Palladium nanoparticles exposure: Evaluation of permeation through damaged and intact human skin☆

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ABSTRACT

The intensified use of palladium nanoparticles (PdNPs) in many chemical reactions, jewellery, electronic devices, in car catalytic converters and in biomedical applications lead to a significant increase in palladium exposure. Pd can cause allergic contact dermatitis when in contact with the skin. However, there is still a lack of toxicological data related to nano-structured palladium and information on human cutaneous absorption. In fact, PdNPs, can be absorbed through the skin in higher amounts than bulk Pd because NPs can release more ions. In our study, we evaluated the absorption of PdNPs, with a size of 10.7 ± 2.8 nm, using intact and damaged human skin in Franz cells. 0.60 mg cm^{-2} of PdNPs were applied on skin surface for 24 h. Pd concentrations in the receiving solutions at the end of experiments were 0.098 ± 0.067 μg cm^{-2} and 1.06 ± 0.44 μg cm^{-2} in intact skin and damaged skin, respectively. Pd flux permeation after 24 h was 0.005 ± 0.003 μg cm^{-2} h^{-1} and 0.057 ± 0.030 μg cm^{-2} h^{-1} and lag time 4.8 ± 1.7 and 4.2 ± 3.6 h, for intact and damaged skin respectively.

This study indicates that Pd can penetrate human skin.

1. Introduction

Palladium nanoparticles (PdNPs) production and use are increasing due to the high catalytic activity that permits many promising industrial applications, including oxidation and coupling reactions, electrocatalysis and fuel cell technology. PdNPs principal use is in automobile catalytic converters and is present in airborne particulate matter (Ravinda et al., 2004; Zereini F. et al., 2004; Kalavrouziotis and Koukoulakis, 2009).

PdNPs are also successfully used in end-of-pipe technologies to control emissions of pollutants, such as halogenated compounds and drugs (Mackenzie K. et al., 2006; Kim et al., 2004; Long et al., 2013).

Its increasing use in jewellery (replacing nickel following EU Directive), dental alloys, and electronics suggests attention to this metal as a main potential allergen of the 21st century (Faurschou et al., 2011). Pd in contact with the skin can induce sensitization and can cause allergic contact dermatitis (Faurschou et al., 2011, Muris et al., 2015a,b). Increased sensitization to Pd was reported by Larese in 2003 in a long term survey on patch tested patients. In general, Pd allergy is associated to Ni allergy, probably due to a cress reaction between Pd and Ni.

Respiratory symptoms as asthma and rhinitis were associated with exposure to Pd salts only (Daenen et al., 1999).

The higher surface/mass of the PdNPs can lead to higher biological activities respect to bulk palladium, as well as an easy release of reactive metal ions (Larese et al., 2004; Ponti et al., 2009), with great potential skin permeation compared to bulk material.

Preliminary studies on PdNPs cell lines, indicated that the risk correlated to metallic palladium (De Windt et al., 2006), Pd ions (Frazzoli et al., 2007) or palladium/magnetite is rather low for particles 60–100 nm sized (Hildebrand et al., 2010). However, Speranza et al. (2010) showed that Pd-nanoparticles (5–10 nm) can effectively modify kiwifruit pollen entering inside the grains in higher and quicker amounts than soluble Pd(II). Wilkinson in 2011 carefully evaluated the toxicity of Pd nanoparticles (10.4 ± 2.7 nm).

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They demonstrated that the PdNPs cellular uptake occurs in human primary bronchial epithelial cells, with a dose-dependent effect on release of cytokines. PdNPs caused a lower responsiveness of human epithelial cells to TNF-α, and an induced apoptosis in human primary bronchial epithelial cells. Remarkably, these particles caused in lung epithelial cells a decrease in PGE2 secretion, when administered at non-cytotoxic doses. This could suggest an enhancement of airway inflammation. Furthermore, Boscolo in 2004 demonstrated that Pd salts inhibit cytokines' release in primary human peripheral blood mononuclear cells, whereas PdNPs enhanced the release of IFN-gamma with immunomodulatory effect. Reale et al. (2011) demonstrated that palladium ions and PdNPs exert different effects in vitro on the expression and release of cytokines from peripheral blood mononuclear cells on Pd-sensitized vs non-sensitized women. In a very recent work, Petrarca et al. (2014) proved that PdNPs modified the cell cycle in peripheral blood mononuclear cells, suggesting that ions, per se or released by NPs, could be the inducers of Pd toxicity.

With the aim to a better investigation on the potential in-vitro PdNPs skin absorption, permeation experiments with human skin were carried out using the Franz cell method (Franz, 1975). We investigated the total amount of Pd permeating through human skin during a 24-h period. We used the protocol defined during the European project EDETOX (Evaluations and predictions of DErmal absorption of TOXic chemicals, 2001–2004) and used for other experiments to investigate the skin absorption of other metal nanoparticles such as silver, gold and cobalt (Larese et al., 2009, 2011, 2013).

2. Materials and methods

2.1. Chemicals

We purchased palladium (II) chlorite, ammonium hydroxide (25% w/v), sodium hydroxide, sodium chloride, ethanol, hydrochloric acid (37% v/v), polyvinylpyrrolidone PVP (K30, average Mw 40,000) from Sigma Aldrich (Milan, Italy); lactic acid (90%) from Acros Organics (Geel Belgium); hydrogen peroxide (30% v/v), disodium hydrogen phosphate, potassium dihydrogen phosphate from Carlo Erba (Milan Italy). We prepared synthetic sweat using 0.5% sodium chloride, 0.1% lactic acid, 0.1% urea in milliQ water (using a Millipore purification pack system). pH was adjusted at 4.5 with ammonia. We prepared physiological solution with 9.00 g of NaCl, 2.38 g of Na_2HPO_4 and 0.19 g of KH_2PO_4 into 1.00 L of milliQ water to obtain a final pH of 7.35. All reagents used were analytical grade.

2.2. Nanoparticles synthesis and characterization

We synthetized PdNPs in vinylpyrrolidone (PVP) as suggested by Peng Choo et al. (2002) to obtain a molar ratio of 1:20 between Pd and. In detail, we dissolved 166.23 mg of $PdCl_2$ in 0.36 mL of HCl 37% under sonication and 4.163 g of PVP into 35 mL of water. We added Pd solution to PVP solution with 35 mL of ethanol. The suspension was refluxed for 3 h, than evaporated to remove ethanol. We dissolved the final product in 30 mL of H_2O and we adjusted to pH 5.5 by addition of NaOH 1 M. We added 50 mL of water to obtain a nominal metal concentration of 2.0 g/L.

To verify the presence of Pd in ionic form in the PdNPs suspension we purified 4.00 mL of NPs suspensions by ultrafiltration with Centrifugal Filters Amicon® Ultra-4, MWCO 10 kDa (Millipore) (Marassi et al, 2015). The recovered suspension was diluted to the original 4.00 mL with milliQ water. We monitored total metal concentration during the whole process by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy).

To evaluate NPs shape and size and aggregation/agglomeration in donor solutions we used a Dynamic Light Scattering (DLS) and a Transmission Electron Microscopy (TEM) before and at the end of the experiment.

2.3. Preparation of skin membranes

Human skin pieces obtained as surgical waste (Ethical Committee authorization n. 236/2007), were cut in 4 $\times$ 4 cm^2 pieces and, after the subcutaneous fat removal and hair shaved, were mounted on the Franz diffusion cells, previously treated with Aqua Regia, second with nitric acid and finally rinsed for 3 times with milliQ water to avoid metallic contamination. The mean exposed skin area was 3.29 cm^2 and the average membranes thickness was 0.9 mm. Four different donors, male and female, (50–70 years-old) were used. To evaluate skin integrity we use a conductometer (Metrohm, 660, Metrohm AG Oberdorfstr. 68 CH-9100 Herisau) at 300 Hz using two stainless steel electrodes (Fasano et al., 2002). In accord with Davies et al. (2004), we rejected cells with a resistance lower than 3.95 $\pm$ 0.27 kΩ cm^{-2}.

2.4. In vitro diffusion system

We used Franz static diffusion cells (Franz, 1975) with a receptor compartment with a mean volume of 14 mL thermostated at 32 °C to mimic hand temperature.

In Experiment 1 the donor chambers of 4 Franz cells were filled with 3.0 mL of freshly prepared donor solution (0.60 mg cm^{-2} of PdNPs) After 2, 4, 8, 12, 20 and 24 h, 1.5 mL of the receiving solutions was collected for the analysis and replaced with an equal volume of physiological solution.

At the end of experiment donor and receiving solutions were collected and ultrafiltered using Amicon® Ultra-4 to evaluate Pd ionization in 24 h.

In Experiment 2 we mounted 4 Franz cells with skin pieces that were marked 20 times in one direction and 20 perpendicular with 19-gauge hypodermic needle as suggested by Bronaugh and Steward (1985) to mimic damaged skin. In each experiment, we added a blank cell that was treated as the others without the use of PdNPs.

We repeated each experiment twice using four different donors to obtain 8 cells with intact skin, 8 cells with damaged skin and 4 blank cells.

2.5. Skin digestion

At the end of the experiment, we removed skin samples from the Franz cells. We immersed intact skin in water at 60 °C for 1 min to separate epidermis and dermis. We stored all skin samples in freezer at −25 °C until the day of the analysis. Skin samples were dried for 2 h at room temperature, weighted and digested using a solution with 10.0 mL of HNO_3 69% v/v and 2.0 mL of H_2O_2 (30%), agitated for 24 h and heated at the boiling point to obtain 2.0 mL that was used diluted to a volume of 10.0 mL for chemical analysis.

2.6. Analytical measurements

We used an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS 7500 CE Agilent Technologies Inc., Santa Clara, CA, USA) to measure the total palladium concentration in the skin and in the receiver solutions. The instrument was calibrated against standard solution from 0 to 10 $\mu g\ L^{-1}$, ion mass selected: 105 and 108 u.m.a. and the limit of detection was 0.05 $\mu g\ L^{-1}$.

We used an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, Spectroflame Modula E optical plasma

interface (OPI) SPECTRO, Germany) to evaluate Pd concentration in receiving solutions.

The instrument was calibrated using a standard curve from 0 to 10 mg L^{-1} and the limit of detection was 0.05 mg/L (at the wavelength of 340.458 nm). The repeatability (RDS%) for the analysis was less than 5% (precision of measurements).

2.7. Data analysis

The penetration of Pd through the skin was calculated considered Pd concentration in receiving solution (μg cm^{-3}) converted to the amount that penetrated (μg cm^{-2}), with a correction to consider the dilution due to sample removal.

We summarized data as mean ± standard deviation (SD) and we evaluated differences between independent data using the non-parametric Mann-Whitney and Kruskal Wallis tests, because data was not normal distributed. We use the statistical software SPSS for Windows (version 15.00) for the analysis and we considered as significant a p value of 0.05.

3. Results

Representative TEM images of PdNPs, diluted in synthetic sweat, and their size distribution are presented in Fig. 1A, B and 2, respectively. The mean size of the metal core was 10.7 ± 2.8 nm (number of measured NPs = 140).

The mean size distribution of the starting aqueous dispersion of PdNPs, obtained by DLS (Fig. 3), was 168 nm and the dilution with synthetic sweat resulted in a decrease of the mean diameter (85 nm, $p < 0.001$). No significant modification of the size distribution was observed after 24 h in synthetic sweat.

These results must be discussed considering the different response of the two investigation techniques. TEM evidences the size of each single metal particle, being the protective organic layer very difficult to observe. On the contrary, DLS analyzes the hydrodynamic diameter of the objects in solution, including the protecting organic layer. A significant difference between particle size by TEM and DLS has been frequently reported in the literature (Gasilova et al., 2010; Heinrich et al., 2012; Mauro et al., 2015; Dauthal and Mukhopadhyay, 2016).

The obtained results indicate that the donor solution contains agglomerates of Pd NPs (maintaining their original size around 10 nm) wrapped by PVP chains. These agglomerates are dynamic objects and their shape and size can change with time and solution environment. In agreement with previous DLS study on polymer-stabilized metal particles (Gasilova et al., 2010), bimodal distribution can be observed in DLS results. The smaller particles consist of single Pd NPs surrounded by PVP while the larger particles contain many Pd NPs enveloped by the polymer chains. We would like to stress that this situation is completely different from aggregates of Pd NPs, in which the metal nanoparticles are considered to be merged in a single, larger object without the possibility to be re-dispersed.

Metal NPs covered by PVP are stabilized by steric repulsion and without significant surface charging. The changes observed in the DLS particle size distribution after dilution with synthetic sweat can be reasonably explained by a contraction in the PVP bundle due to the introduction of ions into the solution, as previously observed for PVP-stabilized Pt and Rh NPs (Mauro et al., 2015). Recently, Morioka et al. (2016) suggested that PVP forms globular structures in water solution (avoiding interaction of the apolar chain with water) and interacts with metal particle surface by adsorption through the C=O group. Considering this, it is reasonable that addition of electrolytes into the solution media results in the shrinkage of these globular structures, finally leading to decrease of the hydrodynamic radius.

The metal concentrations in the ultrafiltered donor solutions collected at the end of the 24 h were always less than 0.1% of the starting suspensions, indicating a very low ionization of the NPs during the exposure time.

Permeation data of Pd are reported in Table 1 and profiles are shown in Fig. 4. Pd permeated the intact skin reaching an amount in dermal bathing solutions of 0.098 ± 0.067 μg cm^{-2} after 24 h. The absorption of Pd significantly increased on damaged skin ($p = 0.02$), reaching an amount in dermal bathing solutions of 1.06 ± 0.44 μg cm^{-2}. Flux through the skin increased of an order of magnitude between intact (0.005 ± 0.003 μg cm^{-2} h^{-1}) and damaged (0.057 ± 0.030 μg cm^{-2} h^{-1}) skin, with a lag time of

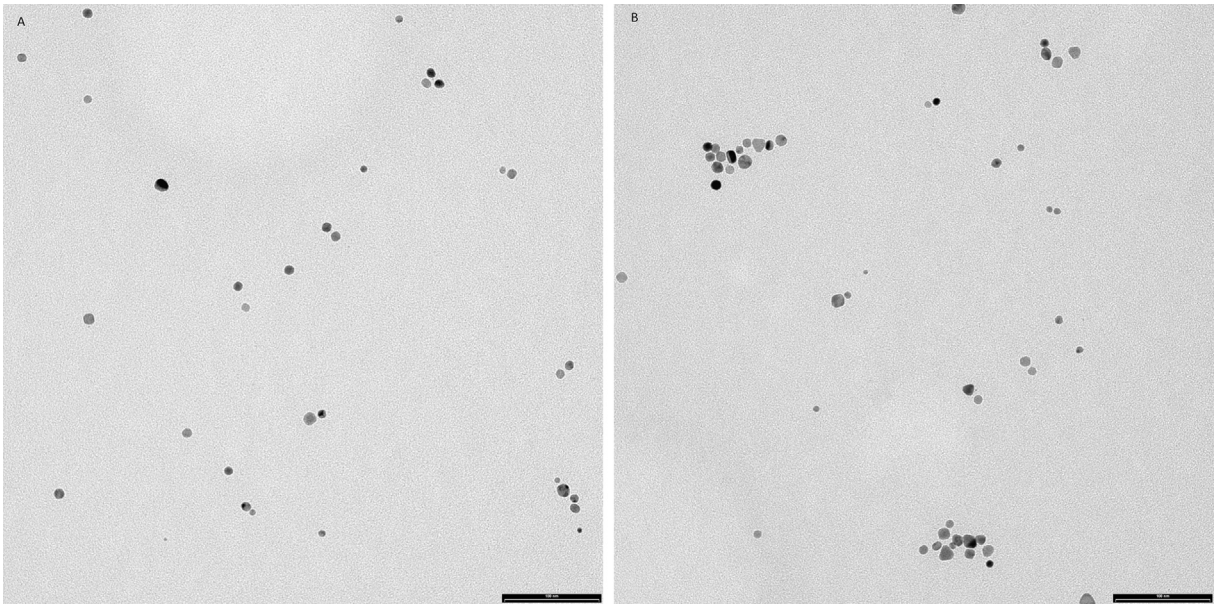

Fig. 1. Representative TEM images of PtNPs suspension in synthetic sweat (bar 100 nm) and size distribution of the metallic core before and after the experiments in donor phases.

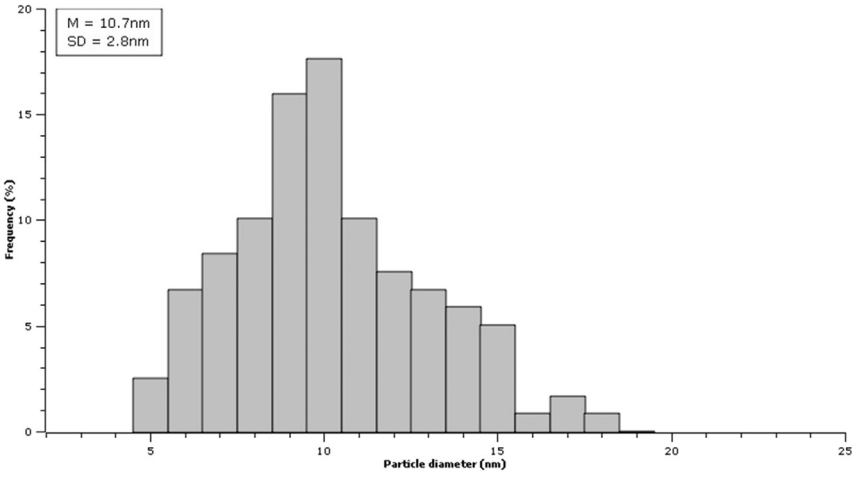

Fig. 2. Size distribution of the metallic core.

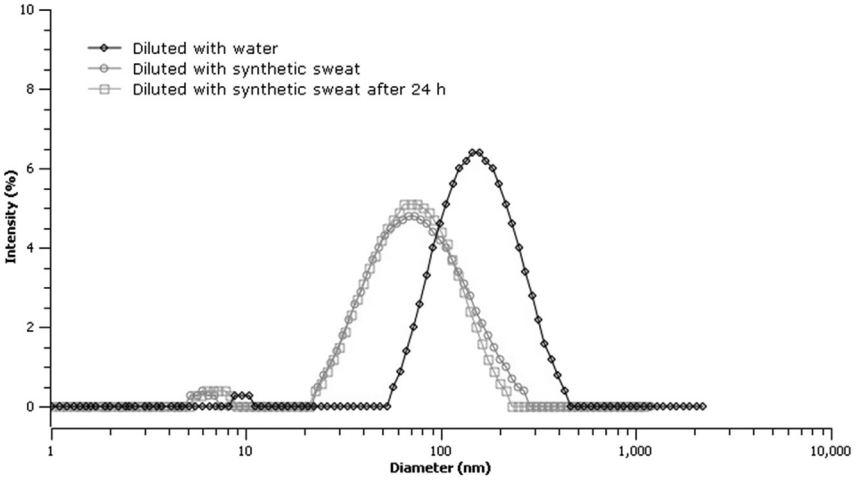

Fig. 3. DLS particle size distribution for PdNPs in different environments.

Table 1
Pd skin permeation and retention in intact and damaged fresh skin (mean ± standard deviation in μg/cm^2)

Time (h)	Intact skin	Damaged skin
0	0.000 ± 0.000	0.000 ± 0.000
2	0.009 ± 0.002	0.010 ± 0.002
4	0.014 ± 0.005	0.077 ± 0.046
8	0.032 ± 0.013	0.281 ± 0.112
12	0.048 ± 0.033	0.397 ± 0.134
20	0.074 ± 0.056	0.750 ± 0.334
24	0.098 ± 0.067	1.06 ± 0.44
Flux	0.005 ± 0.003	0.057 ± 0.030
Lag time (h)	4.8 ± 1.7	4.2 ± 3.6
Skin content	0.69 ± 0.36	0.93 ± 0.41

No Pd was detected in blanks cells (<limit of detection).

4.8 ± 1.7 h and 4.2 ± 3.6, respectively. To discriminate the nature of the species responsible for skin permeation (Pd NPs or Pd ionic species eventually leached during contact with skin), ultrafiltration test have been performed on the dermal bathing solution. Notably, the obtained results completely lack of reproducibility, resulting from the very low Pd content in the bathing solution in combination with the significant release of interfering compounds from the skin (i.e. proteins, lipids etc.) that block the filter.

The total Pd amount assessed in the tissue after mineralization was slightly lower (but not statistically significant) in the intact skin with respect to the damaged skin, with a mean value and standard deviation of 0.69 ± 0.35 and 0.93 ± 0.41 μg cm^{-2} μg cm^{-2} respectively, as shown in Fig. 5.

The palladium content in intact skin (mean and standard deviation) decreased significantly ($p < 0.05$) from the epidermis (0.65 ± 0.34 μg cm^{-2}) to the dermis (0.04 ± 0.01 μg cm^{-2}) as shown in Fig. 6.

4. Discussion

We investigated the skin penetration and permeation of PdNPs using full thickness (epidermis and dermis) fresh human skin using in-vitro diffusion cell system (Franz cells) with intact and needle damaged skin. The findings indicate that human skin absorption of PdNPs is indeed possible in both intact and damaged skin. However, skin lesions increased significantly skin absorption with higher Pd content into the skin and in receiving solution. The chemical analysis performed using ICP-AES confirmed the presence of Pd in both the epidermis and the dermis with a higher content in epidermis.

These results are well in accordance with studies on others

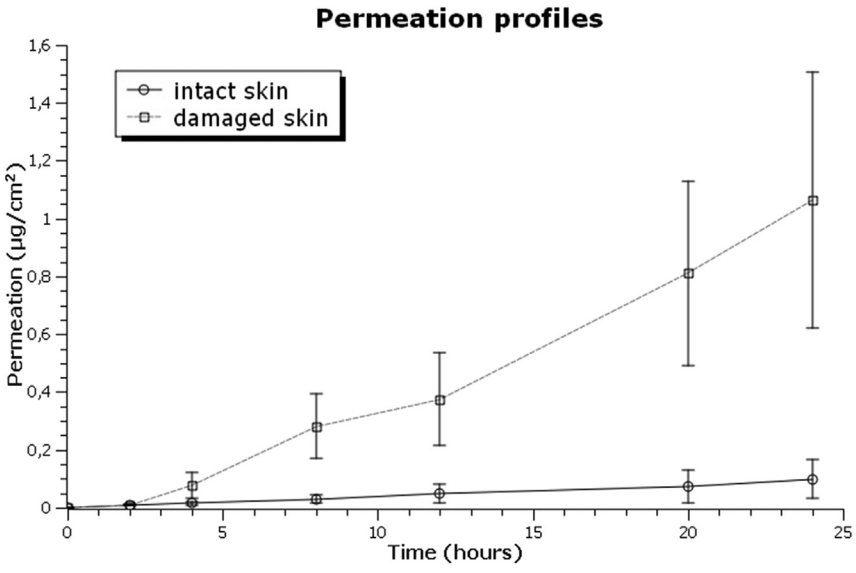

Fig. 4. Permeation profile of palladium after skin application of PdNPs solution (means and standard deviations).

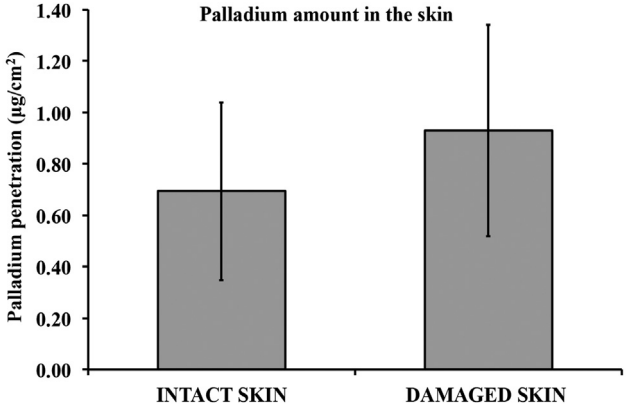

Fig. 5. Mean values and standard deviations of palladium amounts (µg/cm^2) in intact and damaged skin.

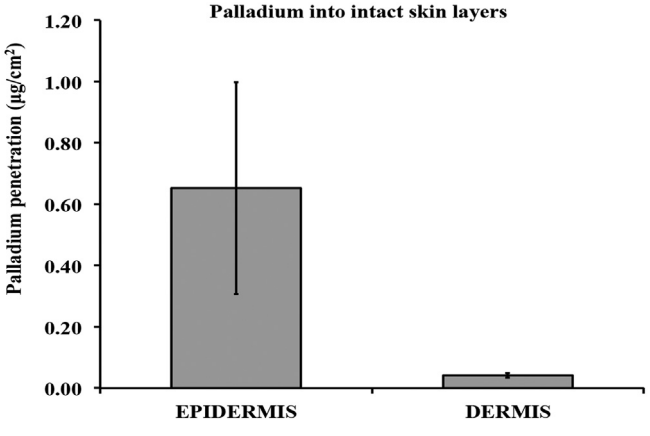

Fig. 6. Mean values and standard deviations of palladium amounts (µg/cm^2) in epidermis and dermis of intact skin.

metal NPs such us silver (Larese et al., 2009; Samberg et al., 2010), gold (Larese et al., 2011; Sanovane et al., 2008) and cobalt (Larese et al., 2013). Similarly, Baroli et al. (2007) showed that iron nanoparticles can penetrate the skin through the stratum corneum lipidic matrix and hair follicle orifices, but these nanoparticles did not pass through the skin. It is important to note that in the case of base transition metals (i.e. Ni and Co), NPs skin absorption can be related to the presence of metal ions that are formed by dissolution of metal. This process is obviously favored in nanosystems due to their high surface area with respect to bulk material (Larese et al., 2013). In the case of noble metals, such as the present Pd, the release of metal ions, even from NPs, is expected to be negligible or very low. In accordance, we measured very low presence of Pd ions in physiological condition, as demonstrated by the ultrafiltration test in donor solution before and after the experiments. Nevertheless, small NPs can penetrate trough pilosebaceous pores and hair follicles (Schaefer et al., 2009; Meidan et al., 2005), mainly if the particle size is lower than 50 nm. (Vogt et al., 2006; Rancan et al., 2012). The PdNPs, used in this study, are stable and small (10.7 nm), with low tendency to form aggregates, and for this reason can occur via the appendageal route. The chemical analysis demonstrated a time dependent permeation of Pd as well as a presence of the metal inside the skin.

Our findings indicate that PdNPs can permeate the human skin reaching the epidermis and dermis, probably stored in hair follicles. Inside the skin these NPs can be a long-term source of metal that could be involved in the sensitization process, or can be available for systemic diffusion. Kim et al. (2004) demonstrated that NPs administered in the dermis migrated to regional lymph nodes, suggesting an increased risk for sensitization. It is well recognized that Pd is a skin sensitizers (Faurscou et al., 2011; Muris et al., 2015a,b) that can cause allergic contact dermatitis with a delayed mechanism. Furthermore, Boscolo et al. (2004) and Di Gioacchino et al. (2007) confirmed the sensitizing power of Pd(II) and Pd(IV) ions. In 2010, Boscolo et al. observed that PdNPs could induce a secretion of INF-γ, a Th1 cytokine involved in type IV immune reactions. These authors suggested a role for pollutant PdNPs in the raised prevalence of allergic contact dermatitis to Pd in people living in urban areas (Santucci et al., 2010). Moreover for other metals such as berillyum the skin contact could induce respiratory symptoms (Tinkle et al., 2003).

In conclusion, our study demonstrated for the first time that PdNPs could permeate the skin in an in-vitro system. Moreover, the presence of Pd inside the skin suggests a potential long term effect.

This aspect, together with an increased risk of Pd absorption through inhalation route and the well-documented sensitization potential of Pd (Faurschou et al., 2011; Muris et al., 2015a,b; Personen et al., 2014), strongly suggests the need to avoid skin contamination with PdNPs. The implementation of personal protective equipment (PPE) which can minimize the overall exposure including inhalation and ingestion for exposed workers is compulsory. Moreover more studies are needed on the relationship between occupational, environmental and consumers exposure to Pd and skin allergies.

Conflict of interest

The authors declare that there are no conflicts of interest.

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