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Nanoparticles for cosmetics. How safe is safe?

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Resum

L'ús creixent de materials nanoparticulats qüestiona la seguretat d'aquests. Un cas paradigmàtic és la seva aplicació en cosmètica, en principi degut a què aquests materials estan directament en contacte amb el cos i perquè probablement la utilització de la nanotecnologia en cosmètica s'anticiparà a la utilització de nanopartícules en medicina. Fins el moment present, no s'han observat efectes tòxics aguts, i ja s'estan fent servir quantitats elevades de nanopartícules en protectors solars. No obstant, els efectes observats sobre la interacció entre nanopartícules i estructures biològiques requereix una millor comprensió d'aquesta interacció i un desenvolupament prudent dels productes de consum basats en nanopartícules. En aquest article, resumim els fets coneguts en relació a l'ús de nanopartícules en cosmètica.

Paraules clau: nanoparticules · toxicología · biodistribució

Abstract

The increasing use of nanoparticulate engineered materials posses the question on the safety of those materials. A paradigmatic case is their use in cosmetics, in principle because those materials are in direct contact with the body and because probably cosmetic usage of nanotechnology will anticipate the use of nanoparticles in medicine. Non acute toxic effects have been observed so far and some tones of some nanoparticles are already used in sunscreens. However the observed effects of interaction between nanoparticles and biological structures calls for a better understanding of that interaction and a prudent development of consumer products based on nanoparticles. In this paper we summarize the known facts regarding the use of nanoparticles in cosmetics.

Keywords: nanoparticles · toxicology · biodistribution

1. Nanoparticles and Cosmetics

Nowadays, there are almost 600 manufacturer nanotechnology-based consumer products on the market. Last year, nanotechnology put $30 billion of manufactured products (a number predicted to grow to $2.6 trillion by 2014) on the market and moreover, the National Science Foundation estimates that by 2015 the nanotechnology sector will employ more than 2 million workers. Products ranging from computer chips to automobile parts and from clothing to cosmetics, dietary supplements, wound dressings, dental-bonding agents, fuel cells, tires, optics, clothing, and electronics[10]. In some of these products, such as skin creams and toothpastes, NPs are in direct contact with the body or can enter the environment on a continual basis from washing off these products [4].

In the cosmetic industry luxurious raw materials and active principles as gold, caviar or silk are used because they sound more attractive than bovine placenta or cream of snail slime. At the same time high tech products are also employed, as delivery systems, second-skin technology, botox-like product, Derma Membrane Structure, etc., being the marketing strategies very important. In such a way that while combining new and high tech products can be appealing and have a positive result in the market, it may increases the health risks in this sector.

Regarding conventional cosmetics, the main materials of pigments which are used in most products, including lipsticks (from where they can reach the mouth), include about 90 kinds of tar pigments separated and synthesized from petroleum. Also, metal compounds are used as pigments to develop colors, and examples thereof include heavy metal compounds such as lead, cadmium, iron oxide. These pigments show excellent color development, thus leading to clear colors, and some of these pigments have good thermal resistance and light resistance. But these metals are insoluble in water and soluble only in some solvents. Also, they are generally present in a dispersed form, but are difficult to maintain in the form of a stable dispersion for a

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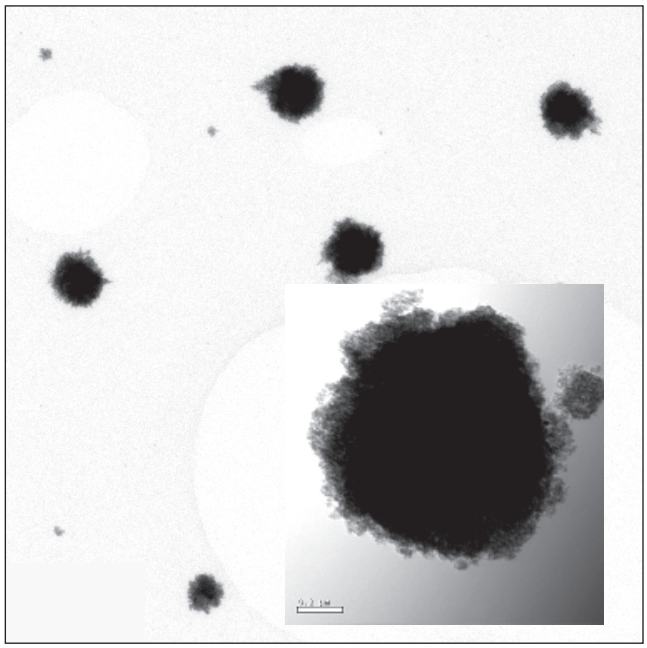

Figure 1. Commercial NPs in Sunscreens. Transmission electron image of a commercial base for sunscreens observed in our lab. Inset scale bars is 200 nm.

long period of time due to the cohesion between pigment particles or the settlement of the pigment particles. In addition, these pigments are substances harmful to the human body, which can cause side effects such as skin diseases.

Now, nanometer sized materials have characteristics that improve the existing cosmetics materials, or at least the product appeal. A material which has raised a number of concerns and deserves special attention is TiO_2 (and ZnO) present in sunscreens. TiO_2, also used to mineralize many undesired organic pollutants, is considered to be a safe physical sunscreen agent which reflects and scatters both UVB (290-320 nm) and UVA (320-400 nm), the principal cause of skin cancer. However, it also absorbs substantial UV radiation which, in aqueous media, yields to hydroxyl species. These species may cause substantial damage to DNA [5,8], therefore raising concerns on the overall effects of sunscreens [13]. Significant efforts to limit DNA damage based on particle surface modification have been developed [20]. This data appears in controversy to the dermatological tests and the ubiquity of TiO_2 NPs in sunscreens or white pigments since ancient times [1], and the answer lays on the aggregation state of the nanometric grains (Fig.1). A part from TiO_2, Au is other of the proposed molecular carriers. Gold nanoparticles show some facilities compared with the other type of carriers. For example, they present control delivery properties, rich functionality, good biocompatibility and they also can be easily prepared and size-controllable [3]. Commonly, this can be achieved by the derivatization of the gold nanoparticles surface. In general, gold nanoparticles are considered to show low toxicity (for example, they have a high LD50, or LD80, at a concentration level of 1mg/ml, while cationic carriers have 10 µg/ml)[24]. Despite all this, you also have to take into account that they are not biodegradable. Not being biodegradable supposes a great advantage during the whole

Figure 2. Cosmetic Magic Colors. Lead-based chemistry, which was initiated in Egypt more than 4000 years ago, could result in the synthesis of lead sulfide nanocrystals by the judicious combination of naturally available minerals with oils. With a diameter of about 5 nm, the appearance of these crystals is quite similar to PbS quantum dots synthesized by modern materials science techniques (the picture does not correspond to the cited materials).

delivery process, but a very important problem in terms of the nanoparticles being accumulated in cells or circulate in plasma, as individual particles or agglomerates.

In nanotechnology, there is a significant effort to obtain isolated NPs because the appealing properties of NPs progressively disappear as NPs agglomerate. Indeed the revolutionary control of matter properties (optical, mechanical, magnetic, electrical, chemical…) are for individual particles with sizes below 100 nm [11], being them single crystal or polycrystalline (off course, the properties will be affected by the existence of grain boundaries). In fact, NPs have to be in no-physical contact to maintain their special nanometric properties (what in turn can be used as a molecular agglomeration test, as in the case of complementary DNA [16]. Therefore, many strategies are being developed to have isolated particles during longer periods, based on the steric or the electrostatic effects [17]. This may be one of the critical differences with the previously existing NPs, since natural and unintentional occurring NPs tend to agglomerate readily. This, the degree of agglomeration, will have determining consequences of the final behaviour of the nanomaterial. For example, looking at NPs used for sunscreens under the microscope, we can observe large agglomerates made of nanometric domains (Fig.1). In this case, penetration of such particles beneath the external skin should be very difficult due to the irreversibility of NP agglomeration and consequently the incorporation of such aggregates into the skin. It is true that the agglomerates are still efficient sun blockers, however their optical properties are less intense than the individual NPs.

One may think that NPs are new things in cosmetics. Thus, these days we are debating if in sunblock and toothpaste are safe. The ancient Greeks and Romans didn't know about such things — but they already used nanotechnology in their cosmetics. A group of researchers in France showed that lead-based chemistry, which was initiated in Egypt more than 4000 years ago, could result in the synthesis of lead sulfide nanocrystals by the judicious combination of naturally available min-

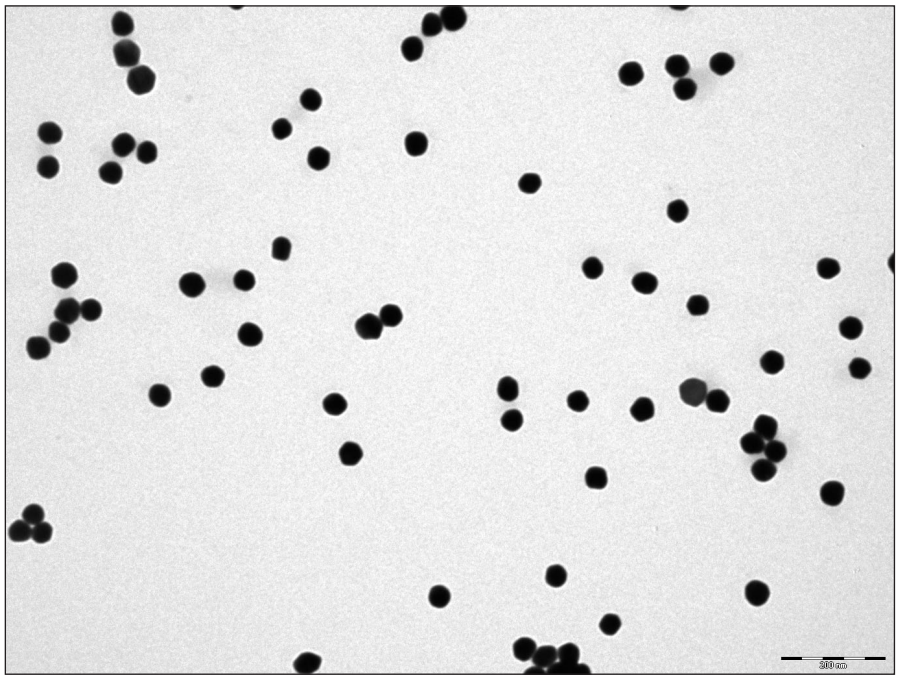

Figure 3. Electron Microscopy of Nanoparticles. 40 nm Au Nanoparticles grown from 5 nm Au seeds by a multiple injection procedure.

erals with oils. With a diameter of about 5 nm, the appearance of these crystals is quite similar to PbS quantum dots synthesized by modern materials science techniques [23] (Fig.2).

2. Nanoparticles and cells

The biological activity and biokinetics of nanoparticles (NPs) depend on different parameters: size, shape, chemistry, crystallinity, surface properties (area, porosity, charge, surface modifications, weathering of coating), agglomeration state, biopersistence, and dose (Fig.3). These parameters are likely to modify biological responses in a yet unknown way. We are therefore in a better safe than sorry scenario.

If NPs enter in contact with human beings, information about the consequences can be extracted from in vitro cell culture models. If we look at Au NPs, which have very often reported to be non toxic, we can find that some in vitro studies show that the interaction of NPs with cells may be deleterious. Tsoli et al. [22] showed that very small Au nanoparticles, approximately 1nm in diameter, can penetrate both the cell and nuclear membranes and become attached to DNA. Hence, this indicate that toxicity may simply result from decreasing the dimensions of a material to the nanometer range, rather than changing its chemical nature.

Nadie Pernodet et al. [15] investigated the effects of citrate/gold nanoparticles at different concentrations and exposure times on human dermal fibroblasts. They found that, as a result of intracellular nanoparticle presence (14-nm particles can easily cross the cell membrane and accumulate into endosomes), actin stress fibers disappeared, thereby inducing major adverse effects on cell viability. Thus, properties such as cell spreading and adhesion, cell growth, and protein synthesis to form the extracellular matrix were altered dramatically. These results suggest that the internal cell activities have been damaged.

Goodman et al. [7] used MTT, hemolysis, and bacterial viability assays to explore differential toxicity among the cell types, using 2 nm particles. These studies show that cationic particles are moderately toxic, whereas anionic particles are quite nontoxic. Toxicity has been observed at high concentrations. Also they have demonstrated that the toxicity of the gold nanoparticles is related to their interactions with the cell membrane, a feature initially mediated by their strong electrostatic attraction to the negatively charged bilayer.

Studies by Kim et al. [9] in mice and pigs with intradermal injected near-infrared quantum dots confirmed that NPs, once cross dermis, will be localized in regional lymph nodes being then useful for *in vivo* imaging. The transport mechanisms to the lymph nodes are via skin macrophages and dendritic (Langerhans) cells [14,19]. From the lymph nodes they may reach the liver and the kidneys by venal translocation. On the other hand, it has to be considered the potential of sensory skin nerves to take up and translocate NPs. This mechanism has been demonstrated for the nasal region. Therefore, it will be interesting to know if this occurs in the dermis layer of the skin with its dense supply of different types of sensory nerves.

3. Nanoparticles under the skin

In cosmetics, the important uptake route is through dermal exposure. [21] The dermis has a rich supply of blood and tissue macrophages, lymph vessels, dendritic cells, and five different types of sensory nerve endings. An increased inflammatory activity and epithelial translocation of manmade 20 and 30 nm solid particles was observed already 20 years ago [6,12]. Broken skin represents a readily available entry even for larger (0.5-7 μm) particles, as evidenced by reports about accumulation of large amounts of soil particles in inguinal lymph nodes from people who often runs or walks barefoot [13]. However reports

show that broken skin is not necessary for uptaking NPs. Tinkle et al. [21] hypothesized that skin when flexed—as in wrist movements—can make the epidermis more permeable to NPs, and then favour uptake into the lymphatic system and regional lymph nodes. In those studies, a solution of buckyball-containing amino acids was placed on small sections of pig skin. In some of the experiments, the skin was held still, and in others it was flexed for either an hour or an hour and a half. Measurements were taken eight hours after exposure and 24 hours after exposure. The more the skin was flexed, the more buckyballs were uptaked and they penetrated deeper. Penetration was also found to be deeper after 24 hours than just after eight hours. Similarly, It has been shown that repetitive movement can speed up the uptake of nanoparticles through the skin [18], as it happens for conventional anti-inflammatory gels, where massaging the affected area translocate the gel to the swollen tissue.

On exposure to the body, particles of different surface characteristics, size and morphology attract different arrays of opsonins. The content and conformation of these hybrids may account for the different patterns in the rate and site of particle clearance. Surface bounded molecules will travel with the NP permanently or not, and may therefore arrive to different places at different times. Agglomeration, a part from embolization, will deactivate the NP or the NP conjugates likely causing either deleterious effects or rapid elimination. Finally, internalization inside the cell may easily trigger cell responses which may result inadequate or hazardous, as inflammation stimulated by the presence of NPs.

Thus, if active molecules are attached to Au NPs, the conjugated molecule will penetrate the cell with the nanoparticle. Altough Au NPs have been demonstrated not to be toxic and on the other hand the bounded active molecules are non toxic, it doesn't mean that the conjugate is not toxic. The main reason is because of the concentration. When administrating a peptide to the body, just a little part enter the cell and the other part has been degraded during the pathway, obtaining a low concentration in the cell. Otherwise, if the peptide is attached to a Au NP, and the nanoparticle is able to enter the cell, then the conjugate will penetrate the cell (NP acts as a Trojan Horse) and result in a high local concentration. In fact, despite the observation that the NPs cause damage when entering the cell, this is not so simply to happen. As we are working with the Trojan Horse trickery, the product you synthesize has to have a very specific characteristic to enter to the cell: it has to be sufficient polar to be easily administered and well distributed in the organism and hydrophobic enough to transverse the lipid bilayer of the cell. Also, a non-specific, electrostatic interaction of the peptide with membrane lipids appears to be crucial [2].

4. Conclusions

In Cosmetics, there is no regulation about the use of NPs, however, the regulation on cosmetic products (RRDD 1599/1997, 2131/2004 y 209/2005) in the article 4 it is said that the commercial cosmetic products distributed in the European community should not harm human health when used in normal and established conditions. One could argue that exist some evidence which points toward potential adverse effects of the use of NPs and therefore the precautionary principle should be applied.

It is a shared opinion that the use of nanoparticles in cosmetics should be evaluated on a case-by-case, taking into account factors like size, shape, concentration, dispersion and distribution, the physical and chemical characteristics of nanomaterials used and interaction with the cells. In the case that NPs enter the systemic system (through the linfatic system for example), the stability and reactivity of the NPs should be determined and their metabolization and expulsion process studied. Similarly, the effect of accumulation in some places of the body as the liver or after repeated exposure are key parameters also. Regarding skin creams, the penetration rate versus the skin tissue turn over rate also has to be assessed, since the skin regenerates itself in the matter of days or weeks. Fortunately, inorganic NPs normally possess signatures which allow their easy monitorization in organic/biological environments and in-vitro models are very useful to test some of these hypothesis. Another comment is that concenrns of the use of nanoparticles in cosmetics should expand to all the other materials, as metal cations and organic solvents, that have been traditionally used in cosmetics and that are known to be non safe despite their accepted massive use.

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About the authors

Authors work at The Inorganic Nanoparticles group at the ICN-CIN2 on the synthesis, characterization and bio-applications of engineered inorganic nanoparticles (NP), mixing expertise from physics, chemistry and biochemistry on the design of inorganic nanoparticles conjugates for their controlled interaction with biological systems. Understanding that the inorganic NPs during thier synthetic process or when exposed to the working environment are coated with organic molecules. This fact can be exploited to control the size, structure and shape of the inorganic core, its stability and the minimal interparticle distance upon collapse. On the other side, the fact of linking an active molecule to an inorganic surface allows to modify the molecule activity. Therefore, the design of NP - organic molecule conjugates pretends to take advantage of both, controlled properties of the inorganic core and controlled properties of the coating molecules.