

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

**INVESTIGATION OF MICRONIZED TITANIUM DIOXIDE
PENETRATION IN HUMAN SKIN XENOGRAFTS AND
ITS EFFECT ON CELLULAR FUNCTIONS**

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INTRODUCTION AND AIMS

Introduction

Titanium dioxide (TiO_2) is an ubiquitously used material in everyday life. It can be found in white paints, household products, plastic goods, medications, orthopedic implants, etc. However, despite its wide array of common applications, its pathogenetic role was also suggested under certain conditions. For example, some lung diseases have already been associated to TiO_2 exposure: it has been shown that inhaled inorganic particles can induce alveolar inflammation, fibrosis, and have a pathogenetic role in pneumoconiosis and related entities. TiO_2 particles, especially the ultrafine (nanometer range) ones, have also been demonstrated to cause lung cancer in rodents. After the administration of particles to animals by inhalation, ultrafine particles were identified in the cytoplasm of all types of cells of the lung tissue in a non-membrane-bound manner, which finding was also reproduced in cell culture. Other authors suggested TiO_2 uptake to alveolar macrophages via MARCO (macrophage receptor with collagenous structure), in an unopsonized manner.

From a dermatological point-of-view, it is also of great importance that TiO_2 also serves as a physical photoprotective agent and is widely used in various cosmetic products. Here, TiO_2 particles act by scattering sunlight from the skin surface, in contrast to chemical sunscreens that transform the energy of the photons into molecular conformational changes. Although the cosmetical appearance of early physical sunscreens was inferior to chemical ones during its application, TiO_2 is widely used because of its very low allergizing potential compared to chemical UV filters. Moreover, the reduction of TiO_2 particle size to the ultrafine, nanometric range resulted in a better cosmetical appearance and an increased photoprotective effect, making it more acceptable for consumers.

However, it should also be mentioned (possibly also reflecting the above harmful effects) that TiO_2 also possesses a photocatalytic effect; namely, when irradiated by UV light, TiO_2 catalyses the formation of superoxide and hydroxyl radicals on the surface of the particles in aqueous environments. These reactive oxygen species (ROS) cause oxidative DNA damage (8) and may alter cellular functions by causing mutations that

result in cell death or proliferative disorders as a consequence of the loss of cell cycle control. Importantly, it has been suggested that ultrafine particles are more likely to induce inflammation than bigger particles as they have better diffusion capacity. Moreover, the relatively larger specific surface of nanoparticles enables them to react with a higher number of molecules than fine particles, thus producing more ROS.

Up to this moment, no definite pathogenetic role is attributed to TiO₂ in skin diseases. It is believed that this is due to the fact that the matured and cornified keratinocytes of the epidermal stratum corneum (unlike the vulnerable alveolar surface) is an effective barrier against these particles and hence may prevent the *in vivo* penetration and putative cellular effects of TiO₂ particles on the cell populations of the living cell layers.

However, as TiO₂ is the component of many sunscreen products, micronized particles are very often applied under such conditions (e.g. “soaked” skin, sunburned skin) where the integrity of epidermis is impaired. Therefore, the above photocatalytic effect can occur within the “living” layers of the skin itself and hence may potentially damage various populations of skin cells. In addition, although sunscreen manufacturers use different surface coatings to outweigh photocatalytic effect of the particles, it can also be assumed that TiO₂ particles can be internalized by skin cells where they might exert a direct toxic effect and hence further dysregulate the complex mechanism of epidermal barrier formation.

Therefore, there is an emerging demand for the investigation of the barrier function of the epidermis against the topically used TiO₂ particles. Previously, only very few experiments were carried out on the penetration of small-particle-size ultrafine TiO₂ through this cornified envelope, as earlier techniques – such as TEWL (transepidermal water loss) measurement, , tape stripping combined with electron microscopy (EM) and energy dispersing X-ray analysis – were difficult to apply in microelement penetration studies.

Recently, however, the nuclear microscopy methods, such as PIXE (Particle Induced X-ray Emission) STIM (Scanning Transmission Ion Microscopy) and RBS (Rutherford backscattering) have already been established by nuclear physics laboratories to investigate the elemental composition of the skin. In this work, employing the above

techniques, we present an *in vivo* penetration study on skin barrier function using human skin transplanted in severe combined immunodeficiency (SCID) mice. In addition, we also aimed to investigate the effect of nanoparticles on various functional properties of numerous epidermal and dermal cells in culture. Hereby, we demonstrate that nanoparticles *in vivo* do not penetrate through the intact epidermal barrier. However, when TiO₂ is exposed directly to cell cultures *in vitro*, it exerts significant effects on such cellular functions as viability, proliferation, apoptosis, and differentiation.

This work was supported by “NANODERM” EU 5th Framework Program Project (“Quality of skin as a barrier to ultrafine particles”, QLK4-CT-2002-02678), in order to get quantitative information on the penetration of ultrafine particles in all strata of skin as well as their impact on human health.

Aims

1. Is the human epidermal xenograft - severe combined immunodeficiency (SCID) transplant model suitable for ion beam analysis? Are there any similarities between the elemental composition of the epidermis of the human xenografts and the previously studied skin models? In the first phase of our experiments, we aimed to investigate the elemental structure of the human skin xenografts transplanted onto SCID mice using TEM (transmission electron microscopy), ion beam analysis and routine histology. The results were compared to previously published data obtained from healthy volunteers.

2. In the next phase of our experiments, we were eager to find out whether the micronized titanium dioxide penetrates the intact epidermal barrier. For this reason, we carried out an *in vivo* penetration study on the SCID mouse-human epidermal xenograft model using the above detailed techniques, conducted in parallel in different laboratories.

3. Finally, we were eager to find out whether the titanium dioxide nanoparticles exert a toxic effect (as earlier described in pulmonary models), if the direct contact with the skin cells occur. To determine the cellular effects of the

potentially penetrated nanoparticles that can directly contact skin cells, we examined the viability, apoptosis and differentiation markers of epidermal and dermal skin cell cultures exposed to titanium dioxide nanoparticles.

MATERIALS AND METHODS

Titanium dioxide formulation

For the penetration studies, a commercially available sunscreen product containing micronized titanium dioxide in a hydrophobic emulsion („TiO₂-emulsion”) was used (Anthelios XL SPF 60, La Roche Posay, La Roche Posay, France).

For experiments in cell culture, the anatase powder form (with a diameter of 9 nm) dispersed in culture medium was employed (obtained as a generous gift from Prof. Z. Stachura, Krakow, Poland).

Animals

All animal experiments were performed as accepted by our institutional ethical committee. Experiments on SCID mice were carried out at the Department of Dermatology, University of Debrecen, Medical and Health Science Center (UDMHSC, Debrecen, Hungary) under serum pathogen free conditions. Human foreskin grafts were obtained from circumcision and transplanted onto the backs of SCID-mice as described before by Jánossy and colleagues. The grafts on the mice were treated with TiO₂-emulsion at 2 mg/cm² concentration in occlusion for 24 hours along with untreated control. After exposure time, animals were euthanized. From each graft area, 6 mm diameter skin punch biopsies were taken.

Nuclear microanalysis

Skin punch biopsies were quench frozen by immersing into liquid isopentane chilled by liquid nitrogen. The biopsies were sectioned into 14 µm thick sections at

– 25°C, and, after freeze-drying, sections were brought to room temperature. Adjoining sections were provided for microprobe analysis and were stained with haematoxylin–eosin (HE) for histological examination. Sections aimed for nuclear microprobe analysis were mounted on the sample holders in self-support mode glued by the edges on carbon tape strips.

The nuclear microanalyses were performed on the Debrecen scanning ion microprobe at the Institute of Nuclear Research of the Hungarian Academy of Sciences, Debrecen, Hungary. To analyze the samples, the bio-PIXE setup was used applying combined ion beam analytical techniques as described earlier. In brief, samples were irradiated with a proton beam focused to 2µm x 2µm, and the investigated area was scanned systematically. The morphology and the area density of the samples were determined by Scanning Transmission Ion Microscopy (STIM), while elemental concentrations and maps (for elements $Z > 5$) were obtained from the emitted characteristic X-ray spectra, using Particle Induced X-ray Emission (PIXE) method. A few additional measurements were carried out in the laboratory of Ph. Moretto (Bordeaux, France) and on the Ljubljana scanning ion microprobe at the Jožef Stefan Institute, Ljubljana, Slovenia.

Transmission electron microscopy

Skin biopsies (6mm in diameter) were chemically fixed during 1h at 4°C in 2.5% glutaraldehyde. After rinsing the samples twice with 0.1M sodium cacodylate buffer (pH 7.4 and 5 min for each rinsing), post-fixation was performed for 2h at room temperature using a 1% osmium tetroxide solution in 0.1M sodium cacodylate buffer at pH 7.4. After two 5 min additional rinsing steps in the same type of buffer, dehydration was carried out in an ethanol series at room temperature. Then the specimens were immersed twice in propylene oxide (30 min each step) before embedding them in a mixture (1:1) of propylene oxide and Epon 812 for 2h and then in pure Epon for 6h. Polymerisation took place at 60°C overnight.

Either semi-thin (500 nm) or ultra-thin (50-100 nm) sections were cut from the embedded skin samples using an ultramicrotome equipped with a 35° diamond knife. The cutting direction was from inside to outside in order to avoid contaminations from

residual formulations residing on top of the SC. Semi-thin sections were collected on microscopy slides. Afterwards, sections were stained with toluidine blue for examination under a light microscope. Ultra-thin sections were collected on formvar/carbon coated copper grids, counterstained with lead-citrate and uranyl-acetate and were investigated in a transmission electron microscope with an 80kV acceleration voltage.

Cell culture

The human immortalized HaCaT keratinocyte cell line was a kind gift of Prof. N. E. Fusenig. Cells were cultured in 25-cm² or 75-cm² tissue culture flasks (if not indicated otherwise) in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS), 2 mM/L-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 1.25 µg/ml fungizone at 37°C in a 5% CO₂ atmosphere.

Human dermal fibroblasts (HDFs) were obtained from de-epidermized dermis using enzymatic digestion as described before and were cultured in the above supplemented DMEM.

The human immortalized sebaceous gland cell line SZ95 was cultured in Sebomed[®] medium supplemented with 10% FCS, 1 mM CaCl₂, 5 ng/ml human recombinant epidermal growth factor, 50 U/ml penicillin, 50 µg/ml streptomycin.

The primary human melanocytes were cultured in DMEM supplemented with 10% FCS, 2 mM/L-glutamine, 50 U/ml penicillin, 50 µg/ml streptomycin, 1.25 µg/ml fungizone.

Determination of cellular proliferation

The number of viable cells was determined by measuring the conversion of the tetrazolium salt MTT to formazan. Cells were plated in 96-well multiter plates (5000 cells/well density) in quadruplicates and were treated with different concentrations of TiO₂ for the time indicated. Cells were then incubated with 0.5 mg/ml MTT for 2 hrs, and the concentration of formazan crystal (as the indicator of number of viable cells) was determined colorimetrically according to the manufacturer's protocol. Data are expressed as mean +/- SEM.

Determination of apoptosis

Cells were collected by trypsinization and were incubated with 1 ml fluorescein isothiocyanate (FITC)-conjugated annexin V for 10 min in darkness. Cells were then measured by a flow cytometer and the percentage of apoptotic cells compared to total cell number was determined.

Western blot analysis

Cells were washed with ice-cold phosphate-buffered saline (PBS), harvested in homogenization buffer and disrupted by sonication on ice. The protein content of samples was measured by a modified BCA protein assay. The samples were subjected to SDS-PAGE (8% gels were loaded with 20–30 mg protein per lane) and transferred to nitrocellulose membranes. Membranes were then blocked with 5% dry milk in PBS and probed with the appropriate primary mouse antibodies against the differentiation marker involucrin or the cell adhesion molecules desmoglein-1 and P-Cadherin. A peroxidase-conjugated goat anti-mouse secondary antibody was then applied and the immunoreactive bands were visualized by an ECL Western blotting detection kit on light-sensitive films. To assess equal loading, nitrocellulose membranes were stripped in 200 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 2% SDS and 0.1% β -mercaptoethanol at 65°C for 1 h and were re-probed with a mouse anti-cytochrome-c antibody followed by a similar visualization procedure as described above.

Calcium imaging

Changes in intracellular calcium concentration ($[Ca^{2+}]_i$) were detected as described in our earlier report. In brief, a calcium sensitive probe was introduced into the intracellular space by incubating the keratinocytes with 5 μ M fura-2 AM for 1 h at 37°C. Before each measurement, the cells were kept at room temperature (22–24°C) in normal Tyrode's solution for a half an hour to allow homogeneous distribution of the dye. The coverslips, containing the fura-2 loaded cells, were then placed on the stage of an inverted fluorescence microscope. Excitation was altered between 340 and 380 nm using a dual wavelength monochromator. The emission was monitored at 510 nm with a

photomultiplier at an acquisition rate of 10 Hz per ratio, and the fluorescence ratio (F_{340}/F_{380}) values were determined.

RESULTS

TiO₂ particles do not penetrate through the stratum corneum of human skin transplants

To check if nanoparticles reach the living cells (i.e. stratum granulosum, spinosum, and basale) of the intact skin *in vivo*, human skin xenografts were treated with TiO₂ emulsion for 1, 24, 48 hours using occlusive bandage (to facilitate the putative penetration). Biopsies were then taken from the treated regions ion microscopy and TEM was employed to determine the penetration depth of TiO₂ through various layers of the skin.

We first verified whether the elemental composition of the xenograft samples correspond to that previously described on human skin samples obtained from healthy volunteers. It is generally accepted that mass distribution maps and spectra, obtained using nuclear microscopy, represent the organic mass of skin (which can be compared to HE stained sections) and, furthermore, that the distribution of organic mass and single elements vary in function of the depth and skin layers. For example, the increased organic mass, sulphur, sodium peak and high calcium levels are characteristic of the stratum corneum. Similarly, the high phosphorus levels indicate the layer of stratum granulosum whereas other layers also represent characteristic elemental distribution patterns. Using ion beam analysis on non-treated (hence control) skin punch biopsy samples, we found that the stratum corneum and stratum granulosum can be identified due to their clearly distinguishable elemental distribution spectra. These elemental distribution patterns and elemental maps were completely identical to those of other skin models earlier demonstrated. Importantly, no TiO₂ signal was detected in the non-treated samples.

Subsequently, skin elemental composition pattern was obtained after TiO₂ treatment for 1, 24,48 hours. The TiO₂ signal peak was detected at the same areas as the high concentrations of sulphur, potassium, calcium elements, characteristic features of the elemental composition of the stratum corneum. Notably, no TiO₂ signal was identified in the “deeper” layers of the skin after 24 hours of titanium-dioxide exposition.

After treatment with nanoparticles in occlusion for 48 hours, a penetration of particles was detected in the deeper layers of stratum corneum, not reaching the stratum granulosum. The same results were obtained with TEM analysis. This finding also correlates with our previous results on other skin types and also those of other PIXE groups. We can conclude, therefore, that TiO₂ nanoparticles do not reach “living” cell layers in human skin xenografts.

TiO₂ nanoparticles are internalized by in vitro cultured fibroblasts and melanocytes but not by keratinocytes and sebocytes

The above findings clearly demonstrated that the stratum corneum functions as an effective barrier against micronized TiO₂ in intact skin. Very often, however (as introduced above), TiO₂ exposure occurs under such conditions where the skin barrier function is impaired (e.g., sunburn, micro-injured or “soaked” skin) and hence TiO₂ may directly “contact” the skin cells. Therefore, in the next phase of our experiments, we intended to define the direct effect of TiO₂ on various cellular functions of several cultured human skin-derived cell types.

We first determined whether skin cells are able to internalize the TiO₂ nanoparticles. TiO₂ (15 µg/cm²) was added to non-confluent cell cultures of keratinocytes, sebocytes, melanocytes, and fibroblasts for 24 hours and then internalization was visualized by phase contrast microscopy. Particles were observed in the cytoplasm of fibroblasts and melanocytes where an enrichment was seen in the perinuclear area leaving the nucleus free of particles. Interestingly, however, there was no cellular uptake of TiO₂ by keratinocytes or sebocytes.

TiO₂ nanoparticles elevate [Ca²⁺]_i in fibroblasts and melanocytes but not in keratinocytes and sebocytes

To measure if TiO₂ (internalized or not) induces cellular signalization pathways, we investigated whether exposure of cells to nanoparticles causes an increase in the [Ca²⁺]_i in different cell types. Using a calcium-sensitive probe to detect intracellular ion levels, we found that 15 µg/cm² TiO₂ nanoparticles induced a relatively slow (onset cca. 45 minutes after exposure) yet partly or fully reversible elevation in [Ca²⁺]_i in fibroblasts and melanocytes. In contrast, in those cells which did not internalize the TiO₂ (i.e.,

keratinocytes and sebocytes), no calcium response was detected. It seems, therefore, that the internalization of TiO₂ induced changes in the calcium-handling of the cells.

TiO₂ exposure differentially affects cellular proliferation and apoptosis in various human skin cells

We then measured the effect of TiO₂ nanoparticles on cellular proliferation. As assessed by a colorimetric MTT assay detecting the viable cells, TiO₂ decreased the cellular growth of all examined cell types (i.e., even of keratinocytes and sebocytes which did not internalize the nanoparticles, see above) in dose- and time-dependent manners. It was also important to observe that, in the cases of keratinocytes, sebocytes, and melanocytes, the decrease in cellular proliferation was not accompanied by cell death. In other words, none of the TiO₂ treatment protocols (concentrations, durations) decreased the viable cell number “below” the control values measured at day 2. As a marked contrast, however, at days 3 and 4, 15 µg/cm² TiO₂ not only prevented the proliferation of fibroblasts but also decreased the viable cells number when compared to control data at day 2.

This latter finding suggested that TiO₂ caused cell death in human dermal fibroblasts. To assess whether apoptosis is involved in the process, we determined the expression of Annexin-V, a hallmark of apoptosis. In good accordance with the proliferation data, TiO₂ induced apoptosis of fibroblasts in dose- and time-dependent manners. Of great importance, however, no significant increase was observed in the percentage of Annexin-V positive apoptotic cells in keratinocytes, melanocytes, and sebocytes arguing for that the apoptosis-inducing effect of TiO₂ was restricted to the dermal fibroblasts.

In keratinocytes, TiO₂ exposure decreased the expression of differentiation markers and cell adhesion molecules

In the final stage of our *in vitro* experiments, we measured whether the inhibition of proliferation of keratinocytes by TiO₂ exposure also affected the expression of certain molecules which were shown to be involved in cell differentiation. Keratinocyte cultures (80-90 % confluence) were therefore exposed to TiO₂ at 15 µg/cm² for 48 hours and then

Western blot analysis was performed. TiO₂ significantly decreased the expression of the keratinocyte (late) differentiation marker involucrin and the levels of the cell adhesion molecules desmoglein-1 and P-Cadherin which (besides functioning as key components of cell-to-cell contacts in the epidermis) were described to be highly expressed in the more differentiated keratinocytes of the human skin. Taken together, these data suggest that the inhibition of proliferation of keratinocytes by TiO₂ was also accompanied by the suppression of the differentiation process as well.

DISCUSSION

In our experiments, we provide clear evidence that the stratum corneum of the human epidermis is indeed an effective barrier defending the cells from the "harmful" contact with micronized TiO₂. However, of great importance, we also show that nanoparticles may significantly (yet differentially) disturb numerous cellular functions when *directly* contact (seen for example upon disruption of the skin barrier) different cell populations of the skin.

Investigation of skin barrier function is a key concept in dermatological research and has important consequences on human health. However, techniques applied earlier have several disadvantages: namely, TEWL measurement gives indirect information about skin barrier function without showing the position of nanoparticles whereas tape stripping penetration study techniques show the particles apart from their original anatomic environments. Therefore, these techniques do not give information about particle penetration and skin barrier function at the same time.

In this study, we show that nuclear microscopy techniques are much more suitable for investigating microelement penetration. In fact, we demonstrated that nuclear microscopy methods have the sensitivity and specificity to reproduce the anatomical structure of the examined skin samples (with much more topographic relevance than other approaches) hence the identification of skin layers could be carried out according to the previously used standard characteristics for human skin. Moreover, result obtained with these methods also proved that the SCID mouse - human prepuce skin transplant model is indeed useful for transepidermal penetration studies and therefore may offer a

handy solution for the problem of recruiting healthy human volunteers for penetration studies requiring skin biopsies.

Using this model, we clearly demonstrate that there is a crucial role on the barrier function of the epidermis and especially the stratum corneum against the TiO₂ nanoparticles. Indeed, nuclear microscopy was able to detect very small amounts of TiO₂ but only in the layers of the stratum corneum. Of great importance, in good accord with our previously published results on pig skin and human xenografts, no penetration of TiO₂ nanoparticles was observed after titanium-dioxide exposure to the deeper part of the epidermis, even by the penetration forcing effect of occlusion in skin grafts.

However, the integrity of the protective skin barrier is very often impaired by numerous factors (e.g. injury, sunburn, extreme bathing, certain skin diseases). Moreover, it can also be assumed that certain agents applied topically on the skin surface may reach the deeper epidermal layers and (even the) dermis via transfollicular and/or transglandular penetration. Therefore, we also investigated the direct effect of exposure by TiO₂ nanoparticles on functions of numerous cell populations the human skin.

Of great importance, we found that TiO₂ do not behave inertly (similar to those observed in pulmonary models and indeed markedly (yet, notably, differentially) affect certain cellular mechanisms. Namely, particles were internalized by fibroblasts and melanocytes but not by epidermal keratinocytes and sebocytes. In addition, we also found that the internalization of TiO₂ nanoparticles is accompanied by the elevation of [Ca²⁺]_i in fibroblasts and melanocytes (but not in the other two cell types). This finding suggests an alteration in the cellular ionic homeostasis, a key regulator of most cellular mechanisms.

It was intriguing to observe, however, that for the development of the cellular effects of TiO₂ nanoparticles, the internalization and the modification of [Ca²⁺]_i are not compulsory processes. Namely, TiO₂ exposure was able to induce a dose-dependent cessation of cell growth in all cell types, i.e. even in those keratinocytes and sebocytes which did not internalize TiO₂ and in which [Ca²⁺]_i was not raised upon exposure. Of further importance, we also found that TiO₂ nanoparticles selectively induced apoptosis in dermal fibroblasts but not in melanocytes (which also internalized and responded with [Ca²⁺]_i elevation to TiO₂ application), keratinocytes or sebocytes. Finally, we were also able to show that the inhibition of proliferation in keratinocytes was accompanied by

suppression of expression of certain differentiation markers and adhesion molecules which may result in an abrupt differentiation and an abnormal cytoskeleton and desmosome structure, defective intercellular adhesion, thus abnormal barrier function.

Collectively, these data unambiguously argue for that, in the case of intact epidermal barrier, TiO₂ nanoparticles *in vivo* do not penetrate the stratum corneum and hence do not reach the deeper, “living” cell layers of the human skin. However, in the cases of impaired barrier function, damaged stratum corneum or putative penetration via the appendages of the skin, it can be assumed that TiO₂ nanoparticles may be internalized by some skin cells; they may dysregulate the complex mechanism of epidermal differentiation; and/or they may exert toxic effects. These alterations in cellular functions then may further “weaken” the epidermal barrier and (e.g. with dermal fibroblast apoptosis) induce consequent elastotic changes in the dermal collagen.

Along these lines, our current novel findings will hopefully inspire one to systemically explore in future, clinically-oriented trials whether there is indeed a risk from micronized TiO₂-containing products on skin with an impaired stratum corneum barrier function. To support such investigations, in our ongoing experiments, we currently investigate the *in vivo* penetration (as well as *in vivo* and *in vitro* cellular effects) of micronized TiO₂ on tape-stripped, soaked, and abraded healthy human skin transplanted to SCID mice, as well as on transplanted skin samples obtained from atopic dermatitis patients (with severely impaired cutaneous barrier functions).

NOVEL FINDINGS

1. The SCID mouse-human epidermal xenograft model is suitable for skin penetration studies.
2. The elemental composition (mass distribution, Ca, K, P, S, Na, Cl elements) of the xenografts did not differ from that of healthy human skin.
3. The nanoparticles do not penetrate the intact stratum corneum layer. It can be assumed, that the nanoparticles potentially reach deeper epidermal layers via transfollicular or transglandular route.

4. The fibroblasts and melanocytes internalize micronized titanium dioxide, where an increase in the intracellular Ca^{2+} levels can be detected.
5. The nanoparticles cause proliferation arrest in sebocytes, melanocytes and keratinocytes..
6. The micronized titanium dioxide particles induce apoptosis in fibroblasts.
7. Exposure of titanium dioxide nanoparticles decreases the expression of keratinocyte differentiation markers in HaCat keratinocytes.

PUBLICATION LIST

Publications on which the Ph. D. thesis is based:

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activity of the retinoid X receptor/PPAR gamma heterodimer *J. Biol. Chem.*, Vol. 282, Issue 52, 37738-37746, December 28, 2007 IF 5.808

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