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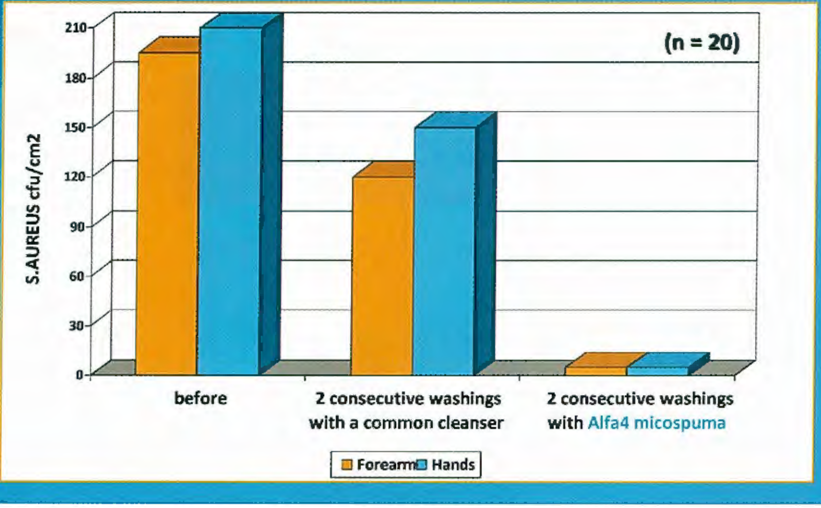

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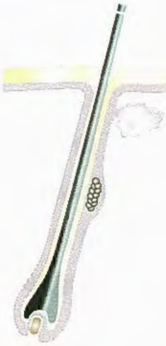

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The Future of Nanotechnology

The potential of nanotechnology stretches across all sector of society, from electronic to medicine. Nano-materials for energy production and storage, nano-particles for drug delivery and biosensors for diagnostics are, however, some of the emerging nano-technologies.

As a relatively new field, the use of research results is in this field still in its early stages with few identified good practices, especially from a multidisciplinary perspective (research centers, venture capitalists and expert intellectual protection). Thus, crystalline silicon (c-Si), wafer-based technology has the most promising potential for significantly reduce the cost of solar cells; organic nano-wires open the possibilities for future electronic and optoelectronic components, nickel-titanium shape memory alloys (SMAs) have been mostly explored and applied in biomedicines because of their excellent flexibility and deformation behavior similar to that of living tissue, as well as nanostructured olysaccharides, as chitin, have been used as drug and gene delivery systems and as scaffold for skin, cartilage and bone replacement therapies.

However, in recent years, novel nano-structured chemicals and devices have been greatly developed, because their potential to improve the quality of life, throughout the world has captured the imaginations of many. The size of nano-products is measured, in fact, in nano-meters (a nanometer is about one billionth of a meter) and they are built from individual atoms. Thus, for example devices, constituted from individual atoms and called nano-machines are studied to enter living cells for combating disease. Moreover, according with Eric Drexler, if the researchers succeed, nano-structured products "can be used to get rid of poverty by obviously converting dirt into food". The idea is to differently assemble the molecules from raw materials by reorganizing atoms to obtain useful products, as happens in nature.

Proteins are the bio-molecular motors which generate forces and movement within cells, transforming chemical energy into mechanical energy. But they act also like an immigration control mechanism of the cell determining what its membrane filter allows in or not. Understanding these processes will help engineers to design special software and hardware necessary for intelligent robots or to create new materials and sensors for biologists and pharmacologists capable to physiologically interact with the cell-structures at the nano-scale, enhancing for example the immunity of the general population. In fact, a drug, to be effective, must first target the appropriate cells and normally gain access to their inside.

Thus, the potential for hard and soft nano-structures is rapidly expanding with new applications in many different areas, such as neurobiology, cardiology and cosmetic dermatology.

Nano-particles are, in fact, of great scientific interest, being, in reality, a bridge between bulk materials, and atomic or molecular structures. A bulk material should have constant physical properties regardless its size, but at the nano-scale this is often not the case. Therefore, these nano-structures often have unexpected visual properties because they are small enough to confine their electrons and produce quantum effects. For example, gold nano-particles appear deep red to black in solutions so that nano-clusters of this metal, used for the sensitive selective detection of DNA, have been demonstrated to be effective in detecting anthrax.

A new EU project is developing targeted nano-systems for improving photodynamic therapy and diagnosis of cancer. The researchers "are creating a nano-particle drug delivery system that behaves like a tiny, cancer-seeking homing missile, attaching itself to the tumors and then delivering the drug payload". Another EU group is focused on "identification and validation of new breast cancer biomarkers based on integrated metabolomics".

While all the signalling molecules cytokines play a crucial role triggering nano-inflammatory elements, their observation and control is very difficult to obtain. Thus special micro probes have been developed to stimulate single neuronal cells and detect inflammatory signalling compounds in real time. To obtain a more sensitive miniaturized probe, capable to detect from the cell the excreted proteins, palladium nanostructures were introduced into the tip of the electrode surface to increase its conductivity.

Differently a EU team is analyzing disease-associated genes, and identifying groups or modules of interacting genes with distinct biological functions. These multi-layered modules integrate information about disease-associated changes on the DNA, RNA at protein levels to identify possible diagnostic markers. In short, the challenge of defining diagnostic markers for personalized medication has been successfully overcome.

Genetics, nano-technology and advanced computer-systems are changing the way to produce medicines. Tailor-made drugs and chemicals target wellbeing and illness more precisely and efficiently and are, therefore, on the way to becoming reality. However, the potential of nanotechnology stretches across all sectors of society and according with Prof. Andrew Richardson: "today tiny polymer gears are able to move bacteria. Tomorrow hybrid biological and micromechanical machines could repair damaged tissue and help heal the wounded".

In any way contrary to popular belief, modern technological materials increasingly used in a wide range of medical devices implants or as raw materials in the cosmetic field are entirely biocompatible and should not induce health problems. Appraising the risk that may arise from nanotechnology requires, however, a new multidimensional and multidisciplinary approach in order to provide a clear understanding of all the factors involved and to facilitate debate among stakeholders.

This special issue has the intention to clarify some nano-technological aspects involving Cosmetic Dermatology.

P. Morganti
Editor-in-Chief

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Nanotechnology for Topical Application in Cosmetic Dermatology

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Key words: *Nanotechnology; Cosmetic dermatology; Skin;*

Summary

Many skin conditions, such as dry skin, oily skin, hyper-, hypo- pigmentation, vascular deformities, photoaging, are major concerns in cosmetic dermatology. A variety of products have been formulated to protect the human skin. However, traditional topical cosmetic formulations have their limitations. For example, traditional formulations employed to absorb or block UV radiation are so thick that they do not blend well into the skin and are cosmetically unappealing. There is a requirement for the traditional sunscreens to be opaque, biologically and chemically inert, stable when applied on the skin, and resistant to water.

Nanotechnology has been introduced into new cosmetic products in recent years. Nanotechnology refers to the precise manipulation of matter at the nanometer scale, i.e., materials are manufactured to ultrafine nanoparticles (NPs) with a diameter of about 100 nm.

Due to the surface physical-chemical activities, NPs have diversified properties which are often distinct from their counterpart macroparticles and is of particular interest to the skin care industry. Using nano-sized particles in cosmetics, products with improved texture, more vibrant color and greater skin penetration have been generated. However, there has been controversy regarding the safety of topical application of NPs. The potential harms are evading from immunologic defense mechanisms, forming complexes with proteins, inducing the formation of free radicals, damage on living cells, et al.

This paper provides an overview of the ongoing research on nanotechnology for topical application in cosmetic dermatology.

Riassunto

Molte sono le condizioni cutanee che interessano la Dermatologia Cosmetologica quali, la cute secca, oleosa, affetta da iper ed ipopigmentazioni, da alterazioni del microcircolo superficiale e/o fotoinvecchiamento. Molti sono i prodotti formulati per risolvere questi problemi.

I classici prodotti cosmetici però hanno ancora molte limitazioni. Ad esempio, alcune formulazioni utilizzate per neutralizzare o respingere le radiazioni solari, sono molto grasse e pastose e non rispondono perfettamente né in termini di compliance cosmetica né in termini di efficacia (non sono in grado di eliminare totalmente sia gli UVB che gli UVA). Si sente, quindi, la necessità di avere protettivi solari più stabili, biologicamente e chimicamente inerti e resistenti al lavaggio.

Recentemente nelle formulazioni dei prodotti cosmetici è stata introdotta la nanotecnologia . Questa branca tecnica si occupa della utilizzazione e della manipolazione di materiali su scala nanometrica che rendono le materie prime ultrafini, perchè di dimensioni pari a circa 100 nanometri.

Per le loro particolari caratteristiche chimico-fisiche, queste nanoparticelle hanno proprietà totalmente diverse rispetto alle macroparticelle dello stesso ingrediente chimico. Pertanto rivestono un interesse particolare per l'industria cosmetica. Utilizzando nano particelle e nano polveri, i cosmetici assumono caratteristiche migliori in termini di consistenza, gradevolezza, aspetto del colore e capacità di penetrazione.

Esistono, comunque, alcune perplessità nell'uso di questi nuovi materiali circa la loro sicurezza nei confronti delle difese immunologiche in quanto potrebbero generare complessi con le proteine cellulari, generando anche un maggior numero di radicali liberi .

Questo articolo intende riportare le ultime ricerche condotte sulle nanotecnologie per fornire al lettore un quadro quanto più completo possibile sugli attuali usi dei nano materiali nella Dermatologia Cosmetologica.

ACTIVE INGREDIENTS AND CARRIER SYSTEM IN NANOTECHNOLOGY

There are two critical factors in nanotechnology: active ingredients and carrier system. Active ingredients such as zinc oxide (ZnO), titanium dioxide (TiO_2), chitin nanofibrils (CN), methoxycinnamate (MC), Coenzyme Q(10) (Q(10), retinoids, vitamin E, and ascorbyl palmitate (AP) are the elemental components of cosmetic formulations.

Carrier system including nano-lipid delivery system, NPs suspension, nanoemulsion (NE), polymer NPs, and magnetic NPs, etc. can load the active ingredients into NPs. The nano-lipid delivery system for topical applications has been abundantly described during the recent decades. These include liposomes (2), oil-in-water emulsions (3), multiple emulsions (4) and microemulsions (5).

With the purpose of increasing physicochemical stability of both active ingredients and the system itself, solid lipid nanoparticles (SLN) (6) and nanostructured lipid carriers (NLC) (7) have been developed.

Zinc oxide (ZnO) and titanium dioxide (TiO_2)

ZnO and TiO_2 have traditionally been used in sunscreens because of their ability to filter ultraviolet (UV) light. In recent years, nanosized forms of ZnO and TiO_2 have been made in order to produce more transparent, less viscous, and easier blending effects, while keeping their screening potency toward UV irradiation. However, the small particle size of the NP may have different chemical, optical, magnetic, and structural properties; consequently, they also may possess differential toxicity profiles than normal size.

Studies have concentrated on the penetration of nano-sized physical sunscreens to justify the safety. The less content of ZnO or TiO_2 penetrates the epidermis, the more safety would be considered. Cross SE (8) evaluated the extent to which topically applied NPs ZnO sunscreen formulation can penetrate human epidermis using Franz-type diffusion cells, 24-hour exposure and an electron microscopy.

The results was that less than 0.03% of the applied ZnO penetrated the epidermis and no particles could be detected in the lower stratum corneum or viable epidermis, indicating that rare NPs penetration occurs through the human epidermis.

Zvyagin AV. et al. (9) addressed the subdermal absorption of ZnO NPs in human skin in vitro and in vivo using multiphoton microscopy imaging with a combination of scanning electron microscopy and an energy-dispersive x-ray technique. The overall outcome revealed that ZnO NPs stayed in the stratum corneum and accumulated into skin folds and/or hair follicle roots of human skin.

Another study conducted by van Der Merwe D. et al. (10) applied a nanocrystalline TiO_2 and magnesium oxide mixture to the skin surface as a dry powder, as a water suspension, and as a water/surfactant suspension, for 8 hours.

There was no detectable dermal absorption of the nanocrystalline through human skin with intact and functional stratum corneum. If the NP in sunscreens applied to the skin penetrates the dermis, there is a concern for systemic absorption of these particles that have potential inflammatory and carcinogenic effects.

Fortunately, the in vivo studies reviewed here suggest that nanosized ZnO or TiO_2 particles do not penetrate the stratum corneum.

However, if the nano-sized TiO_2 was exposed directly to cell cultures in vitro (11), it exerted significant and cell-type dependent effects on cellular functions such as viability, proliferation, apoptosis and differentiation (12). If the TiO_2 NP was exposed to human dermal fibroblasts, cell surface area, cell proliferation, mobility, and ability to contract collagen would be decreased.

Individual particles were shown to penetrate easily through the cell membrane in the absence of endocytosis. Once inside, the particles were sequestered in vesicles, which continue to fill up with increasing incubation time till they rupture. Due to the extremely small size of the ZnO NPs or TiO_2 NPs, there is a concern that they may interact directly with macromolecules such as DNA and produce reactive oxygen species (ROS) at the same time.

Sharma et al. study (13) demonstrated that ZnO NPs possessed a genotoxic potential in human epidermal cells which may be mediated through lipid peroxidation and oxidative stress. Antioxidants such as phenylalanine, sodium ascorbyl phosphate, and ascorbyl palmitate can scavenge the oxidizing species photogenerated by nano-TiO_2 as well as inhibit UVB-induced peroxidation (14).

Vileno B. et al. (15) also found that low-intensity UVA (8 and 20 mW/cm^2) could induce low concentration of nanoTiO_2 (4 microg/mL) generated ROS and thus induce the decrease of cell stiffness (human skin fibroblasts). In the presence of a well-established antioxidant, as beta-carotene, the photo-oxidative stress was cancelled. Therefore, more attentions should be paid to the potential biological hazard of this nano material application. Fortunately, the addition of free radical scavengers such as antioxidants can effectively decrease the peroxidation risk induced by UV irradiation.

Sporadicly, Warheit B.'s study (16) examined other possible side effects of nano-TiO_2 including the acute dermal irritation and dermal sensitization response. On the one hand, ultrafine TiO_2 particles were applied to the shaved intact skin of male rabbits for 4 h. The rabbits exhibited no dermal irritation 0, 1, 24, 48, and 72 h after test substance removal. On the other hand, female mice were dosed for 3 consecutive days with ultrafine TiO_2 particle-types on both ears. On test day 5, the cell proliferation in the draining auricular lymph nodes of the ears significantly increased at the 50% and 100% test concentrations and the stimulation indices were all less than 3.0 at test concentrations of TiO_2 particle-types. Under the condition of Warheit's study, the TiO_2 NP were not considered to be skin irritant in rabbit skin and did not produce a dermal sensitization response in mice.

Chitin nanofibril (CN)

In chemical terms, chitin is a natural polysaccharide and has the ability to link water for long periods and neutralize free radicals.

The CN is an alpha-nanocrystalline form separated from the raw material chitin. According to Morganti P., CN is able to activate fibroblast proliferation and cytokine production, favoring the giant cell migration, macrophage activation and neovascolarization (17). When CN is used in the right way and in the right emulsion, it can positively affect the partition coefficient between formulation and skin, and thus increase the clinical efficacy of the active ingredients (18).

In vitro and in vivo studies (19, 20), CN was found to produce higher level of fibroblast proliferation, collagen production of fibroblast, skin hydration, surface skin lipids, and lower level of transepidermal water loss (TEWL) and lipids peroxide, compared to controls.

Moreover, the CHROMAMETER VALUE a* of CN treated skin areas significantly decreased comparing to untreated areas, indicating a good whitening activity of CN.

Interestingly, when CN was combined with antioxidant and immunomodulant, the clinical efficacy could be further enhanced. Skin hydration increased from 40% (CN treated group) to 80% (CN + Melatonin-Lutein-Ectoin treated group), superficial skin lipids increased from 35% to 68%, TEWL decreased from about 50% to 72% and lipid peroxides decreased from 35% to 66%.

Methoxycinnamate (MC)

Octyl or ethylhexyl MC (OMC) is an active ingredient in sunscreen products.

In vivo, Calderilla-Fajardo SB. (2006) evaluated the percutaneous penetration of OMC formulated in NE and nanocapsules. The results showed that NE OMC formulated with sucrose laureate exhibited the highest penetration in the stratnm corneum compared to the control and the nanocapsule formulations. The NE OMC containing sucrose laureate showed a two-fold increase in OMC skin deposition compared to the control.

The data suggested the nanocarrier such as NE and the enhancer such as sucrose laureate can promote the amount of OMC detected in the stratnm corneum and the penetration depth. Whereas other nanocarrier, such as nanocapsules and other sucrose ester used in this study, showed no obvious effects. So the nanocarrier's nature and the choice of enhancer can influence percutaneous penetration of active ingredient.

In another study conducted by Puglia C. et al. (21), the percutaneous absorption of two combined sunscreen formulations were evaluated. The concurrent application of two suscreens often increases their systemic absorption, compromising the safety and efficiency of the cosmetic product.

Puglia incorporated OMC and diethyltolnamide into SLN and used differential scanning calorimetry analysis to determine the percutaneous absorption. The data highlighted that OMC and diethyltoluamide modified the temperature and the enthalpy change associated to the calorimetric peak of SLN. The concurrent presence of the two compounds in the SLN caused a synergic effect and the particles were able to reduce the skin permeation of the two cosmetic ingredients in comparison with a control emulsion.

Puglia's study provided evidence that SLN as carriers for topical administration could reduce the systemic absorption, and enhance the safety and efficacy.

Photostability is the most important characteristic of effective sunscreens. When exposed to sunlight, OMC can change from the primary trans-form to cis-form and the isomerization conducts to a reduction of the UVB filtering efficiency because of a higher extinction coefficient.

Vettor M. et al. (22) investigated emulsion-gels containing different percentages of OMC free or loaded in poly (D,L-lactide) NPs.

The results revealed that free OMC in different formulations has different photoisomerization degree and formulations containing sunscreen loaded in NPs showed a significant reduction in photoisomerization degree, highlighting that the encapsulation was a suitable strategy to improve OMC photostability.

Perugini P. et al. (23) investigated the influence of NP-based systems on the light-induced decomposition of trans-2-ethylhexyl-p-methoxycinnamate (trans-EHMC). The photodegradation of the trans-EHMC-loaded thepoly-D,L-lactide-co-glycolide NPs was reduced by 35.3% compared to 52.3% for free trans-EHMC, whereas the Ethylcellulose NP system had no significant improvement effect in the photostability. The result indicated that different type of NPs played various roles in the photostability of sunscreen.

Coenzyme Q(10) (Q(10)

Q(10) is a naturally occurring antioxidant and has been applied for cosmetics for its' various biological effects. It is a lipid-soluble provitamin and can be incorporated into lipids. Junyaprasert VB (24) loaded the NE of medium chain triacylglycerols (MCT) and NLC of cetyl palmiate/MCT to Q(10) and characterized for their stability before and after incorporation into xanthan gum hydrogels.

Similar results were found in xanthan gum-based hydrogels containing NE or NLC. After storage at 4, 25 and 40 degrees C, the particles remained in the nanosize range for 12 months. The Q(10) entrapped in NLC and NE remained higher than 90% at all temperatures for 12 months but dramatically decreased when exposed to light. In Teeranachaideekul V.'s study (25), Q(10) was incorporated in NLC composed of cetyl palmitate with various amounts of caprylic/capric triacylglycerols. NE composed of liquid lipid was prepared for comparison. In this study, NLC and NE provided a good physical stability of the formulations. Otherwise, Q(10)-loaded NLC possessed a biphasic release pattern, in comparison to Q(10)-loaded NE possessing a nearly constant release, and the NLC release patterns were defined by an initial fast release in comparison to the release of NE followed by a prolonged release. Different NPs produced different release pattern.

Retinoids

Various natural and synthetic retinoids have been explored for the treatment of skin aging or improve various hyperpigmented lesions. However, irritant reactions such as burning, scaling or dermatitis limit their acceptance by patients.

This problem is more prominent with tretinoin, whereas retinaldehyde and retinol are considerably less irritating. In order to improve the efficacy and minimize these side effects, various novel drug delivery systems including NPs have been developed.

The study by Yamaguchi Y. et al. (26) observed the effect of nano-scale tretinoin (all-trans retinoic acid, atRA) on photo-damaged skin. They found irritation and inflammation associated with nano-scale atRA therapy were substantially reduced due to the complete encapsulation of the carboxylic function. The nano-atRA developmentally improved the permeability to the stratum corneum and resulted in the remarkable pharmacological effects such as thickening of epidermis, overexpression of mRNA for heparin-binding epidermal growth factor as the provocation epidermal hyperplasia and boosting in production of hyaluronan among the intercellular spaces of the basal and spinous cell layers in epidermis. So the nano-atRA could efficiently regulate keratinocyte cell proliferation and differentiation.

The study of Jenning V. et al. (27) tested glyceryl behenate SLN loading vitamin A (retinol and retinyl palmitate) with respect to their penetration into porcine skin. Excised full thickness skin was mounted in Franz diffusion cells followed by the application of formulations for 6 and 24 h. High retinol concentrations were found in the upper skin layers following SLN preparations, whereas very low levels of vitamin A were found in the deeper regions.

The penetration of retinyl palmitate was even more influenced by SLN incorporation, and the enhanced uptake seemed to be derived from specific SLN effects. This in vitro study indicated that SLN can promote vitamin A penetrated into porcine skin.

Pople PV. (28) conducted both in vitro and in vivo studies to investigate the effects of topical application of SLN loaded vitamin A palmitate gel on skin. In vitro release profile showed prolonged drug release up to 24 hours, which could be owing to embedment of drug in the solid lipid core. In vitro penetration studies showed almost 2 times higher drug concentration in the skin with lipid NPs-enriched gel as compared with conventional gel, thus indicating better localization of the drug in the skin. In vivo skin hydration studies in albino rats revealed increase in the thickness of the stratum corneum with improved skin hydration. The developed formulation was nonirritant to the skin with no erythema or edema. Thus the SLN used in Pople PV.'s study was concluded to be a promising particulate carrier having controlled drug release, improved skin hydration, and potential to localize the drug in the skin with no skin irritation.

Another clinical study of Sato K. et al. (29), however, exhibited only a little promising results for nano-atRA. Sato evaluated whether combine use of inorganic-coated atRA nanoscale particles gel, 5% hydroquinone and 7% lactic acid ointment can improve the efficacy and produce less adverse effects. Sato's study revealed that the nano-atRA gel can improve hyperpigmentation in 84 of 88 lesions (95.5%) and almost eliminate hyperpigmentation in 52 lesions (59.1%), to a similar extent as conventional atRA gel.

The study also revealed the nano-atRA gel caused exfoliation and scaling similar to that seen with conventional nano-atRA, whereas the erythema appeared to be weaker. So nano-atRA gel used in this study seemed to have no advantage over conventional nano-atRA gel on efficacy and some irritant reactions (exfoliation and scaling), with the only advantage of weeker erythema.

Vitamin E

Vitamin E is able to extinguish single oxygen species as well as to terminate free radical chain reactions, and is considered as the principal lipid-soluble antioxidant.

It can be divided into two families of compounds, the tocopherols and the tocotrienols. Limited studies of nano-sized vitamin E are focused on the tocopherols.

Teo BS. et al. (30) investigated the potential of tocopherol acetate-loaded palm oil esters-in-water NE for nanocosmeceuticals. The best formulation was found to be the composition of 10% vitamin E, 10% Palm Oil Esters (POEs), 24% Tween 80, 2.4% Pluronic F-68 and 53.6% deionized water. Those compositions are considered to be the best as a nanocosmeceutical product due to the small particle size (94.21 nm), low occurrence of Ostwald ripening and the stability at different storing temperatures (5, 25 and 45 □) for four weeks.

Kotyla T. et al. (31) compared the transdermal application of a nano-sized emulsion versus a micron-sized emulsion preparation of delta tocopherol on the shaven dorsal area of hamsters. Compared to baseline, hamsters that were applied with the nano-sized emulsion had a 36-fold and 68-fold significant increase of plasma delta tocopherol at 2h and 3h post-application, versus the micron-sized emulsion with only 9-fold and 11-fold increase, respectively. This study suggests that nano-sized emulsions significantly increase the bioavailability of transdermally applied delta tocopherol.

Moddaresi M. et al. (32) evaluated the effect of a lipid nanocarrier system loaded with tocopheryl acetate in the short-term (3 h) and long-term (4 weeks) trials. The study showed that the lipid nanocarrier system enhanced the skin hydration, comparing to non-lipid nanocarrier systerm. While the skin viscoelastic parameters and the antioxidant efficacy showed no statistic differences So tocopheryl acetate -loaded lipid nanocarrier system exhibited the ability to enhance skin hydration, while their effect on skin biomechanical properties and on antioxidant efficacy could not be enhanced.

Ascorbyl palmitate (AP) and others

AP is known as an effective antioxidant that protects tissue integrity similar to vitamin C and its moisturizing activity in conventional topical formulations was found to be high.

Uner M. et al. (33) investigated the moisturizing potential of AP in SLN and NLC incorporated into hydrogel. AP incorporation moisturized skin

CONCLUSIVE REMARKS

As one of the fastest growing technologies, nanotechnology has been widely applied in the pharmaceutical industry, medicine, and engineering technology during the past two decades.

It is also frequently used in Cosmetic Dermatology.

Recently, however, scientists and organizations have raised the safety concerns about nanotechnology.

Therefore, more elaborate clinical and experimental studies are required to confirm their advantage and weight the safety issue.

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Safety of Nanotechnology in Sunscreens and Personal Care Products

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Summary

Personal care product or sunscreen formulations may contain nano-sized components including nanoemulsions and microscopic vesicles consisting of traditional cosmetic materials.

Numerous studies suggest that vesicle materials do not penetrate human skin beyond the superficial layers of the *stratum corneum*. When compared with the skin absorption / penetration of ingredients of traditional formulations (solution, gels, creams), nano-sized formulations may enhance or reduce skin absorption of ingredients, albeit at a limited scale.

Modern sunscreens contain solid titanium dioxide (TiO_2) or zinc oxide (ZnO) nanoparticles (NP) that filter UV more efficiently than larger particles.

A series of studies suggest that insoluble NP do not penetrate into or through normal or compromised human skin. *In vivo* toxicity tests confirmed that TiO_2 and ZnO NP are non-toxic, biologically inert and have a good skin tolerance.

A series of *in vitro* and *in vivo* cytotoxicity, genotoxicity, photo-genetic, pulmonary or acute toxicity, sensitisation and ecotoxicity studies on TiO_2 NP found no difference in the safety profile of micron- or nano-sized materials, all of which were non-toxic.

Some *in vitro* investigations on TiO_2 or other insoluble particles reported cell uptake, oxidative cell damage or genotoxicity; however, such data should be interpreted with caution since they may be secondary to phagocytosis of cells exposed to excessive concentrations of insoluble particles.

Data from studies on surgical implant-derived wear debris nano- and micro-particles or other insoluble particles support the traditional view of toxicology that their toxicity is generally related to their chemistry, rather than their particle size.

At present, there is no evidence for a rule that adverse effects of particles to the skin or other tissues increase with smaller particle size or produce novel toxicities. Overall, the weight of current scientific evidence suggests that nano-sized personal care or sunscreen ingredients pose no or negligible potential risk to human health, whereas their use in sunscreens has significant benefits including protection of human skin against skin cancer.

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[1] *Abbreviations: NP = nanoparticles; NM = nanomaterials; TiO_2 = titanium dioxide; ZnO = zinc oxide.*

Riassunto

I cosmetici utilizzati per la formulazione dei prodotti per la cura della persona e per la fotoprotezione della cute possono contenere componenti nanostrutturati, quali nanoemulsioni e particelle di grandezza microscopica contenenti, a loro volta, ingredienti cosmetici tradizionali.

Numerosi studi evidenziano come queste nanoemulsioni non sembrino penetrare oltre lo strato corneo e, paragonate ai materiali tradizionali, quali le soluzioni, i gel o le creme, possono esaltare o ridurre l'assorbimento dei diversi ingredienti di uso corrente.

La moderne formulazioni dei filtri solari contengono nanoparticelle di biossido di titanio (TiO_2) o ossido di zinco (ZnO) in grado di respingere gli UV molto più efficacemente di quanto facciano se utilizzati nelle loro dimensioni tradizionali.

Molti studi condotti *in vitro* e *in vivo* sulla citossicità, genotossicità, tossicità fotogenetica, sia acuta che cronica, insieme ad altri studi di ecotossicità condotti su nanoparticelle di TiO_2, non hanno fatto riscontrare differenze significative sulla sicurezza d'uso di questi materiali nanostrutturati rispetto ai materiali di uso corrente, essendo risultati tutti non tossici.

Anche se alcuni studi *in vitro* condotti su TiO_2 o su altre nanoparticelle insolubili hanno descritto alcuni danni cellulari da ossidazione o alterazioni riferibili a processi genotossici, è necessario interpretare i risultati con molta cautela perché secondari a fagocitosi o a cellule esposte a concentrazioni eccessive di particelle.

Altri dati provenienti da studi relativi a impianti di chirurgia ripartiva evidenziano come il grado di tossicità delle micro e nano particelle sia generalmente legato alle specifiche caratteristiche chimiche del materiale utilizzato e non sia imputabile alla dimensione delle particelle stesse.

Al momento non vi è alcuna evidenza sperimentale che mostri i maggiori effetti collaterali o tossici provocati sulla cute o su altri tessuti dai prodotti nanostrutturati rispetto agli stessi materiali di dimensioni tradizionali.

Le attuali evidenze scientifiche pongono in risalto come l'uso appropriato di questi filtri nanostrutturati inseriti nei prodotti solari abbia profondamente incrementato i loro effetti protettivi nei confronti dei tumori cutanei a fronte dei potenziali rischi risultati, al contrario, trascurabili per la salute umana.

INTRODUCTION

There is a broad consensus that the principal potential human health risk of nanoparticles (NP) or nanomaterials (NM) may be due to inhalation (1); however, concerns have also been raised about the risks of the contact of NP/NM with human skin. The possibility that topically applied NP may penetrate human skin and thereby produce a systemic health risk has been suggested in reviews by Hoet et al. (2), Oberdörster et al. (3) or the US EPA Draft White Paper (4), the latter suggesting that *nanoparticles may penetrate the skin and distribute throughout the body once translocated to the circulatory system.*

Therefore, the key questions that must be asked for NP / NM applied to the skin is a) are they absorbed and b), if so, are they intrinsically toxic? Specific questions concerning the safety of NP and NM in cosmetic products and sunscreens include the following:

a) Do cosmetic formulations containing nano-sized features (vesicles or droplets) enhance the skin penetration of cosmetic ingredients, thereby increasing the risk of human skin sensitisation or systemic exposure?

b) Do nano-sized cosmetic formulations pose new risks when compared with those of traditional cosmetic products?

c) Do topically applied insoluble NP remain on the skin surface or are they able to pass the skin barrier of normal or compromised skin to gain access to systemic compartments of the organism?, and

d) Are insoluble NP in sunscreens intrinsically more hazardous than larger particles, i.e. micro-particles or bulk material?

Therefore, in the present review, an attempt is made to summarise the current knowledge about the safety of cosmetic nano-formulations and insoluble nano-particles used in sunscreens.

NANO-SIZED FORMULATIONS AND SOLID NANOPARTICLES

Modern personal care or sunscreen products may contain nano-sized components, including nano-sized formulations or insoluble solid particles in the nano-range, i.e. 1 to 100 nm in diameter. Nanoemulsions are transparent and have unique tactile and texture properties. Nanosomes, niosomes or liposomes are small vesicles (range: 50 to 5000 nm) consisting of traditional cosmetic materials, and are mainly used to protect light- or oxygen-sensitive cosmetic ingredients. Given that such formulations are inherently unstable and rely on liquid / liquid interactions, it is unclear whether they should be qualified as genuine nanoparticles. However, numerous studies have shown that vesicle materials generally do not seem to penetrate human skin beyond the superficial layers of the *stratum corneum* (5). When compared with the skin penetration of ingredients in traditional formulations (solution, gels or creams), nano-sized cosmetic formulations may enhance or reduce skin absorption or penetration, albeit at a limited order of magnitude (6,7).

Although very recently novel vesicular pharmaceutical formulations ("transferosomes") have been developed that appear to produce a genuine and significantly increased percutaneous drug delivery of drug ingredients, these pharmaceutical formulations are still under development (8).

Modern sunscreens contain insoluble titanium dioxide (TiO_2) or zinc oxide (ZnO) nanoparticles (NP), which are colorless and filter UV light more efficiently than micron-sized (>100 nm) particles.

In addition, the combination of particle-based and organic UV filters has a synergistic effect of UV scattering (particles) with UV-absorption (organic UV filters), which permitted the development sunscreen with high (>30) sun protection factors (SPF).

SKIN PENETRATION AND TOXICOLOGICAL PROPERTIES OF INSOLUBLE NANOPARTICLES

All available evidence, including numerous studies on skin penetration in human or animal skin as well as human subjects, demonstrated that TiO_2 or ZnO NP do not penetrate into or through normal as well as compromised human or animal skin (6,7,9,10,11). Very recent data suggested that ZnO or TiO_2 nanoparticles do not penetrate into normal or sunburned skin of minipigs (12).

The absence of skin penetration of insoluble nanoparticles is supported by decades of experience of transdermal drug delivery research, which has demonstrated that significant passive skin penetration may only be achieved for substances that combine a low molecular weight (<500 Dalton), low melting point (<200 °C) and a favorable logPO/W (between -1 and +3), i.e. properties that exclude insoluble nanoparticles (13).

The results of oral and topical *in vivo* toxicity tests showed that TiO_2 and ZnO NP have low toxicity and are well tolerated on the skin. Subcutaneous or intravenous administration of ZnO or TiO_2 nanoparticles produced no toxicity or unexpected body distribution (14, 15, 19). Cytotoxicity, genotoxicity and photo-genotoxicity studies on TiO_2 NP found no difference in the safety profile of micro- or nano-sized materials, all of which were non-toxic or non-genotoxic (16).

A recent study compared toxicological and ecotoxicological aspects two types of TiO_2 nanoparticle materials with those of a pigment-grade (micron-sized) materials using a battery of tests, including acute toxicity, sensitization, pulmonary toxicity, genotoxicity and toxicity to algae, Daphnia and fish. There was no difference in acute, inhalation, genotoxicity and ecotoxicity between the materials tested; they were all equally non-toxic (17). Although ZnO particles have been suspected to be photo-genotoxic *in vitro*, recent results demonstrated absence of a genuine photo-genotoxic potential (18).

A very recent study showed that single intravenous doses of ZnO nanoparticles, micro-particles and zinc sulphate in rats produced similar effects and were essentially non-toxic (19).

The toxicity of small insoluble and inert particles to mammalian cells, whether micron- or nano-sized, tends to be correlated with their cell uptake. Many mammalian cells *in vivo* or when cultured *in vitro* have a capacity for endocytosis or phagocytosis of small, insoluble particles. During phagocytosis / endocytosis, materials ingested are progressively enclosed by the cell membrane which eventually detaches to form an endocytic vesicle, whereas phagocytosis is a receptor-mediated characteristic for neutrophils, macrophages and dendritic cells and may result in active ingestion of insoluble particles up to 3 μm (20). Cells that phagocytose small insoluble particles will release reactive oxygen species and lysosomal enzymes in order to destroy or degrade the ingested particles, attempting to convert them to an ineffective, safer form (21). When phagocytosed particles cannot be degraded, they may accumulate in the cell resulting in oxidative cell damage, inhibition of cell proliferation and, ultimately, cytotoxicity and provoking a physiological response termed *activation*; the non-proliferating cell may release numerous inflammatory factors, resulting in inflammatory responses in adjacent tissues and stimulation of fibroblasts for fibrogenesis (22).

This well-known sequel, a normal physiological response of cells to an excessive amount of insoluble particles, may result in oxidative cell damage, such as lipid peroxidation, cytotoxicity and DNA damage, ultimately resulting in genotoxic effects (21, 23, 24, 25). Therefore, in order to avoid false positive artefacts, international guidelines for *in vitro* genotoxicity testing in mammalian cell cultures recommend testing of insoluble compounds only up to the lowest precipitating concentration (26).

Consequently, reports claiming the discovery of active penetration of nanoparticles into mammalian cells *in vitro* should be interpreted with great caution, since such findings may often be more convincingly explained by the phagocytic activity of treated cells.

Given that human keratinocytes have considerable phagocytic capacity (27), oxidative stress, antioxidant depletion and cytotoxicity in keratinocytes treated with carbon nanotube materials, such as reported by Shvedova et al. (28) or Monteiro-Riviere et al. (29) are not surprising and may be a normal response of mammalian cells to foreign and insoluble materials.

Similarly, findings suggesting *in vitro* genotoxic effects of small, insoluble particles in mammalian cells do not necessarily suggest an intrinsic genotoxic activity of these materials, but may be due to the same mechanism.

For example, the results of a recent report on the uptake of TiO_2 NP by murine brain microglia cells and subsequent generation of reactive oxygen species (30), do not necessarily mean that TiO_2-particles in sunscreens are potentially neurotoxic or may cause brain damage in people, as suggested by some NGOs, but could more convincingly be attributed to the known phagocytic capacity of microglia, which has been well characterised (31).

Our view is supported by a recent report suggesting that the principal route of uptake of quantum dots by mammalian cell cultures was via endocytosis (32).

On the other hand, a recent study suggested that the uptake of very small TiO_2 particles (22 nm-agglomerates of smaller particles) by lung macrophages and human blood cells may be via a non-phagocytotic mechanism (33).

More research may be needed to clarify the mechanisms of cellular entry and subsequent adverse effects of NP in cultured mammalian cells prior to applying such test results to a hazard identification of NP.

Results of studies on wear debris particles that include nanoparticles from surgical implants and other toxicity studies on insoluble NP support the experience of traditional toxicology that the toxicity of small particles is mainly related to their chemistry, rather than their particle size (22, 23, 32).

CONCLUSION

Overall, there is no evidence supporting a general rule that effects of particles to the skin or other tissues increase with smaller particle size, or produce novel toxicities when compared with those of micro-sized materials.

The current weight of current scientific evidence suggests that the use of nano-sized cosmetic or sunscreen ingredients poses no or negligible potential risk to human health (34).

The safety of insoluble nanoparticles in sunscreens has been reviewed and confirmed by several, international regulatory authorities (6, 7, 35, 36).

In summary, concerning the exposure of human skin and the human organism to nano-materials originating from topical application of cosmetics or sunscreens, the present knowledge may be summarized as follows:

- *Soluble nano-scale materials:* given that vesicle- or emulsion-type nano-sized formulations of cosmetic ingredients are intrinsically instable and rely on physical / chemical liquid / liquid interactions, it is uncertain whether they should be classified as genuine nanoparticles. Such formulations may produce reduced or enhanced skin uptake when compared with that from solutions, although at a limited scale. Therefore, the potential human skin and systemic exposure from nano-scale vesicle-type formulations should be rated similar to those of solutions of the respective ingredients.

Skin penetration of vesicle materials has been shown to be negligible.

- *Insoluble nanoparticles:* At present, the current scientific evidence suggests that insoluble nanoparticles used in sunscreens do not penetrate into or through normal or compromised human skin and produce no human local or systemic exposure or adverse health effects. Intravenous toxicity studies on TiO_2 and ZnO nanoparticles showed low intrinsic toxicity and no evidence of new or unexpected toxicities.

In conclusion, the weight of evidence suggests that nano-materials currently used in cosmetic preparations or sunscreens pose no risk to human skin or human health, in the contrary: they provide a large benefit to human health by protecting human skin against the adverse effects of UV radiation, including that of skin cancer.

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Safety assessment of cosmetic products containing nanomaterials. Current research trends and challenges

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Summary

Nanomaterials (NMs) may exert biological effects that differ from their macroscale counterparts. The combination of small particle size, large surface area, and ability to generate reactive oxygen species has been demonstrated to be a key factor in induction of cell injury following exposure to certain engineered NMs. Nanostructure can be associated with modification of biological properties and toxicological effects of ingredients used in cosmetics. However, the issue is controversial. Contrasting results have been obtained with widely used agents such as TiO_2 and ZnO.

From a regulatory perspective, there are two major uncertainties that impact on safety assessment strategies for nanotechnology-derived products. The first is as to whether nano-sized particles have to be considered new chemicals, or whether the use of existing materials at the nano-level should be considered a "new use" of an existing chemical. Addressing this question is of crucial importance to define adequate strategies and establish whether NM-tailored testing methods should be added to conventional toxicity testing protocols to comply with regulatory demand. A second issue is whether the classical toxicity testing methods and strategies that are currently used in the hazard evaluation of macroscale chemicals are adequate when applied to NMs.

NMs used in cosmetics can be divided into two groups: labile nanoparticles which disintegrate upon application to skin into their molecular components (e.g. liposomes microemulsions, nanoemulsions) and insoluble nanoparticles such as TiO_2, fullerenes and quantum dots. It is currently believed that while conventional risk assessment methodologies may be adequate for labile nanoparticles, specific toxicological and physicochemical parameters must be investigated to establish safety characteristics of insoluble particles. At the present time, the production and commercialisation of manufactured NMs do not trigger additional safety testing. However, the European regulation of chemical substances (REACH), which effectively shifts responsibility from authorities to industry to assess safety of chemical substances, is likely to represent a significant challenge in resolving the conflict between progress and protection of cosmetics containing NMs.

Riassunto

I Nanomateriali (NM) possono causare effetti biologici diversi rispetto a quelli prodotti dai corrispondenti composti esistenti su scala macrometrica. Studi sperimentali hanno dimostrato come la combinazione di tutte le peculiari caratteristiche chimico-fisiche, quali la dimensione estremamente ridotta delle particelle combinata ad un'ampia area di superficie, e l'abilità di generare specie reattive all'ossigeno e indurre stress ossidativo, sia un fattore chiave nel determinare fenomeni di insulto cellulare dopo esposizione a certi NM ingegnerizzati. La nanostruttura di questi materiali di nuova sintesi, come nel caso di vari ingredienti utilizzati nella produzione di cosmetici, è spesso associata a modificazioni delle proprietà biologiche e degli effetti tossicologici.

Comunque, l'argomento è ancora controverso; risultati sperimentali contrastanti sono stati ad esempio ottenuti con due composti "nano" ampiamente utilizzati nel mercato della cosmesi: titanio biossido (TiO_2) e zinco biossido (ZnO).

Dal punto di vista regolatorio, esistono a tutt'oggi due grosse incertezze sulle strategie di studio per valutare la sicurezza dei prodotti ottenuti con nanotecnologie. La prima è se una particella con dimensione "nano" debba essere considerata come un nuovo composto chimico, o se, piuttosto, l'utilizzo di materiali, già esistenti, su scala nanometrica debba essere considerato semplicemente un "nuovo utilizzo" di un composto chimico pre-esistente. Affrontare questo problema è di fondamentale importanza per poter definire strategie di studio adeguate e stabilire se nuovi test messi a punto ad hoc per i NM debbano essere aggiunti ai tradizionali metodi d'indagine per la caratterizzazione tossicologica, al fine soddisfare le emergenti esigenze regolatorie.

La seconda questione è se i test tossicologici tradizionali e le strategie correntemente utilizzate nella valutazione della pericolosità dei composti chimici su scala macrometrica siano adeguati allorché applicati ai nuovi NM.

I NM utilizzati nella cosmesi possono essere fondamentalmente suddivisi in due gruppi: le nanoparticelle labili, che, subito dopo l'applicazione sulla pelle, si scindono nei loro componenti molecolari (ad es. liposomi, microemulsioni, nano-emulsioni) e le nanoparticelle insolubili come il TiO_2, i fullereni ed i quantum dots. Si ritiene che, mentre per le nanoparticelle labili le convenzionali metodologie di "risk assessment" siano adeguate, specifici parametri tossicologi e fisico-chimici dovrebbero essere invece considerati ed attentamente studiati al fine di stabilire le caratteristiche di sicurezza per le nanoparticelle insolubili.

A tutt'oggi, nonostante la vasta produzione e commercializzazione di NM ingegnerizzati, non si è ancora provveduto alla messa a punto e applicazione di nuovi test adeguati per la determinazione della sicurezza di questi prodotti nanostrutturati. Ciononostante, le recenti direttive europee, con il nuovo regolamento destinato ad aumentare la sicurezza nell'ambito della produzione e dell'utilizzo di sostanze chimiche (REACH), che effettivamente sposta la responsabilità del "safety assessment" delle sostanze chimiche, sia pre-esistenti sia di nuova produzione, dagli organi regolatori direttamente alle imprese, sembra rappresentare un significativo passo avanti nella risoluzione del conflitto fra progresso e protezione nell'ambito del mercato dei prodotti cosmetici contenenti NM.

INTRODUCTION

Cosmetics and pharmaceutical products

That a cosmetic must be inactive with no biological or physiological interactions with cells and tissues is an ambiguous concept often contrasting with scientific evidence (31). The simplest cosmetic emulsion composed of oils and water is known to induce physiological skin modifications and is effective in increasing biological activity or promoting absorption of other active ingredients.

Experimental and clinical studies of cosmetic products have indicated a range of biological activities that are compatible with their observed (desired or adverse) effects. In principle, the ability to modify a biological function may be interpreted as an indicator of possible pharmacological activity. However, accurate studies of the mode of action and evaluation of effects occurring at various levels of biological complexity may valuably contribute to distinguish a cosmetic from a pharmaceutical product (31).

Application of *omics* and other special molecular techniques offer new opportunities to characterise biological effects of cosmetics and determine whether these biological activities do not reflect unacceptable toxicity and are compatible with the intended cosmetological applications. In the last years, molecular biology techniques have increasingly been used to assess the mode of action of ingredients that are currently found in the cosmeceutical marketplace such as retinoids, B vitamins, peptides, antioxidants, and polyhydroxy acids. These methods can also be used to define the biological effect profile of other emerging topical agents such as peptides, growth factors, nanotechnology-derived products, and a range of products proposed for natural skin defence, lightening and depigmentation. Findings obtained by these techniques clearly indicate that the boundaries between cosmetics and dermatological products are fading away rapidly (57). Certain cosmetics have been proposed in the treatment of minor skin disorders and mild skin abnormalities based on the hypothesis that they may be effective as adjuvant of physiological processes by mechanisms not implying direct pharmacological action. These dermatological applications should be decided case by case based on prudent evaluation of safety and risk-benefit issues.

In minor skin disorders, cosmetic treatment has been proposed as a valuable alternative to anti-inflammatory agents, antibiotics and other potentially dangerous drugs (56, 55, 30, 26, 31). Nanotechnology is expected to make it even less marked the difference of certain cosmetic products from pharmaceuticals.

Nanomaterials and cosmetol ogical applications

A future in which the ability to understand and control matter at the nanoscale is expected to represent a revolution in technology, medicine and industry that will benefit society (32, 50, 34). Today, NMs are present in a broad range of consumer products including colloidal health drinks, carbon fibre sport equipment, electronic products, as antibacterial components of toys, and cooking products. NMs have also entered numerous personal care products on the market, including sunscreens and cosmetics (43). Common nano-ingredients in cosmetic products include metal oxides such as titanium dioxide (TiO_2) and zinc oxide (ZnO), nanoemulsions and nanoencapsulated delivery systems (Table I).

However, concern has been expressed about the potential of adverse and unanticipated toxic effects of NMs on human health, apparently due to their unique and tunable chemical, mechanical, electrical, optical, or magnetic properties. These physico-chemical properties can lead to

NMs used in cosmetics can be classified into two general groups: (i) labile nanoparticles (e.g. liposomes, microemulsions, nanoemulsions), which disintegrate upon application to skin into their molecular components, (ii) insoluble or persistent particles such as TiO_2, fullerenes, and quantum dots. It is primarily for the insoluble particles that health concerns related to dermal contact and possible absorption arise. Should they become systemically available, translocation/transportation and eventual accumulation in secondary target organs may occur. This feature does become important with repeated application of cosmetic products.

According to the EU's statement in 2008 (European Commission, 2808), "current legislation covers to a large extent risks in relation to nanomaterials; these risks can be dealt with under the current legislative framework".

This concept, however, has become a matter of considerable debate among experts in the nanorisk community (5). In particular, many have questioned how the safety can be assessed given important knowledge gaps, as mentioned above, (Table II).

With respect to NMs used in cosmetics, it is generally believed that, for the labile NPs, conventional risk assessment methodologies based on mass metrics may be adequate whereas other metrics, such as number, surface area and size distribution of particles, should be adopted for testing insoluble particles.

In toxicological studies, it is crucial to assess transfer of nanoparticles across biobarriers and their possible uptake by tissues and cells following exposure by the intended route (dermal). Exposure via inhalation (occupational setting), ingestion (accidental exposure), conjunctival and mucosal surfaces may should be considered as relevant aspects for risk characterisation.

There are two controversial issues that significantly impact on regulatory aspects and safety assessment strategies for NMs used in cosmetology. The first is as to whether these nano-sized particles have to be considered as "new chemicals", or whether the use of existing materials at the nano-level should be considered a "new use" of an existing chemical.

Addressing this question is of crucial importance to define adequate testing strategies and establish whether NM-tailored studies should be integrated into conventional toxicity assessment to comply with regulatory demand.

A second issue is whether the classical toxicity testing methods and strategies that are currently used in the hazard evaluation of macroscale chemicals are adequate when applied to NMs.

In vitro toxicology has developed significantly in recent years, based on the 3Rs strategy: refinement, reduction, replacement put forward by Russell and Burch, 1959. *In vitro* assays are expected to become an essential component of toxicological and risk assessment research paradigms for chemicals, including pharmaceuticals, consumer products, and fine and ultrafine particulates. *In vitro* (cell culture) testing is also an essential element in all tiered approaches currently proposed for toxicity assessment of NMs.

In perspective, a great power is attributed to high-throughput systems that can be used for rapid and cost-effective screening of chemical hazards and identification of toxicity mechanisms evaluated at subcellular and molecular levels (4, 15). However, despite considerable growing application of *in vitro* systems to NM toxicity assessment (6, 24), large data gaps and methodological uncertainties remain with respect to evaluation of NMs in cosmetic products. In particular, only a limited number of validated *in vitro* methods exist that are applicable for regulatory purposes in risk assessment of cosmetics.

The validated *in vitro* assays currently in use are listed in Commission Regulation EC No 440/2008 of 30 May 2008, laying down test methods pursuant to Regulation EC No

1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) that came into force in June 2007.

Use of these methods is required for the safety assessment of cosmetic ingredients, according to the recent EU Cosmetic Product Regulation (EC 1223/2009), aiming at the complete phasing out of animal testing as indicated in the last 2010/63/EU. Validated alternative methods are currently available for assessing acute and short-term effects but not repeated-dose or long-term toxicity (Table III).

Notably, all these methods were originally developed for application to safety assessment of traditional chemicals and therefore still require rigorous scrutiny and validation studies to determine their general applicability to nanotoxicology. Other *in vitro* assays that could become a valuable tool for testing of NMs include the bovine cornea opacity permeability screening test (BCOP) to assess eye corrosives and irritants, the isolated chicken eye test (ICE), as well as IRE (isolated rabbit eye test) and HET-CAM (hen's egg test - chorioallantoic membrane). These approaches are not yet recognized officially by regulatory bodies for quantitative risk assessment.

To be acceptable for comprehensive and scientifically sound toxicity assessment of NMs, an *in vitro* testing strategy should take into careful consideration a set of pivotal toxicological endpoints, namely (i) penetration across physiological barriers (e.g. skin, lung, oral route), (ii) uptake and translocation, (iii) biochemical and functional cytotoxicity, (iv) induction of cellular stress with emphasis on oxidative stress and inflammation, (v) mutagenicity/genotoxicity.

TABLE III

Validated in vitro testing methods applicable to nanotoxicology.

Enpoint	***Methods***
– Skin irritation	Human skin models (Episkin™)
– Skin corrosion	– Transcutaneous Electrical Resistance Test (TER) – Human skin models (Episkin™ and Epiderm™
– Skin absorption	Franz cell using human/pig skin
– Phototoxicity	3T3 NRU
– Genotoxicity/Mutagenicity	– Bacterial reverse mutation test – Gene mutation test in *Saccharomyces Cerevisiae* – Sex-linked recessive lethal test in *Drosophila melanogaster* – Mammalian cell gene mutation test – Micronucleus test – Mammalian chromosome aberration test
– Embryotoxicity	– EST (embryonal stem cell test) – MM (micromass assay) – WEC (whole embryo culture)

A variety of techniques have been proposed in studies of dermal skin penetration of chemicals (49). However, due to the specific characteristics of NMs, doubts have been raised about the applicability of these techniques to topical formulations of cosmetics containing NMs, such as liposomes.

Useful information from *in vitro* studies can be obtained by morphological methods examining the post-treatment skin, such as bright-field microscopy, high resolution transmission electron microscopy and autoradiographic methods (1, 2). The requirements for testing the mutagenicity/genotoxicity potential of NPs do not differ from those regarding other agents. However, the peculiar properties of NPs may require further considerations.

Genotoxicity of NPs can be assessed *in vitro* using mammalian cells provided that exposure of the nucleus at relevant times for each assay is demonstrated. Validated *in vivo* assays can also be used, even though current experience with these assays applied to NM-containing cosmetics is limited and, in addition, it is difficult to establish whether the model would cover the expected target organ(s) for the NP of interest.

The EU Directive EC 1223/2009, adopted from 30 November 2009, is the first piece of supranational legislation that incorporates rules relating specifically to the use of NMs in any commercial products. The Directive (provisions of which will be applicable from 11 July 2013) confirms the ban of animal testing for the safety assessment of cosmetics, in accordance to the 7th Amendment.

An exception from this rule, which should be justified by specific regulatory needs, is the provision regarding strictly controlled *in vivo* assays of acute- and repeated-dose toxicity, reproductive toxicity and toxicokinetics. Since no alternatives are yet available, these studies are permitted, provided that limited numbers of laboratory animals are used. Experiments must be designed in order to minimize suffering of animals (2010/63/EU), after taking into account (i) any existing human and/or animal data on toxicity and toxicokinetics of the test substance or related materials, (ii) possible structure activity relationships (SAR), and (iii) results from *in vitro* or ex vivo tests. On these grounds, a tiered toxicity testing strategy has recently been developed in our laboratories as a preliminary research tool applicable to engineered nanomaterials (Table IV) (9).

TABLE IV

A proposed tiered toxicity testing strategy as a preliminary research tool applicable to engineered nanomaterials.

- Physico-chemical characterisation
- Evaluation of existing toxicological data
- In *silico* (SAR modelling, read-across, computational data gap filling, etc)
- *In vitro* (cell/tissue cultures)
- *In vitro* ex vivo
- Limited, justifiable *in vivo* testing
- Overall product evaluation and risk-benefit analysis

In summary, a number of validated methods may be used for toxicity assessment of NMs in cosmetics, according to the guidelines proposed by various European organisations such as the Scientific Committee for Consumer Safety (SCCS), ECVAM (European Centre for the Validation of Alternative Methods) and OECD (Organisation for Economic Cooperation and Development) guidelines.

However, despite a large body of research in this field, no methods are still available which could reliably predict the reactions of the intact organism exposed the cosmetics containing man-made nanoparticles.

CONCLUSIONS

Based on current information, some NMs that have been adopted by the cosmetics industry raise little concern. Other could present risks because of their new properties, such as resistance to degradation or tendency to cause oxidative stress. The growing production and commercialisation of engineered NMs poses the urgent need to develop effective testing strategies that can be applied for risk assessment purposes.

The challenge is developing a battery of tiered *in vitro* assays that can replace or reduce the application of existing *in vivo* protocols to fully understand the effects and mechanisms of toxicity of NMs.

The European regulation of chemical substances (REACH), which effectively shifts responsibility from authorities to industry to assess safety of chemical substances, is certainly a real incentive to promote scientific research aimed at protecting consumers against possible risks associated with NM-containing cosmetics.

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PaO (period after opening)

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Summary

The fundamental aim of the 2003/15/EC Directive is actually to guarantee the safety of the cosmetic products on the basis of new scientific progress in safety matters pertaining to the life-time of cosmetic products. In order to enhance consumer information PaO (Period after Opening) indication on the label aims to inform the consumer about the stability of the cosmetic, as it indicates the period of time after opening during which the product can be used without harmful effects.
For PaO (period after opening) determination of cosmetic products, no specific validated scientific methods exist, nor any standardized protocols (characterizing the most suitable analysis for the different products), or references that accurately document protocols of this kind (there are no recognized bibliographical references describing the effective correspondence between the residence time in thermostated rooms and the effective aging of the product). The evaluation must reasonably take into account the physical-chemical characteristics of the products and the normal or expected conditions of use. The environmental persistence test is proposed here for the determination of PaO of two commercial cosmetic products, a face powder and a daytime cream at the time of opening, and after accelerated artificial ageing in the artificial aging device in order to estimate the possible degradation.

Riassunto

La Direttiva 2003/15/CE del 27-2-03 che regolamenta i prodotti cosmetici, più nota come VII Modifica, sancisce l'obbligo per tali prodotti di indicarne nella confezione il PaO (Period after Opening) cioè il tempo di stabilità e quindi di sicurezza d'uso a partire dal momento di apertura della confezione. Per la determinazione del PAO dei prodotti cosmetici non esistono metodi scientifici specifici e validati, né protocolli standardizzati (che individuino la tipologia di analisi più idonea per i diversi prodotti), né riferimenti bibliografici che documentino con precisione protocolli di questo genere. La valutazione deve tener conto delle caratteristiche fisico-chimiche dei prodotti e delle normali o ragionevolmente prevedibili condizioni d'uso. In questo lavoro si propone un metodo per la

determinazione del PAO nei prodotti cosmetici; si applica il test di persistenza ambientale a due cosmetici commerciali (cipria e crema da giorno) all'atto dell'apertura, e dopo l'invecchiamento artificiale accelerato in veterometro, pervenendo alla conclusione che tale risposta può essere diagnostica ai fini della sicurezza del consumatore.

INTRODUCTION

Stability and state of preservation of cosmetic products

The European Union (EU) has set up a rapid alarm system for non food products, including cosmetics, which poses a serious risk to public health (RAPEX), together with provisions for withdrawal from the market of products representing a threat to consumers' health and safety.

In Italy the cosmetics sector is regulated by law no. 713 of 11 October 1986 and subsequent modifications (Decree law no. 300 of 10 September 1991, Decree law no. 126 of 24 April 1997, Decree law no. 87 of 15 February 2005).

In the early 1970s the Member States of the European Union decided to harmonize their legislation governing cosmetic products in order to avoid the circulation inside the Community of non controlled products.

Following a wide-ranging discussion among experts from all the Member States a Directive (76/768/EEC) was adopted on 27 July 1976. The principles underlying this Directive are related to consumer's rights, the encouragement of trade exchanges and the elimination of the trade barriers.

For example, a product that is to circulate freely in Europe must have the same type of packaging, label and safety rules.

The fundamental aim of the Directive is actually to guarantee the safety of the cosmetic products.

On the 27 February 2003 this Directive was further completed and modified (2003/15/EC) on the basis of new scientific progress in safety matters.

As a first consequence of this Directive, on the 5 September 2003, the European Commission issued important safety regulations (Directive 2003/80/EC) pertaining to the life-time of cosmetic products in order to enhance consumer information.

Directive 2003/15/EC of 27 February 2003, incorporated into Italian legislation by DLgs no.50 of 15 February 2005 (1) provided for the introduction of two new labelling regulations and laid down that, as from the 11 March 2005, any cosmetic product that does not comply with the new regulations cannot be manufactured in or imported into the European Community. However, no provision was made to limit transfer to the final customer to whom cosmetics complying with the pre-existing regulations may legitimately be sold provided they were marketed prior to 10 March 2005:

1. For production with a minimal duration greater than 30 months after opening, better known as PaO (Period after Opening), the indication consists of a small open-jar symbol accompanied by a figure indicating the number of months and the letter "M" inside or close to the jar symbol. It was adopted throughout the European Union with the Directive 2003/80/EC of 5 September 2003:

This symbol is particularly important for products that, once opened and coming into contact with the environment, could be subjected to degradation and are likely to become dangerous (for example, due to microbiological contamination).

The PaO must be indicated on the primary and secondary packaging of the cosmetic (primary packaging is the container in direct contact with the product, while secondary packaging may be, for example, the envelope containing it). On some products, for example, single-use disposable products or those for which, owing to their composition or method of manufacture, display practically zero risk of alteration (for example, products that do not allow direct contact between the contents and the outside environment, such as spray products under pressure), the symbol will not appear as it is not necessary.

2. the inclusion in the list of ingredients of the presence of one or more of the 26 molecules identified by the Scientific Committee for Products destined to Consumers (SCCP) as an important cause of allergic reactions due to contact among consumers allergic to perfumes.

PaO indication on the label aims to inform the consumer about the stability of the cosmetic, as it indicates the period of time after opening during which the product can be used without harmful effects.

The PaO symbol must be present on the labels of all the cosmetic products, with the exception of:

- products with a durability of less than 30 months, as these products already carry a "best use before" date;
- single-dose products (for example, free samples);
- products manufactured in a such way as to avoid contact between the cosmetic and the surrounding environment (for example, aerosols);
- products for which the producer certifies that the formula is such as to prevent any risk of deterioration having a negative effect on the product's safety over time.

The new PaO symbol have been progressively introduced: all the products affected by the Directive are labelled with this symbol since 11th March 2005.

Products without PaO, already marketed before this date, can be continued to be sold. In order to clarify the meaning and interpretation of the post-opening period, it is necessary to specify that:

- PaO is an indicative period of time established on the basis of the knowledge acquired from the manufacturers concerning their products;
- a cosmetic is considered "opened" when it is used for the first time. The post-opening period must therefore be computed from this first use;
- the information justifying the presence or absence of PaO is accessible to the control authorities.

Toxicity

Art. 3 of the Directive 92/32/EEC of 30 April 1992 regulating the classification, packaging and labelling of dangerous substances marketed in European Union countries provides for the "determination and the evaluation of the property of the substances" through toxicological tests involving experiments on animals. According to the results of the experiments, a substance will be classified in one of the following categories:

- very toxic
- toxic
- injurious
- not dangerous.

A standard protocol provides for the study of:

1) Short-term toxicity, subdivided into the study of:
   - acute toxicity, normally carried out on mouse or rat, using LD_{50}.
   - irritation of the eyes, skin and mucous membrane, usually carried out on albino rabbits by means of "Draize tests" in cosmetics and aimed at assessing the tolerability of the skin or mucous membrane to contact with the substance under investigation.
   - sensitization, normally carried out on guinea pigs to assess the potential of the chemical substance to induce allergic or immune responses as a result of multiple administrations.
2) Repeated toxicities, the assessment of which is carried out through the study of:
   - sub-acute, sub-chronic and chronic toxicity, usually performed on two species, one of which a rodent (normally rat), and mon-

key or dog. The duration of the studies varies from two to four years; the administration method is that of exposure.

- oncogenesis.

3) Reproductive toxicity and teratology in order to detect any interference by the new substance in the reproductive sphere and on offspring; the studies are subdivided into three groups:
   - Fertility and reproduction
   - Teratology.
   - Peri-post natal toxicity studies

In short-term toxicity (acute effect) the toxicity level is defined on the basis of the lethal amount of the chemical compound and the method of exposure; the Lethal Dose, 50% and Lethal Concentration, 50% used to classify a product as very toxic, toxic or injurious are set out in the following (Table I) (2):

LD_{50} is the amount of substance that, administered in a single dose, causes the death of 50% of the experimental animals; it only indicates the short term toxicity (acute toxicity) of the substance not the long term toxicity (which is the result of contact with small amounts of a given substance over long periods of time); it is usually expressed as the amount of substance administered versus the weight of the sample animal (for instance, mg of substance per 100 grams for small animals or per kg for larger animals); the administration method must be also defined (oral, cutaneous, etc.). An LD_{50} greater than 2000 mg/kg means that the substance tested may be considered as not particularly dangerous.

For oral LD_{50} EU regulations prescribe rat as the experimental animal, while for cutaneous LD_{50} the use of rabbit is also allowed.

LC50 denotes the concentration in the atmosphere that causes the death of 50% of the experimental animals when inhaled for a given period of time. For LC50 EU regulations prescribe the use of rat as experimental animal with an exposure of 4 hours.

The methods or procedures leading to the substitution of an animal experiment or the reduction of the number of animals required, and to the optimization of the experimental procedures in order to reduce animal suffering are alternative methods to experimentation in vivo. This concept corresponds to Russel and Burch's "3Rs" (3): replacement, refinement, reduction:

1) replacement identifies the substitution, where possible, of higher animals with biological materials of lesser complexity (cellular bacteria, cultivations, isolated organs, cultivations in vitro), computerized models, videos, films;
2) refinement refers to the search for increasingly specific experimental procedures that can at least reduce the suffering and stress of the animals used
3) reduction is aimed at limiting the number of animals used for a particular experiment that achieves given study results. This can be achieved by standardizing the animal population, the main factor involved in result variability.

TABLE I

Category	Oral LD_{50} mg/kg	Cutaneous LD_{50} mg/kg	Inhaled LC_{50} mg/L/4 hours
very toxic	<25	<50	<0.5
toxic	25-200	50-400	0.5-2
injurious	200-2000	400-2000	2-20

Methods of the first type allow certain experimental information to be obtained without using animals; methods of the second type are suitable for obtaining comparable levels of information using a smaller number of animals and allow maximum information to be obtained from a single test on animals; methods of the third type all allow suffering and damage caused by the experimental practices to be reduced.

Methods of the first type include "biological substitutive methods" and "non biological substitutive methods"; the former ones consist of "in vitro methods", and make use of various types of biological material (of human or animal origin); the latter ones take advantage of the contributions of sciences such as mathematics, computer science and statistics, etc.

A new experimental approach, in order to be considered alternative to conventional animal experimentation, must be reproducible, reliable, rapid and not more expensive than the one it is intended to replace.

The European centre responsible for enforcing the above parameters by the new method ("validation") is the ECVAM (European Centre for the Validation of Alternative Methods), established by the European Commission in 1991 at the proposal of the European Parliament as part of the "Joint Research Centre" in Ispra (Varese/Italy). ECVAM coordinates the validation of the alternative methods at Community level, and constitutes a point of reference for the exchange of information on the development of these methods by means of a database of available methods (either already validated or in the process of validation) managed by the same centre.

The reliability and importance of a method are established through the validation process. Reliability refers to the reproducibility of results in time and space, that is, both in the same laboratory and in different laboratories (so-called "standardization"); significance measures the usefulness and importance of the method with regard to a given aim.

Validation tests are extremely long (they can even last years) and are designed to verify if, for given substances, a new method produces results similar to those previously obtained by means of animal experimentation.

The ultimate destination of a new method is its inclusion in the international regulations, and the introduction of alternative tests among the guidelines of the OECD (Organization for Economic Cooperation and Development).

The OECD not only gathers together the member countries of the European Union but also the United States, Japan and others; it has the task of harmonizing the different experimental protocols in the form of guidelines. OECD guidelines are periodically modified in order to adapt them to new scientific knowledge and to any legislative changes.

A EU White Book (4) invites the scientific community to make the utmost effort to develop chemical tests that do away with the need for animal experimentation, and that can provide information – particularly danger warnings concerning the toxicity of a compound – in real or quasi real time.

AIM OF THE WORK

For PaO (period after opening) determination of cosmetic products, no specific validated scientific methods exist, nor any standardized protocols (characterizing the most suitable analysis for the different products), or references that accurately document protocols of this kind (there are no recognized bibliographical references describing the effective correspondence between the residence time in thermostated rooms and the effective aging of the product).

The evaluation must reasonably take into account the physical-chemical characteristics of the products and the normal or expected conditions of use.

The environmental persistence test is proposed here (5) for the determination of PaO of two commercial cosmetic products, a face powder (the PaO on the label is 24 months) and a daytime cream (the PaO on the label is 12 months) (both opened and unopened cosmetics) at the time of opening, and after accelerated artificial ageing in the artificial aging device in order to estimate the possible degradation.

Persistence

Persistence is related to the way a substance is used, its toxicity and its life cycle; it is an important index, both positive, regarding the assessment of durability or longevity during the use of a product, and negative, regarding the assessment of chemical pollutant risk, related to the prolonged time of interaction with human organism and ecosystem.

Environmental persistence, in the positive sense of durability, is the ability of a material to maintain its physical – mechanical characteristics over time and its original appearance. Environmental persistence, in the negative sense of environmental risk assessment of chemical substances, is related to the absence of degradability in the environment and indicates that a substance is not easily biodegradable by bacteria, fungi or other natural agents; is the result of either the absence or inefficiency of sinks or of the inability of a substance to reach potential sinks: a substance will only disappear from the environment if it is degradable, the rate of disappearance depending on the kinetics of the process involved.

Ecopersistence is not directly measurable, but can only be inferred from the continuous presence of a substance in the environment or from a lack of laboratory degradation data.

The European Commission (6) defines organic compound persistence in terms of half-life of the single medium or on the lack of any half-life as the result of the studies and biodegradation tests carried out. While some of these tests are suitable for identifying the substances as non persistent (that is substances subjected to rapid and complete biodegradation in all environmental compartments), they could be not utilized to classify a chemical substance as "persistent"; generally speaking, a substance with a half life greater than one year in all environmental compartments is considered "persistent" (7).

Principles of the ecopersistence test method

In the present work, an innovative method is proposed for evaluating environmental permanence or ecopersistence (5); this both positive and negative index is determined using a titanium dioxide photosensor.

Titanium dioxide, TiO_2, is the most important Ti compound with an oxidation degree of +4; it represents one of the more efficient photocatalysts of oxidative degradation of many organic compounds (8-10); it is the most frequently used semiconductor among those available as it is photostable, non toxic ($LD_{50} > 10000$ mg/kg) and insoluble in water. When TiO_2 absorbs light having an energy ≥ 3.2 eV (band gap energy of titanium anatase), electrons and holes are formed in the conduction and valence bands, respectively (e^-_{cb} and h^+_{vb}).

$$\mathrm{TiO_2 + h\nu \rightarrow TiO_2(e^- + h^+) \rightarrow TiO_2(e^-_{cb} + h^+_{vb})}$$

$$\mathrm{e^-{}_{cb} + O_2 \rightarrow O_2^{\bullet -}}$$

$$\mathrm{h^+_{vb} + H_2O \rightarrow OH^\bullet}$$

$$\mathrm{O_2^{\bullet -} + OH^\bullet} + \text{organic substance} \rightarrow \text{organic substance degradation } (\mathrm{CO_2} + \mathrm{H_2O} + \text{mineral acids})$$

Hydroxyl radicals are powerful oxidizing agents and readily oxidize organic pollutants and subsequent intermediates formed during the mineralization process to carbon dioxide.

The superoxide anion radical, formed as a result of oxygen reduction from photogenerated electrons (eq. 4), itself having a relatively low oxidant capacity, can combine with organic peroxy radicals (ROO.) to generate an unstable tetroxide (ROOOOH) which decomposes into reaction products (10-11).

Species such as $HO_2^{\bullet}$, $O_2^{\bullet}$, H_2O_2, are present either at the interface or in solution and can be involved in the complex degradation scheme leading to the final mineralization of organic substrates. TiO_2 due to its structural composition can also act as pH indicator (5, 12).

Several tests were performed to evaluate the performance of TiO_2 as a pH indicator in terms of calibration curve slope (mV/pH), potential (pH) jump on acid and base additions and end-point potentiometric acid-base titrations (5).

The 350 nm UV photoactivated TiO_2 acts as a photocatalyst of organic substance degradation and as a pH material indicator. It therefore allows measurement of the time needed to reach the acidification corresponding to the production of CO_2 due to the decomposition of the considered compound.

The beginning of the process of acidification can be considered as the conclusion of the period of induction; the rate of pH variation over time can be considered as to be calculated starting from this time: higher it is, greater the concentration of carbonic dioxide and carbonic acid in environment and of mineral acids produced by mineralization in unit time.

Before acidification TiO_2 potential (pH) versus irradiation time generally decreases (the pH increases) to a minimum (maximum); after the induction time, it changes slope and begins to increase (the pH decreases).

The time needed to observe the change of slope (induction or delay time) is related to the beginning of the degradation of molecules leading to CO_2, H_2O and mineral acid production; after this time, TiO_2 potential (pH) increases (decreases) as rate of mineralization (Figure 1).

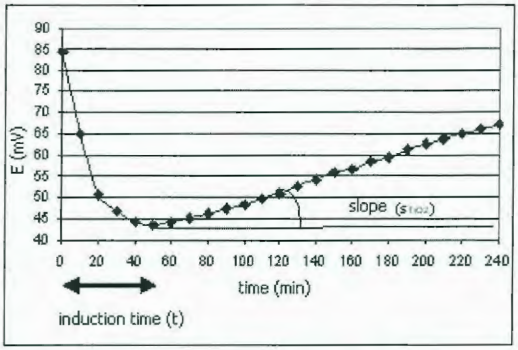

Fig. 1 *Typical trend of titanium dioxide potential versus UV irradiation time (350 nm) for an organic compound.*

We reasonably assume that the index of environmental persistence, P_{env}, or ecopersistence, is assumed to be the ratio between the induction or delay or activation time t, needed to induce the degradation, and the curve (straight line point) slope s of the increasing TiO_2 potential versus irradiation time $sTiO_2$ (the increase of TiO_2 potential with time corresponds to the increase of acidity over time):

$$P_{env} = \frac{t}{s_{TiO_2}}$$

In some cases degradation takes place without any activation time, t=0, therefore $P_{env} = 0$, and $sTiO_2$ is taken as an index of degradability assessment.

Experimental section
Apparatus

UV source: Polilight®, supplied by Rofin (Dingley, Victoria, Australia), equipped with high quality interference filters, at central UV wavelength of 350 nm.

For ecopersistence test: Orion pH Meter (model 420) for the measurement of titanium dioxide potential (uncertainty was ± 0.1 mV).

For artificial weathering of real matrices: Weatherometer QUV Accelerated Weathering

Tester (Q - Panel Company, Cleveland, OH USA) - Model QUV/spray Q-Panel LAB-Products in the following conditions of UV exposure: spectral irradiance 0.6 $W/m^2/nm$, relative humidity 58 %, T = 45 °C, λ = 310 nm.

This apparatus was equipped with 8 mercury lamps (40 W each), perfectly simulating sunlight up to 370 nm and relatively well up to 400 nm.

Commercial cosmetics: face powder and daytime cream.

Ecopersistence measurement

Titanium dioxide phototocatalyst was used in suspension with the suspension acting as electrode. The photoreactor was a 150 mL Pyrex glass beaker. The 350 nm *Polilight®* was placed inside the photoreactor and 0.5 cm away from the free surface of the suspension.

For each analysis the suspension consisted of 50 mg of TiO_2 in 105 mL of

- cosmetic product as is: 5 mL of daytime cream or 1.5 g of face powder;
- aqueous extract of the cosmetic product (just opened vase, artificially aged in a weatherometer in an opened vase, artificially aged in a weatherometer in a closet)
  - 1.5 g of face powder in 300 mL of water,
  - 4 g of daytime cream in 800 mL of water, after two filtrations;

RESULTS

The results of the measurements on just opened cosmetics did not allow the P_{env} value to be estimated because TiO_2 potential continues to decrease for the full duration of the analyses (up to 8h) so lacking the value of the induction time. The difficulties encountered in applying the test could be due to the cosmetics' composition; the presence of the fat component probably renders impermeable the suspension/emulsion of cosmetic in TiO_2/water and prevents the passage of ultraviolet radiation and thus does not allow catalyst activation.

The cosmetic product was then extracted. The results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged, artificially aged in closed pots and in an opened pot up to 200 h, are reported in Table II.

TABLE II

Results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged, artificially aged in closed pot and in opened pot up to 200 h.

Aqueous extracts of cosmetic products	P_{env} (min^2/mV)		
	Just opened and unaged	**Artificially aged in closed pot up to 200 h**	**Artificially aged in opened pot up to 200 h**
Face powder	293±10	290±10	75±5
Cream	114±10	112±10	30±3

The results of ecopersistence tests carried out on aqueous extracts of cosmetic products, just opened and unaged and in an opened pot up to 400 days, are reported in Figure 2 for face powder and in Figure 3 for cream of labelled commercial products.
The values of the time for which P_{env} falls down abruptly are perfectly corresponding with PaO value if to 1 week of artificial ageing in the described conditions we let correspond 1 year of natural ageing.

CONCLUSIONS

PaO values for cosmetics, generally determined by chemical and biological long tests of stability can be rapidly and reliably determined by the proposed photosensor and artificial ageing of the cosmetic samples basing on a correspondance factor of natural and artificial ageing in controlled conditions and on the behaviour of P_{env} during artificial ageing.

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第十届国际美容皮肤科学大会

10th International Congress of International Society of Cosmetic Dermatology

2011年5月30日、31日、6月1日 中国沈阳

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Invited speakers (Listed in alphabetic order)

Leslie Baumann	Baumann Cosmetic & Research Institute, Miami, FL
Anthony V Benedetto	University of Pennsylvania School of Medicine, Philadelphia, PA
Ying-Jui Chang	Strait Exchange Academy for Dermatology and Esthetic Medicine, Taiwan
John ZS Chen	Sheftel Associates Dermatology,Tucson, AZ
Won Soon Chung	Yonsei Star Skin & Laser Clinic, Korea
Richard Clark	State University of New York, NY
Umberto Cornelli	Loyola University School of Medicine, Chicago, IL
Michael H. Gold	Gold Skin Care Center,Tennessee Clinical Research Center, TN
Philippe Humbert	Central Hospital of University of Franche-Comte, France
Sewon Kang	Johns Hopkins University, School of Medicine,Baltimore, MD
Mu-Hyoung Lee	Korean Society of Cosmetic Dermatology, Korea
Henry W. Lim	Henry Ford Hospital,Detroit, MI
Mary Matsui	The Estee Lauder Companies Inc. Melville, NY
Pierfrancesco Morganti	International Society of Cosmetic Dermatology, Italy
Yoon-Kee Park	Korean Society of Cosmetic Dermatology, Korea
Schwartz A. Robert	New Jersey Medical School,Newark, NJ
Michele Verschoore	L'Oreal, France
Huachen Wei	Mount Sinai Medical Center,New York, NY
Manuskiatti Woraphong	Siriraj Hospital,Mahidol University, Tailand
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4. Laser skin rejuvenation.
5. Non-laser skin rejuvenation (BTA, filler...).
6. Beauty from inside out.
7. Pearls in dermatalogic surgery.
8. What's new in photoprotection.
9. Antioxidants (Dialogue between the East and the West).
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