_{2+\delta}$ NWs ≈ $H_yTiO_{2+\delta}$ NTs. In addition, also $Na_xTiO_{2+\delta}$ nanotubes (NT) were tested. As in Fig. 2a the HCl-treated nanofilaments were more toxic than the Na^+ forms and the nanotubes were slightly more toxic than the nanowires. However, the differences between tubes and wires were smaller than differences between HCl-treated (H^+) and Na^+ forms. Tween-80 was used as the dispersing agent, since Tween-80 containing solutions can be stored for prolonged periods (> 1 yr). In gelatin-containing solutions the proteinaceous parts start to decompose resulting in non-specific growth-inhibiting effects seen in the MTT assays when comparing old (> 6 months) with freshly prepared (< 1 month) gelatin-containing solution, even in the absence of nanofilaments (data not shown).



Supplemental Figure 5: Morphology of H596 lung carcinoma cells treated with TiO_2-based nanofilaments in the Na^+ forms (nanotubes: NaT; nanowires: NaW). All experimental details were identical to the ones used to obtain the images of the H^+ forms of nanofilaments presented in Fig. 3. Also in cultures treated with the Na^+ forms of the nanofilaments, a decrease in cell-cell contacts was the most obvious observation. The main difference compared to cells exposed to the H^+ forms was a decreased fraction of cells showing morphological alterations such as giant cells with lobulated or fragmented nuclei, cells with vacuole-like structures, pycnotic nuclei, and nuclei with multiple nucleoli. However, with respect to the intracellular distribution, the $Na_xTiO_{2+\delta}$ nanomaterials were similarily distributed as the $H_yTiO_{2+\delta}$ nanomaterials. In the 2 insets, examples of cells with 2 nuclei possibly as the result of aberrant mitosis are shown. In the example treated with NaW, a small piece of the nucleus has budded off (arrow). Scale bar: 20 µm.