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Hydrophilic/hydrophobic features of TiO_2 nanoparticles as a function of crystal phase, surface area and coating, in relation to their potential toxicity in peripheral nervous system

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ABSTRACT

The hydrophilic/hydrophobic properties of a variety of commercial TiO_2 nanoparticles (NP), to be employed as inorganic filters in sunscreen lotions, were investigated both as such (dry powders) and dispersed in aqueous media. Water uptake and the related interaction energy have been determined by means of adsorption microcalorimetry of H_2O vapor, whereas dispersion features in aqueous solutions were investigated by dynamic light scattering and electrokinetic measurements (zeta potential). The optimized dispersions in cell culture medium were employed to assess the possible *in vitro* neuro-toxicological effect on dorsal root ganglion (DRG) cells upon exposure to TiO_2-NP, as a function of crystal phase, surface area and coating. All investigated materials, with the only exception of the uncoated rutile, were found to induce apoptosis on DRG cells; the inorganic/organic surface coating was found not to protect against the TiO_2-induced apoptosis. The risk profile for DRG cells, which varies for the uncoated samples in the same sequence as the photo-catalytic activity of the different polymorphs: anatase–rutile > anatase ≫ rutile, was found not to be correlated with the surface hydrophilicity of the uncoated/coated specimens. Aggregates/agglomerates hydrodynamic diameter was comprised in the ~200–400 nm range, compatible with the internalization within DRG cells.

1. Introduction

Titanium dioxide is present in nature mainly in three crystalline forms: rutile, anatase and brookite [1]. Thanks to its unique properties [2,3], TiO_2 is widely used in industry in a variety of applications: (i) as white pigment in paints, food and pharmaceutics; (ii) as heterogeneous photo-catalyst to remediate polluted soil, aqueous and atmospheric ecosystems; (iii) to make self-cleaning surfaces; and (iv) as UV blockers in sunscreen lotions. An almost exhaustive list of applications of TiO_2 materials is reported by Carp et al. [1]. It has been also reported that a combination of anatase and rutile polymorphs (e.g., P25 Aeroxide from Degussa) exhibits enhanced photo-catalytic features [2,4–8].

As for the use in cosmetics applications, TiO_2 powders have been considered since the early fifties [9], as excellent candidates to act as physical filters in sunscreen lotions, because of their absorption/scattering properties and cosmetic acceptability [3]. The required feature for inorganic filters is to screen/block UV solar radiation over the whole UVA/UVB range, while the cosmetic acceptability of sunscreens requires the employment of powders opaque to ultraviolet but transparent to visible radiation. In order to fulfill this latter requirement, TiO_2 powders for sunscreen lotions are preferentially made up of primary nanoparticles the optimal size of which is comprised in the 20–50 nm range [3,10,11].

It is generally accepted that the biological activity of TiO_2 is enhanced by sunlight exposure [3,10,12–15], in that TiO_2 is photo-activated by UV radiation generating radicals such as $^{\cdot}OH$ and $O_2^{\cdot-}$, and other reactive oxygenated species (ROS), such as H_2O_2 and singlet oxygen [3,10,16–18]. For instance, photo-generated ROS and $^{\cdot}OH$ radicals are reported to be responsible for TiO_2-NP toxicity in eukaryotic and bacterial cells [19,20], $^{\cdot}OH$ radicals for lipid peroxidation, membrane destabilization and eventually cell death [21]. It is, however, worth mentioning that radical reactions potentially leading to biomolecules oxidative damage have been reported to occur at the TiO_2 surface also in the absence of UV illumination [20,22].

Micrometric-sized TiO_2 is generally considered a safe material, and it has been largely employed as a negative control in experi-

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mental studies aimed at assessing the toxicity of mineral dusts [23]. Moreover, thanks to their biocompatibility with the human body, TiO_2-based biomaterials are also largely employed as bone substitutes and reinforcing mechanical supports [24–26].

Conversely, in recent years, some concern has progressively arisen over the adverse biological effects, with potential health damage risk to humans, caused by the exposure to nanometric-sized TiO_2 particles (TiO_2-NP). In fact, the biological effects caused by finely divided solid materials depend on their surface chemistry, which in turn depends upon the "form" of the particulate, including crystal phase and morphology [27]. In particular, in TiO_2 case, the size of primary particles and the amount of structural defects, which increases as far as the size of the particles decreases [1,2,28–30], are expected to play a major role in both chemical and biological reactivity of the sample.

Several papers have been published over the years regarding the nanosized TiO_2 particles toxicity [31,32], but the results are still controversial. In particular, (i) nanosized TiO_2 particles under illumination are strong oxidizing agents, capable of reacting with a wide range of organic and biological molecules [3,10] and are expected to be more toxic than the (biologically inert) micron-sized TiO_2 particles [31–34]; (ii) epithelial cells oxidative damage and lipid peroxidation in experiments conducted in the dark were found to depend upon crystal phase and particles size [35,36]; (iii) cellular behavior is influenced by both TiO_2 crystal phase and morphology: anatase is more toxic than rutile, and the reactivity of both increases with increasing exposed surface area [17,20,22,37–39]; (iv) according to Fenoglio et al., anatase samples made up of either micron- or nanosized particles, but exposing the same total surface area, were found to react similarly in free-radical generation [17,22]. This datum does suggest that the reduction in the particle size to nanometric scale does not generate *per se* a new kind of reactive TiO_2 surface sites; and (v) in summary, Warheit et al. [39] claim that specific surface chemistry properties of the different forms of TiO_2 are determinant in developing toxicity.

TiO_2-NP for cosmetic applications are often coated with either organic (e.g., silane) or inorganic (e.g., alumina, silica) materials, which are expected to reduce the oxide photoreactivity [17,40], and, mostly in the case of silane-coated TiO_2, to enhance the compatibility with the lipophilic components of the cosmetic preparations. As a consequence of the changed physico-chemical features of the coated TiO_2-NP surface, the biological activity is expected to be dramatically different from that of the uncoated TiO_2-NP [31,32].

As for the interaction of the (nano)particles with living bodies, it is worth mentioning that it is generally accepted that sunscreen lotions TiO_2-NP do not penetrate the stratum corneum of the intact skin [41]. However, it cannot be definitely discarded the possibility for NPs to penetrate sunburnt, broken or abraded skin [31,32], to translocate within the body and to interact with nerve terminals of sensory neurons [42–44]. On the other hand, some authors [41] claim that also hairy skin follicles allow NPs to penetrate the skin. Also in this latter case, a fraction of NPs may reach the sensory endings. An interesting and instructive review on nanoparticle functionality and toxicity on the central nervous system is reported by Yang and coauthors [45]. Very little is known on TiO_2-NP neurotoxicity [46], and, to the best of our knowledge, virtually nothing is reported about the effects on sensory neurons, the nerve endings of which are considered potential targets of TiO_2 nanoparticles present in cosmetic preparations.

On the other hand, the affinity toward water, which is ubiquitous in physiological fluids, is expected to influence the interaction between inorganic (nano)particles and cells [27,47,48]. (Nano)materials in contact with physiological fluids create a (nano)-bio-interface, as described by Nel et al. [49], which comprises the following: (i) the primary nanoparticle surface, the features of which depend on chemical composition, crystal structure and morphology of the solid material; (ii) the solid–liquid interface, which is influenced by the chemical composition of the aqueous medium leading to the primary particles aggregation; and (iii) the solid–liquid interface in the proximity of the biological substrate region, made up of (nano)particles aggregates interacting with the cell membrane components.

The main issues of the present work are to describe the hydrophilic/hydrophobic properties of a variety of commercial TiO_2-NP (to be employed as inorganic filters in sunscreen lotions), both as such (dry powder) and dispersed in aqueous media, and to assess whether the investigated surface features of the materials are somehow correlated with their *in vitro* possible neuro-toxicological effects. Water uptake and the related energy of interaction with surface sites have been determined by means of adsorption microcalorimetry of H_2O vapor. Dispersion features in different aqueous media have been carefully investigated in order to optimize the TiO_2-NP dispersions, to be employed in experiments aimed at evaluating dorsal root ganglion (DRG) cells survival when exposed to TiO_2-NP.

2. Experimental

2.1. Materials

2.1.1. TiO_2 specimens

All TiO_2 specimens, with the exception of one sample synthesized *ad hoc,* are commercial samples that were kindly supplied by different companies (*vide infra*). In Table 1, the uncoated and

Table 1

Uncoated TiO_2 samples: (i) anatase (A8) provided by A.C.E.F. (Piacenza, Italy); (ii) non-commercial sol gel anatase (A72) as a model system; (iii) MT 500 B pure rutile (R) provided by LCM-Trading (Milano, Italia); (iv) Aeroxide P25 pyrogenic anatase/rutile TiO_2 (AR) produced by Degussa–Evonik Goldschmidt (Frankfurt, Germany). *Coated TiO_2 samples*: (i) Max Light F-TS20 (A-S), supplied by Showa Denko K.K. (Tokyo, Japan); (ii) Eusolex T AVO (R-S) supplied by Merck; (iii) UV-Titan M262 (R-Alu-D), supplied by Kemira (Helsinky, Finland); (iv) PW Covasil S1 (AR-SL1), supplied by LCW-Sensient (Saint Ouen L'Aumone, France); (v) Tego Sun T805 (AR-SL2), produced by Degussa–Evonik Goldschmidt; (vi) Tego Sun TS Plus (AR-SL2-S), produced by Degussa–Evonik Goldschmidt. TMCS: tri-methoxy-caprylyl-silane. The symbols (A) and (R) in the fourth column refer to anatase and rutile polymorphs crystallites, respectively; ur = unresolved, due to the low intensity of the corresponding diffraction pattern.

Commercial name	Nomenclature	Polymorph	Crystallites size (nm)	SSA_{BET} (m^2/g)	Coating
Anatase ACEF	A8	Anatase	215 ± 17 (A)	8	–
Sol gel anatase	A72	Anatase	11 ± 1 (A)	72	–
MT 500 B	R	Rutile	31 ± 2 (R)	39	–
Aeroxide P25	AR	Anatase 80%; rutile 20%	30 ± 4 (A); 50 ± 6 (R)	53	–
Max Light F-TS20	A-S	Anatase	14 ± 1 (A); ur (R)	55	SiO_2 21%
Eusolex T AVO	R-S	Rutile	26 ± 2 (R)	63	SiO_2 20%
UV-Titan M262	R-Alu-D	Rutile	24 ± 4 (R)	58	Al_2O_3 5.9% Dimethicone 1.2%
PW Covasil S1	AR-SL1	Anatase 80%; rutile 20%	26 ± 1 (A); 44 ± 8 (R)	43	TMCS 5–2%
Tego Sun T805	AR-SL2	Anatase 80%; rutile 20%	21 ± 2 (A); 20 ± 3 (R)	47	TMCS 1–5%
Tego Sun TS Plus	AR-SL2-S	Anatase 80%; rutile 20%	19 ± 4 (A); 34 ± 3 (R)	60	TMCS 1–5% SiO_2 10–25%