ARTICLE

Cellular Toxicity of TiO_2-Based Nanofilaments

Arnaud Magrez,[†,*] Lenke Horváth,[†,‡] Rita Smajda,[†] Valérie Salicio,[‡] Nathalie Pasquier,[§] László Forró,[†] and Beat Schwaller[‡,*]

[†]Institute of Physics of Condensed Matter (IPMC), Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland, [‡]Unit of Anatomy, Department of Medicine, University of Fribourg, Route Albert-Gockel 1, CH-1700 Fribourg, Switzerland, and [§]Laboratoire Cytopath, Avenue Cardinal-Mermillod 36, 1227 Carouge, Switzerland

ABSTRACT At present, nanofilaments are not exclusively based on carbon atoms but can be produced from many inorganic materials in the form of nanotubes and nanowires. It is essential to systematically assess the acute toxicity of these newly synthesized materials since it cannot be predicted from the known toxicity of the same material in another form. Here, the cellular toxicity of TiO_2-based nanofilaments was studied in relation to their morphology and surface chemistry. These structures produced by hydrothermal treatment were titanate nanotubes and nanowires with a $Na_xTiO_{2+\delta}$ composition. The cytotoxic effect was mainly evaluated by MTT assays combined with direct cell counting and cytopathological analyses of the lung tumor cells. Our work clearly demonstrated that the presence of $Na_xTiO_{2+\delta}$ nanofilaments had a strong dose-dependent effect on cell proliferation and cell death. Nanofilament internalization and alterations in cell morphology were observed. Acid treatment performed to substitute Na^+ with H^+ in the $Na_xTiO_{2+\delta}$ nanofilaments strongly enhanced the cytotoxic action. This effect was attributed to structural imperfections, which are left by the atom diffusion during the substitution. On the basis of our findings, we conclude that TiO_2-based nanofilaments are cytotoxic and thus precautions should be taken during their manipulation.

KEYWORDS: TiO_2 · nanotubes · nanowires · cytotoxicity · MTT · ionic exchange

The new technologies are going to explore the creation of functional devices at nanometer scales, which necessitates the production and handling of components of submicrometer sizes. The past decade has seen the rapid development of various nanostructures, especially nanofilaments (carbon or inorganic nanotubes (NTs) and nanowires (NWs)). Due to their advantageous properties, nanofilaments are foreseen to be the building blocks of new technologies such as flat panel screens, composite materials, or catalyst supports, expected to be applied in medical diagnosis, imaging, and drug delivery. Although these nanofilaments are very promising, one must know the health hazard factors involved in their production and handling.

The question on their toxicity either during the nanomaterial synthesis or after short- or long-term exposure to traces released in the environment has not been addressed in detail, and reports on the putative harm of these manmade materials just start to emerge. On the basis of the fact that the first of these nanofilaments discovered consisted of carbon nanotubes,[1] most studies on the toxicity have almost exclusively focused on carbon nanotubes.[2−8] The toxicity of inorganic nanofilaments (INFs) has not been addressed so far, yet some of those closely resemble asbestos, a material that has been largely abandoned in Western countries due to health hazard problems. The toxicity of inert systems,[9,10] such as iron oxides, gold, or cadmium chacolgenides, has been investigated but is limited to nearly isotropic particles (*i.e.*, with a low aspect ratio compared to fibers). Unfortunately, the toxicity of materials in nanofilament form cannot be predicted from their known toxicity in a different structural appearance. Asbestos is a well-characterized example, where a "benign" silicate is rendered highly toxic in its fibrous form. Inhalation of asbestos fibers is known to induce asbestosis (a progressive fibrotic disease of the lung), lung cancer, and tumors of the pleura (mesothelioma). In some parts of the lung, type I alveolar epithelial cells are almost in direct contact with mesothelial cells of the visceral pleura, only separated by a thin layer of connective tissue. Thus, asbestos fibers may end up in these cells but can also traverse the pleural space and be taken up by mesothelial cells of the parietal pleura. Other identified translocation pathways include pulmonary lymphatics connecting to the pulmonary blood vessels and from there to essentially all organs.[11] After a prolonged incubation period (10−30 years), asbestos (mainly of the amphibole type: amosite, crocidolite) can induce one of the most aggressive tumor types, mesotheliomas.

*Address correspondence to arnaud.magrez@epfl.ch, beat.schwaller@unifr.ch.

Received for review March 2, 2009 and accepted July 13, 2009.

Published online July 17, 2009. 10.1021/nn9002067 CCC: $40.75

Nowadays, INFs can be produced from many materials and include elemental semiconductors, metal oxides, chalcogenides, or pnictides.[12−14] This allows synthesizing nanomaterials with well-defined and desired properties (size, geometry, charges, aspect ratio, *etc.*) but bears the risk of undesired adverse effects on the exposed surfaces of men and animals (*e.g.*, skin, lung). Some of these new materials might even pose health problems, big as or even bigger than those posed by asbestos fibers. Therefore, it is important to systematically assess the toxicity of all INFs before allowing their industrial application and mass production.

Here, we performed studies on lung tumor cells exposed to TiO_2-based nanofilaments. Since decades, significant progress has been made in incorporating TiO_2 nanoparticles for numerous applications. Nanostructured TiO_2 is the most popular catalyst for air and water purification systems, based on the photodegradation of organic pollutants.[15] For instance, glasses or textiles are rendered self-cleaning when coated with TiO_2 nanoparticles.[16] TiO_2 is also widely used as food and pharmaceutical additive,[17] as well as white pigment in the paper industry and in cosmetic products.[18] Modern sunscreens contain insoluble nanostructured TiO_2, which is colorless and filters ultraviolet radiation with high efficiency. Large-scale arrays of TiO_2-based nanofilaments (including nanotubes and nanowires) are being used in photovoltaic cells and in photoelectrolyzer for the production of hydrogen by water splitting.[19] Indeed, TiO_2-based nanofilaments exhibit as well extraordinary properties of lithium intercalation, thus they are foreseen in rechargeable battery applications.[20] Nonetheless, the experimental results concerning the potential adverse effects of nanostructured TiO_2 are still under debate.[21] First, the dermal penetration of TiO_2 nanoparticles is discussed controversially. Once entered in the vascular system, nanostructured TiO_2 could be distributed throughout the body to organs, various tissues and exposed cells.[22,23] Second, while the toxicity of TiO_2 in its isotropic form (particles with diameter ranging from few nanometers to micrometers) has been widely studied,[10,24] the toxic action of TiO_2-based nanofilaments is essentially unknown.

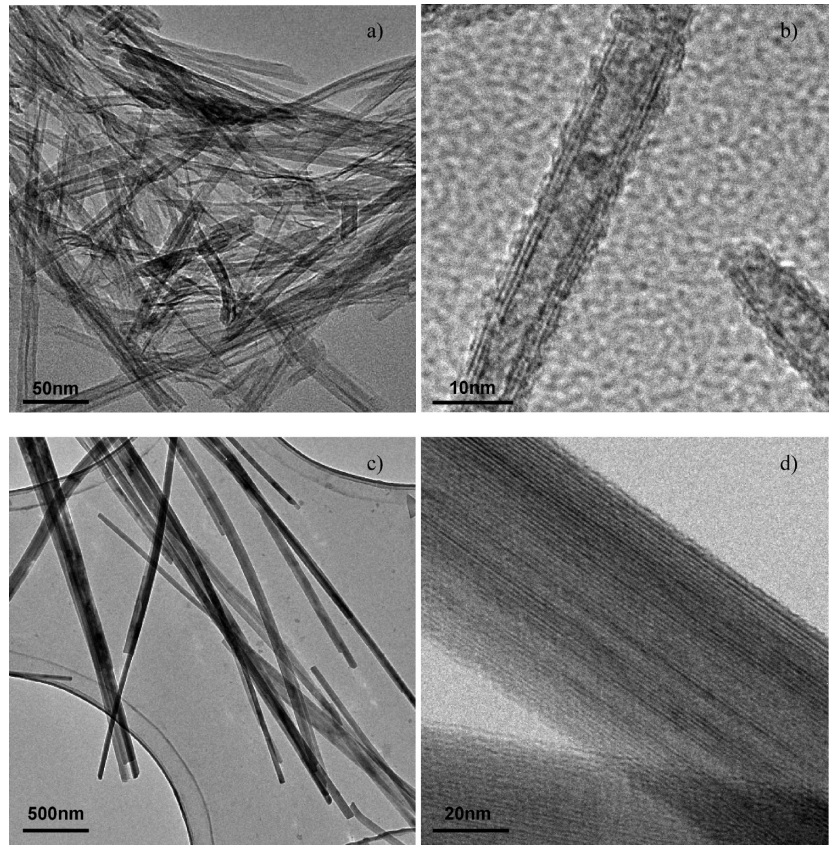

Figure 1. TEM pictures of TiO_2-based nanotubes (a,b) and nanowires (c,d).

RESULTS AND DISCUSSION

On the basis of the fact that the surface cells of the airways, including the epithelial cells of the lungs, are the first cell type to encounter the TiO_2-based nanofilaments released into the environment, we chose to investigate the acute cytotoxicity of different TiO_2-based nanofilaments on lung cells *in vitro*. The different materials were synthesized by hydrothermal treatment from anatase and highly concentrated NaOH solution, producing a large quantity of nanofilaments with $Na_xTiO_{2+\delta}$ composition.[25,26] The Ti−O skeleton of the layered structure consists of TiO_6 octahedrons sharing edges and corners. Either nanowires or nanotubes (Figure 1) can be obtained by tuning the growth conditions (namely, temperature and composition of the starting mixture Ti/Na ratio). Unlike carbon nanotubes, the TiO_2-based nanotubes are not perfect cylinders. They are better described as a scroll of a TiO_2 layer. These TiO_2-based multiwalled nanotubes exhibit an average diameter of around 12 nm with approximately five walls. For comparison, the average diameter of the TiO_2-based nanowires is about 75 nm. Subsequent washing of the $Na_xTiO_{2+\delta}$ materials with hydrochloric acid promotes complete exchange of Na^+ with H^+ to form $H_yTiO_{2+\delta}$ nanofilaments without drastic modification of their morphological characteristics. Chemical, structural, and morphological characteristics of the TiO_2-based nanofilaments are presented in the Supporting Information.

The cytotoxic effect of the TiO_2-based nanofilaments was evaluated by the widely established MTT assay performed with H596 human lung tumor cells. This cell line was selected because, in a previous study with the lung tumor cell lines H596, H446, and Calu-1, H596 cells gave the most consistent and reliable results and

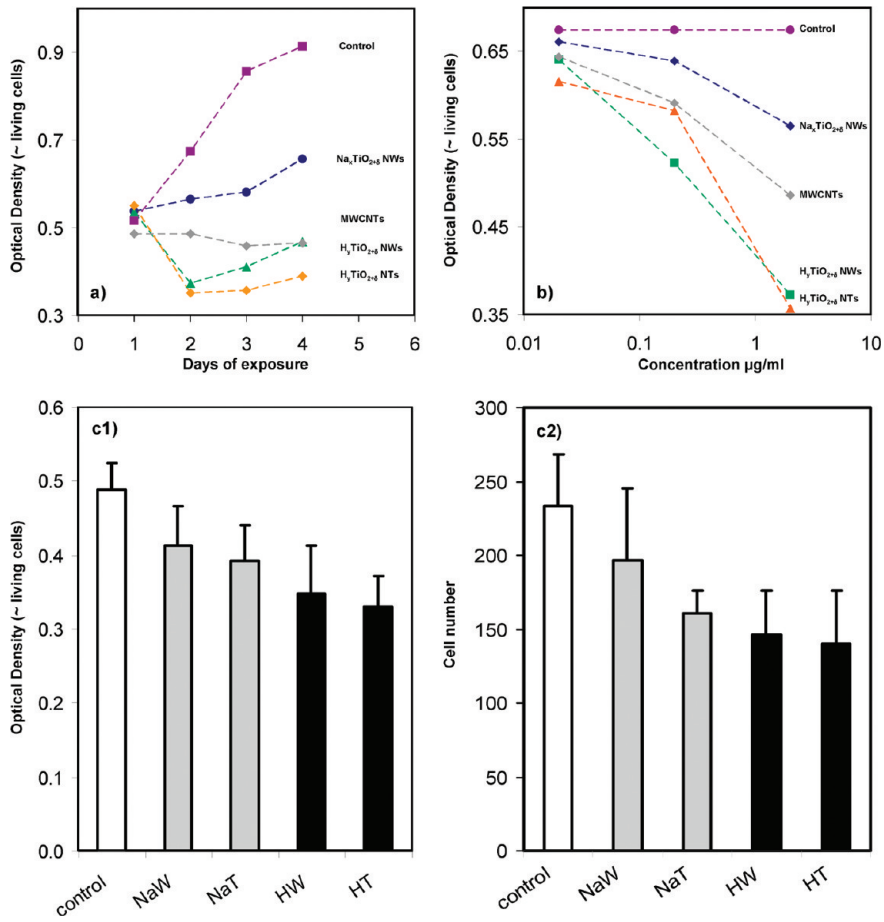

Figure 2. (a) Representative growth curve for H596 cells grown in normal medium (control), or 2 µg/mL of MWCNTs, $Na_xTiO_{2+\delta}$, and $H_yTiO_{2+\delta}$ nanofilaments (NWs, nanowires; NTs, nanotubes). (b) Dose-dependent toxicity of H596 cells exposed to the tested materials for 2 days. (c) Cytotoxicity of TiO_2-based nanomaterials determined by MTT assays (c1) and cell counting (c2). H596 cells were seeded in 96-well plates for MTT assays or on laminin-coated coverslips for cell counting and were exposed to nanomaterials for 4 days (for experimental details, see Experimental Methods). The numbers in c1 and c2 represent averages ± standard deviations from four independent experiments. In the MTT assay (c1), each sample was measured in triplicate, and in c2, the cell number was determined from four microphotographs per sample. Results were analyzed by ANOVA, followed by posthoc analyses by a Tukey test (NaW and NaT, nanowires and nanotubes in the Na^+ form (gray bars); HW and HT, nanowires and nanotubes in the H^+ form (black bars)).

displayed a relative high sensitivity to carbon-based nanomaterials.[27] The MTT assay is routinely used for these types of experiments and reports the combined effects of cell proliferation (cell number) and metabolic activity of cells. Under our assay conditions, that is, dissolving the formazan crystals in a solution containing dimethylsulfoxide (DMSO), irreversible binding of formazan to nanofilaments reported before was found to be negligible.[28] As a result, the method is more sensitive than just counting cells directly in the dishes by microscopic methods or indirectly by measuring the DNA content of the sample.[29] However, in order to validate the results presented in Figure 2a and Supporting Information Figure 4, parallel cultures of H596 cells treated for 4 days with nanomaterials were compared either by determining the intensity of the MTT signals or by directly counting cells from microphotographs of fixed and HE-stained cells (see below and Figure 2c).

An average growth curve of untreated H596 cells for 4 days is shown in Figure 2a (control). Addition of TiO_2-based nanofilaments (2 µg/mL) impaired cell proliferation/cell metabolic activity, and a decrease of the MTT signal was clearly visible 2 days after starting the treatment; the effect was even more pronounced after 4 days (Figure 2a). In order to compare the results on TiO_2-based nanofilaments with our previous results obtained with carbon nanotubes,[27] we selected multiwalled carbon nanotubes (MWCNTs) displaying an intermediate toxicity. The MWCNTs together with the single-walled carbon nanotubes (SWCNTs) are among the most widely studied carbon-based nanomaterials and served as a reference substance to compare the toxicity of TiO_2-based nanofilaments. The MTT signals were clearly smaller in all nanofilament-treated samples, and in all cases, the effect was more pronounced as the nanofilament concentration increased from 0.02 to 2 µg/mL (Figure 2b). In contrast to some of the carbon-based nanomaterials, such as carbon nanofibers (CNFs) and carbon black that significantly decreased the MTT signal already after 24 h,[27] clear differences became visible only after 2 days of treatment. Thus, none of the tested TiO_2-based nanofilaments was as toxic as CNFs and carbon black.

Clearly, $Na_xTiO_{2+\delta}$ nanowires had the least effect on proliferation/activity and were thus considered to be the least toxic at all concentrations and at time points later than 1 day of exposure. Interestingly, the treatment by hydrochloric acid performed on the $Na_xTiO_{2+\delta}$ nanofilaments to exchange Na^+ with H^+ strongly enhanced the toxic action of the TiO_2-based nanofilaments. In particular, at high nanofilament concentrations (2 µg/mL), the cell number/viability decreased in the following sequence: $Na_xTiO_{2+\delta}$ NWs > MWCNTs > $H_yTiO_{2+\delta}$ NWs ≈ $H_yTiO_{2+\delta}$ NTs. In the protonated form, there was no clear difference between TiO_2-based nanotubes as compared to the nanowires with respect to the MTT signal. On the other hand, nanowires in the Na^+ form were clearly less toxic than in the H^+ form. The same was also the case for nanotubes (see Supporting Information Figure 4). This could indicate that the

surface chemistry of the nanofilaments (nanotubes and nanowires) affected more the cell proliferation/activity than their morphological characteristics. Indeed, the ionic exchange performed can induce damage, vacancies, or interstitial atoms within the structure, subsequently to the atom diffusion during the substitution of Na^+ with H^+. These local structure defects are very reactive sites.[30] On the other hand, the $Na_xTiO_{2+\delta}$ nanofilaments might act as a weak base, thus we checked the pH effect of the purified $Na_xTiO_{2+\delta}$ and $H_yTiO_{2+\delta}$ NWs in pure water. When these substances were added to bidistilled water at a concentration of 2 mg/mL, at a 1000-fold higher concentration than used in the experiments, a decrease in pH was observed for $H_yTiO_{2+\delta}$ nanofilaments compared to a suspension of $Na_xTiO_{2+\delta}$ nanofilaments. To exclude the possibility that the increased toxic effect of the H^+ forms of the nanofilaments was due to acidification of the cell culture medium, we measured the pH of the medium before and after adding the highest concentration tested (2 μg/mL). In line with the qualitative observation that the pH indicator present in the cell culture medium did not change color after addition of the nanofilaments, also no change of the pH could be measured using a pH microelectrode. This is likely due to the large pH-buffering capacity of the cell culture medium.

Because the MTT assay measures the combined effects of cell proliferation and metabolic activity of cells and was reported to be prone to artifacts under certain experimental conditions,[28] results presented in Figure 2a and Supporting Information Figure 4 were validated by another method, that is, directly counting the number of surviving cells from microphotographs. For this, the time point (day 4) and nanomaterial concentration (2 μg/mL) were selected, where differences between the various nanomaterials were most evident. In comparison to untreated cells, both the MTT signals (ANOVA; $p < 0.0001$) and number of cells (ANOVA; $p < 0.0001$) were decreased in all nanomaterial-treated samples (Figure 2c). According to posthoc analysis by the Tukey test, the samples could be grouped into three classes (white, gray, and black bars in Figure 2c). Samples with the same shading in Figure 2c1 were significantly different from the other groups (all at $p < 0.05$). The only exception was the Na^+ form of the nanotubes (NaT in Figure 2c2) that was not significantly different from the group of the nanofilaments in the H^+ form. However, the toxicity order, that is, that the Na^+ forms were less toxic than the H^+ forms, was also seen in the direct cell counting. The generally larger size of the error bars in Figure 2c2 as compared to Figure 2c1 is the likely explanation why the difference between NaT and the H^+ forms did not reach statistical significance. In summary, the two methods, MTT assay and cell counting, yielded essentially identical results.

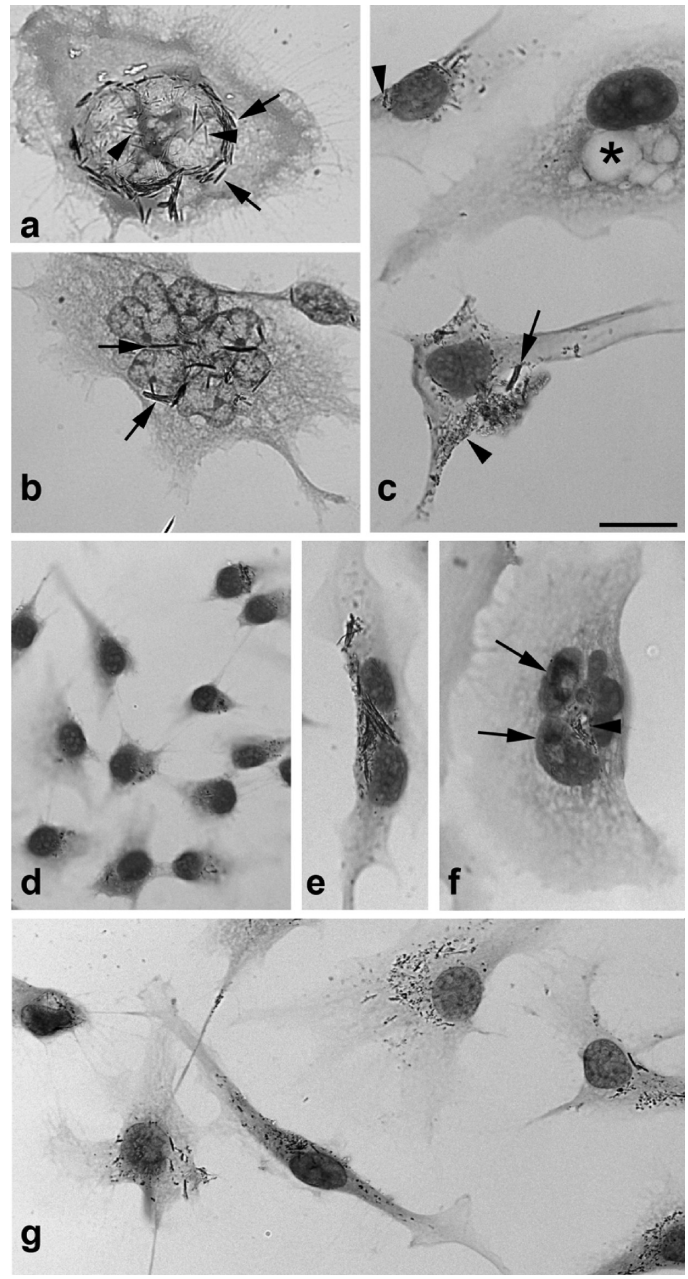

Figure 3. Morphology of H596 lung carcinoma cells exposed to TiO_2-based nanofilaments. Cells were treated for 4 days with 2 μg/mL $H_yTiO_{2+\delta}$ nanotubes (a−f) or $H_yTiO_{2+\delta}$ nanowires (g), fixed and HE-stained. (a) Nanofilaments inside the cells have needle-like structures often concentrated around the enlarged nucleus (arrows), and even thinner nanofilaments are found within the nucleus (arrowheads). (b) In some giant cells, the nuclei are strongly lobulated or possibly fragmented, and the nanofilaments are localized between the lobules (arrows). In (a) and (b), a weaker staining of the nuclei was chosen to better visualize the intranuclear nanotubes. (c) Besides larger fibers (arrow), nanofilaments are also present in the form of small dark particles (arrowheads). In some cells with an altered morphology, vacuole-like structures (*) surrounding the nucleus are seen. (d) A series of cells with small pycnotic nuclei are characterized by the presence of short, granular-like intracytoplasmic nanotubes. (e) An elongated cell with two nuclei is depicted. The needle-like nanotubes are highly concentrated in the region of the two nuclei. (f) A giant cell with several, likely fragmented nuclei also shows multiple nucleoli (dark spots; arrows), and again, the granular nanofilaments are in the center of the nuclear structures (arrowhead). (g) Intracellular distribution of the $H_yTiO_{2+\delta}$ nanowires is not different from the localization of the $H_yTiO_{2+\delta}$ nanotubes (a−f) and also consists of both needle-like and granular nanomaterial. Scale bar in (c): 20 μm.

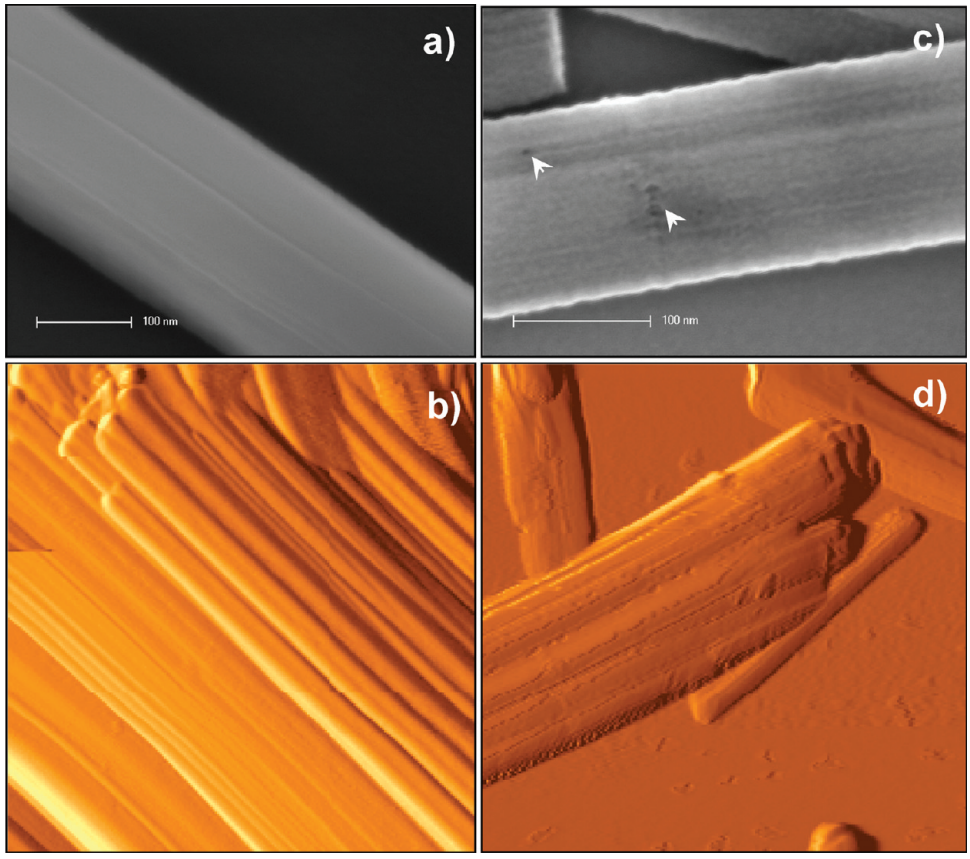

Figure 4. HRSEM and AFM images of $Na_xTiO_{2+\delta}$ (a,b) and $H_yTiO_{2+\delta}$ (c,d) nanowires. During the ionic exchange process, structural point defects as well as extended defects (arrowheads) are induced by the Na^+ and H^+ diffusion within the nanofilament crystal structures. This is confirmed by an increase of the nanowires' surface roughness. $H_yTiO_{2+\delta}$ nanowire surfaces exhibit an average roughness (R_a) of about 2.4 nm in contrast to the smooth surface of the $Na_xTiO_{2+\delta}$ nanowires (R_a = 0.5 nm).

We were also interested whether the TiO_2-based nanofilaments were taken up by the H596 cells or whether their toxic properties were principally mediated by their presence in the cell culture medium. For this, cells were treated for 4 days with the nanofilaments, and the distribution of these compounds was investigated. Clearly, nanofilaments were present inside the treated cells and consisted mainly of rather short (<5 μm) needle-like structures (Figure 3). Qualitatively, the morphological changes in H596 cells treated with the various compounds (2 μg/mL) were very similar; they were most pronounced in cells treated with $H_yTiO_{2+\delta}$ tubes, followed by $H_xTiO_{2+\delta}$ wires, and least in cells treated with $Na_xTiO_{2+\delta}$ wires. Thus, the severity of morphological alterations clearly corresponded to the number/activity of viable cells encountered in the MTT assays or the number of cells directly counted from microphotographs of the HE-stained fixed cells (Figure 2).

Generally the cell−cell contacts were disrupted, particularly in areas comprising cells with large amounts of intracytoplasmic nanofilaments (Figure 3c,d,g). The nanostructures were often clustered and gathered around the cell nucleus (Figure 3a,b,e). Multinucleated giant cells (likely resulting from incomplete mitosis) (Figure 3a,b,f) and apoptotic cells were present in a small percentage (Figure 3d). Apoptotic cells appeared slightly more numerous in cells treated with $Na_xTiO_{2+\delta}$ wires, but we did not attempt to quantify these differences because we estimated these differences likely to be not significant when compared to the other two treatments. Interestingly, we also observed apoptotic cells with no apparent accumulation of nanostructures that could point to an indirect effect possibly due to cytokines or other substances released by the dying cells. Although giant cells can be normally seen in tumor cell lines as a sign of abnormal mitosis, they were more frequently observed in nanofilament-exposed cells and correlated with very strong accumulation of intracytoplasmic nanotubes (Figure 3a,e,f). A moderate anisocaryosis with nucleolus activation was visible and was clearly more pronounced than in the untreated control cells (Figure 3c,f,g). There was some focal intracytoplasmic granular material likely corresponding to aggregates of proteins.

Most of the morphological changes we have observed after the addition of TiO_2-based nanofilaments were similar to the morphological changes induced by carbon-based nanomaterials reported before,[27] but subtle morphological differences specific to each type of substance cannot be excluded. Furthermore, the morphological changes observed in H596 cells treated with the less toxic TiO_2-based fibers in the Na^+ form were not found to be different than the ones seen in cells exposed to fibers in the H^+ form, and typical examples are shown in Supporting Information Figure 5. The main difference was the frequency at which cells with distinct morphological changes were encountered on the glass slides containing the HE-stained cells.

Toxicology studies with asbestos fibers *in vivo* either using inhalation systems or directly injecting fibers into the peritoneal cavity of rodents have demonstrated that the biopersistence of these fibers and their length were important parameters for tumor formation.[31] While fiber length is also important in relation to whether alveolar macrophages are able to take up

these fibers by phagocytosis and thus prevent an accumulation in lung epithelial cells, this appears to be less so for *in vitro* studies using lung tumor cell cultures. In this case, fibers were directly taken up by epithelial cells and uptake was rather efficient with the various materials tested, whether they consisted of carbon-based[27] or TiO_2-based fibers. Although geometry also appears to play a role, that is, the thinner nanotubes both in the H^+ form (Figure 2a,c) and in the Na^+ form (Supporting Information Figure 4) were slightly more toxic than the corresponding nanowires, surface chemistry affected the survival of exposed cells more importantly. In the case of $Na_xTiO_{2+\delta}$, when Na^+ is exchanged for a smaller H^+ ion, the structural imperfections left behind act as chemically active sites (Figure 4) and render the nanostructures more toxic. The importance of surface chemistry had already been observed in a previous report on carbon-based nanomaterials.[27] Even though multiwalled carbon nanotubes have comparable diameter and length as TiO_2 nanofilaments, their toxicity is markedly different. Thus, the chemical composition of nanomaterials also appears to have an effect on cell survival. Whether the toxicity determined in this acute model will also translate to models of chronic toxicity or even tumor development (lung carcinoma or mesotheliomas) needs to be addressed in future studies.

TiO_2 nanoparticles are known to be photosensitizers, generators of free radicals in aqueous media. It is likely that our TiO_2-based nanostructures can also produce reactive oxygen species under illumination. Future studies need to also address the putative phototoxicity of TiO_2-based nanostructures.

EXPERIMENTAL METHODS

The synthesis of the nanofilament was performed by hydrothermal treatment. Nanotubes were prepared by adding 6 g of anatase (Alfa Aesar, 99.9%) to 28 mL of a 15 M NaOH (Aldrich, 99.99%). The mixture was subsequently heated to 150 °C for 72 h. For the synthesis of nanofilaments, a 10 M NaOH solution was used and the temperature was raised to 170 °C, while the amount of anatase and the duration of hydrothermal treatment remained identical. The white precipitate produced was washed with distilled water and dried at 120 °C overnight. The ionic exchange was performed by dispersing, for 2 h, the produced nanofilaments in diluted HCl with a H/Na ratio equal to 100.

The MTT method is based on the accumulation of dark blue formazan crystals inside living cells (but not in dead cells) after their exposure to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). A linear relationship between the number of untreated living cells and the optical density (OD) measured in the MTT assay was established, indicating that the OD values obtained in the MTT assay allowed for an accurate quantification of cell numbers (not shown).

The TiO_2-based nanofilaments were dispersed in a highly diluted gelatin solution to minimize aggregation. Gelatin-containing suspensions of TiO_2-based nanofilaments were stable for more than a month. SEM analysis of nanowires deposited by the boil deposition process onto Si wafers revealed that the nanofilaments were dispersed individually.[32] We had previously demonstrated that the proliferation of H596 cells in a standard medium and in a medium containing the same gelatin concentration as the nanofilament-treated cells was indistinguishable;[27] thus, the addition of gelatin at the concentration used does not affect the cell proliferation. In addition, some of the experiments were repeated using Tween 80 as the dispersing substance, a nondenaturing detergent previously used in several toxicity studies of nanomaterials.[33] Results are shown in the Supporting Information Figure 4.

For the direct counting, H596 cells were seeded on round laminin-coated glass covers, exposed to nanomaterials (2 μg/mL) for 4 days, fixed, and stained with HE (hematoxylin and eosin). Microphotographs (20× magnification) of the stained cells were acquired by a systematic random approach similar to the one described before.[34] Briefly, a region of the round glass cover close to the periphery was randomly selected, and on the acquired image, the numbers of cells were counted. On the basis of this initial starting point, the other three regions of the glass cover to be analyzed were determined by a predefined L-shaped scheme. This prevented a bias toward selecting particular regions on the glass covers. The acquired images were randomly numbered and analyzed by the experimenter not knowing the key until the final calculations. From each glass cover, the number of cells (determined by counting the dark stained nuclei) on each of the four images were added, yielding one value per glass cover. Results are presented in Figure 2c.

Acknowledgment. The work in Lausanne was supported by the Swiss National Science Foundation and its NCCR "Nanoscale Science". We thank the Centre Interdisciplinaire de Microscopie Electronique (CIME) for access to electron microscopes and technical support. L.H. thanks for the support of the Swiss National Foundation (Project Number 205321-125299/1).

Supporting Information Available: Chemical and structural characterization of TiO_2-based nanofilaments. Growth curve of H596 cells in the presence of TiO_2-based nanofilaments in the presence of dispersing agent Tween 80. Morphology of H596 lung carcinoma cells treated with TiO_2-based nanofilaments in the Na^+ forms. This material is available free of charge *via* the Internet at http://pubs.acs.org.

REFERENCES AND NOTES

1. Iijima, S. Helical Microtubules of Graphitic Carbon. *Nature* **1991**, *354*, 56–58.
2. Helland, A.; Wick, P.; Koehler, A.; Schmid, K.; Som, C. Reviewing the Environmental and Human Health Knowledge Base of Carbon Nanotubes. *Environ. Health Perspect.* **2007**, *115*, 1125–1131.
3. Lam, C. W.; James, J. T.; McCluskey, R.; Hunter, R. L. Pulmonary Toxicity of Single-Wall Carbon Nanotubes in Mice 7 and 90 Days after Intractracheal Instillation. *Toxicol. Sci.* **2004**, *77*, 126–134.
4. Warheit, D. B.; Laurence, B. R.; Reed, K. L.; Roach, D. H.; Reynolds, G. A. M.; Webb, T. R. Comparative Pulmonary Toxicity Assessment of Single-Wall Carbon Nanotubes in Rats. *Toxicol. Sci.* **2004**, *77*, 117–125.
5. Shvedova, A. A.; Castranova, V.; Kisin, E. R.; Schwegler-Berry, D.; Murray, A. R.; Gandelsman, V. Z.; Maynard, A.; Baron, P. Exposure to Carbon Nanotube Material: Assessment of Nanotube Cytotoxicity Using Human Keratinocyte Cells. *J. Toxicol. Environ. Health A* **2003**, *66*, 1909–1926.
6. Maynard, A. D.; Baron, P. A.; Foley, M.; Shvedova, A. A.; Kisin, E. R.; Castranova, V. Exposure to Carbon Nanotube Material: Aerosol Release during the Handling of Unrefined Single-Walled Carbon Nanotube Material. *J. Toxicol. Environ. Health A* **2004**, *67*, 87–107.
7. Monteiro-Riviere, N. A.; Nemanich, R. J.; Inman, A. O.; Wang, Y. Y.; Riviere, J. E. Multi-walled Carbon Nanotube Interactions with Human Epidermal Keratinocyte. *Toxicol. Lett.* **2005**, *155*, 377–384.

8. Panessa-Warren, B. J.; Warren, J. B.; Wong, S. S.; Misewich, J. A. Biological Cellular Response to Carbon Nanoparticle Toxicity. *J. Phys.: Condens. Mater.* **2006**, *18*, S2185–S2201.
9. Stern, S. T.; McNeil, S. E. Nanotechnology: Safety Concerns Revisited. *Toxicol. Sci.* **2008**, *101*, 4–21.
10. Lewinski, N.; Colvin, V. L.; Drezek, R. Cytotoxicity of Nanoparticles. *Small* **2008**, *4*, 26–49, and references therein.
11. Miserocchi, G.; Sancini, G.; Mantegazza, F.; Chiappino, G. Translocation Pathways for Inhaled Asbestos Fibers. *Environ. Health* **2008**, *7*, 4.
12. Rao, C. N. R.; Nash, M. Inorganic Nanotubes. *Dalton Trans.* **2003**, 1–24.
13. Patzke, G. R.; Krumeich, F.; Nesper, R. Oxidic Nanotubes and Nanorods—Anisotropic Modules for a Future Nanotechnology. *Angew. Chem., Int. Ed.* **2002**, *41*, 2446–2447.
14. Rao, C. N. R.; Vivecchand, S. R. C.; Biswas, K.; Govindaraj, A. Synthesis of Inorganic Nanomaterials. *Dalton Trans.* **2007**, 3728–3749.
15. Mills, A.; Davies, R. H.; Worsley, D. Water-Purification by Semiconductor Photocatalysis. *Chem. Soc. Rev.* **1993**, *22*, 417–425.
16. Gueneau-Rancurel, L. Chemistry for Architecture: The Self-Cleaning Glass. *Act. Chim.* **2007**, *311*, 6–10.
17. Lomer, M. C. E.; Thompson, R. P. H.; Powell, J. J. Fine and Ultrafine Particles of the Diet: Influence on the Mucosal Immune Response and Association with Crohn's Disease. *Proc. Nutr. Soc.* **2002**, *61*, 123–130.
18. Dransfield, G. P. Inorganic Sunscreens. *Radiat. Prot. Dosim.* **2000**, *91*, 271–273.
19. Shankar, K.; Bandara, J.; Paulose, M.; Wietasch, H.; Varghese, O. K.; Mor, G. K.; LaTempa, T. J.; Thelakkat, M.; Grimes, C. A. Highly Efficient Solar Cells Using TiO_2 Nanotube Arrays Sensitized with a Donor Antenna Dye. *Nano Lett.* **2008**, *8*, 1654–1659.
20. Park, J. H.; Kim, S.; Bard, A. J. Novel Carbon Doped TiO_2 Nanotube Arrays with High Aspect Ratios for Efficient Solar Water Splitting. *Nano Lett.* **2006**, *6*, 24–28.
21. Nohynek, G. J.; Lademann, J.; Ribaud, C.; Roberts, M. S. Grey Goo on the Skin? Nanotechnology, Cosmetic and Sunscreen Safety. *Crit. Rev. Toxicol.* **2007**, *37*, 251–277.
22. Fabian, E.; Landsiedel, R.; Ma-Hock, L.; Wiench, K.; Wohlleben, W.; van Ravenzwaay, B. Tissue Distribution and Toxicity of Intravenously Administered Titanium Dioxide Nanoparticles in Rats. *Arch. Toxicol.* **2008**, *82*, 151–157.
23. Vileno, B.; Lekka, M.; Sienkiewicz, A.; Jeney, S.; Stoessel, G.; Lekki, J.; Forró, L.; Stachura, Z. Stiffness Alterations of Single Cells Induced by UV in the Presence of NanoTiO(2). *Environ. Sci. Technol.* **2007**, *41*, 5149–5153.
24. Warheit, D. B.; Hoke, R. A.; Finlay, C.; Donner, E. M.; Reed, K. L.; Sayes, C. M. Development of a Base Set of Toxicity Tests Using Ultrafine TiO_2 Particles as a Component of Nanoparticle Risk Management. *Toxicol. Lett.* **2007**, *171*, 99–110, and references therein.
25. Armstrong, A. R.; Armstrong, G.; Canales, J.; Bruce, P. G. TiO_2-B Nanowires. *Angew. Chem., Int. Ed.* **2004**, *43*, 2286–2288.
26. Armstrong, G.; Armstrong, A. R.; Canales, J.; Bruce, P. G. Nanotubes with the TiO_2-B Structure. *Chem. Commun.* **2005**, *19*, 2454–2456.
27. Magrez, A.; Kasas, S.; Salicio, V.; Pasquier, N.; Seo, J. W.; Celio, M.; Catsicas, S.; Schwaller, B.; Forró, L. Cellular Toxicity of Carbon-Based Nanomaterials. *Nano Lett.* **2006**, *6*, 1121–1125.
28. Worle-Knirsch, J. M.; Pulskamp, K.; Krug, H. F. Oops They Did It Again! Carbon Nanotubes Hoax Scientists in Viability Assays. *Nano Lett.* **2006**, *6*, 1261–1268.
29. Kaiser, J.-P.; Wick, P.; Manser, P.; Spohn, P.; Bruinink, A. Single Walled Carbon Nanotubes (SWCNT) Affect Cell Physiology and Cell Architecture. *J. Mater. Sci. Mater. Med.* **2008**, *19*, 1523–1527.
30. Contreras, R.; Sahlin, H.; Frangos, J. A. Titanate Biomaterials with Enhanced Anti-inflammatory Properties. *J. Biomed. Mater. Res. A* **2007**, *80*, 480–485.
31. Miller, B. G.; Searl, A.; Davis, J. M.; Donaldson, K.; Cullen, R. T.; Bolton, R. E.; Buchanan, D.; Soutar, C. A. Influence of Fibre Length, Dissolution and Biopersistence on the Production of Mesothelioma in the Rat Peritoneal Cavity. *Ann. Occup. Hyg.* **1999**, *43*, 155–166.
32. Lee, K.; Duchamp, M.; Kulik, G.; Magrez, A.; Seo, J. W.; Jeney, S.; Kulik, A. J.; Forró, L. Uniformly Dispersed Deposition of Colloidal Nanoparticles and Nanowires by Boiling. *Appl. Phys. Lett.* **2007**, *17*, 173112.
33. Wick, P.; Manser, P.; Limbach, L. K.; Dettlaff-Weglikowska, U.; Krumeich, F.; Roth, S.; Stark, W. J.; Bruinink, A. the Degree and Kind of Agglomeration Affect Carbon Nanotube Cytotoxicity. *Toxicol. Lett.* **2007**, *168*, 121–131.
34. Maetzler, W.; Nitsch, C.; Bendfeldt, K.; Raclay, P.; Vollenweider, F.; Schwaller, B. Ectopic Parvalbumin Expression in Mouse Forebrain Neurons Increases Excitotoxic Injury Provoked by Ibotenic Acid Injection into the Striatum. *Exp. Neurol.* **2004**, *186*, 78–88.