Transdermal permeation of inorganic cerium salts in intact human skin

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ABSTRACT

The *stratum corneum* protects the body against external agents, such as metals, chemicals, and toxics. Although it is considered poorly permeable to them, comprising the major barrier to the permeation of such substances, it may become a relevant gate of entry for such molecules. Cerium (Ce) is a lanthanide that is widely used in catalytic, energy, biological and medicinal applications, owing to its intrinsic structural and unique redox properties. Cerium salts used to produce cerium oxide (CeO_2) nanostructures can potentially come into contact with the skin and be absorbed following dermal exposure. The objective of this study was to investigate the percutaneous absorption of three inorganic Ce salts: cerium (III) chloride ($CeCl_3$); cerium (III) nitrate ($Ce(NO_3)_3$) and ammonium cerium (IV) nitrate ($Ce(NH_4)_2(NO_3)_6$), which are commonly adopted for the synthesis of CeO_2 using *in vitro - ex vivo* technique in Franz diffusion cells. The present work shows that Ce salts cannot permeate intact human skin, but they can penetrate significantly in the epidermis (up to 0.29 μg/cm^2) and, to a lesser extent in dermis (up to 0.11 μg/cm^2). Further studies are required to evaluate the potential effects of long-term exposure to Ce.

1. Introduction

In occupational settings, the skin is constantly exposed to chemicals, metals and other contaminants, which is a potential health hazard. Even though the skin constitutes a protective barrier to various xenobiotics, it is well known that it is not a totally impermeable barrier, and can be a route of entry of such molecules. Chemicals and metals can penetrate and permeate the skin inducing local effects such as skin sensitization and potential metal diffusion into the bloodstream (Filon et al., 2016; Brouwer et al., 2016). Percutaneous penetration of metals is closely related to the ability of the sweat to form complexes with or oxidize metal atoms (Julander et al., 2013; Erfani et al., 2015). Cerium (Ce) is an iron-gray, ductile and malleable metal belonging to the lanthanide series, also namely rare earth. It can exist either in the free metal or oxide form, owing the ability to cycle between the (Ce^{3+}) and (Ce^{4+}) oxidation states. The most stable oxide of Ce is cerium dioxide (CeO_2), also called ceria, which has attracted substantial attention in the field of environmental applications, catalysis and more recently in biomedical research, due to its excellent catalytic and antioxidant activity (Sun et al., 2012; Montini et al., 2016). Nanoparticles of cerium dioxide (CeO_2 NPs), as scavengers of reactive oxygen and nitrogen species (ROS/RNS) (Filippi et al., 2019; Hirst et al., 2013), have been proposed as pharmacological treatments in many human diseases associated with a ROS disbalance such as neurodegenerative pathologies, autoimmune diseases, diabetes, cancers and wound repair (Zschauer et al., 2017; Sack et al., 2014; Naseri-Nosar et al., 2017; Chigurupati et al., 2013). The Control of the morphology of these NPs is a critical parameter for their catalytic performance. Recent studies have demonstrated that CeO_2NPs with a spherical shape decreased neuronal cell death and calcium dysregulation in a mouse model of mild traumatic brain injury (mTBI) (Youn et al., 2021). In addition, CeO_2 truncated nano octahedra riching in {111} planes have shown exciting potential for the fast and total degradation of paraoxon, an organophosphorus compound (Trenque et al., 2019). Furthermore, literature reports show that inorganic cerium salts such as: cerium (III) chloride ($CeCl_3$); cerium (III) nitrate ($Ce(NO_3)_3$) and ammonium cerium (IV) nitrate ($Ce(NH_4)_2(NO_3)_6$) may be adopted as cerium precursors for the synthesis of CeO_2 (Phokha et al., 2012; Trenque et al., 2020). These Ce salts are increasingly used in the nanotechnology sector, however to the best of our knowledge, no data on their skin permeability are available. Skin absorption depends on physical chemical characteristics such as morphology, shape, size, and surface composition (Cheng et al., 2013; Kumari et al., 2014), vehicles,

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skin conditions, contaminated areas, and time of contact (Filon et al., 2016). Because a previous work by our research group was based on the skin permeation of CeO_2NPs (Mauro et al., 2019), the current study aimed to investigate the percutaneous absorption of Ce following exposure to three cerium salts: $CeCl_3$; $Ce(NO_3)_3$ and $Ce(NH_4)_2(NO_3)_6$ commonly adopted for the synthesis of NPs. These Ce salts may therefore come into contact with the skin during the various steps of NPs preparation such as weighing, and transfer, representing one of the most probable risks associated with cutaneous exposure. Furthermore, dermal exposure to Ce needs to be explored. Although it is a relatively new concern, the Ce permissible exposure limit for surface contamination in occupational settings over an 8-h period has not yet been established. In the current work, the cutaneous penetration of Ce was investigated *in vitro* using static Franz diffusion cells through intact human skin. Experiments were performed for 24 h and the metal concentration in each layer of human skin was assessed at the end of the experiment.

2. Material and methods

2.1. Chemicals

All chemicals were analytical graded: urea, sodium chloride, sodium hydrogenphosphate, and potassium dihydrogenphosphate were purchased from Carlo Erba (Milan, Italy); ammonium hydroxide (25% *w/v*) was bought from J. T. Baker (Deventer, Holland); lactic acid (90% *v*/v) from Acros Organics (Geel, Belgium); and nitric acid (67–69% v/v) from VWR (Milan, Italy). Cerium nitrate and Ammonium Cerium (IV) nitrate were obtained from Sigma Aldrich (St. Louis, MO, USA), Cerium (III) chloride from chemPUR (Feinchemikalien und Forschungsbedarf GmbH, Germany). Reagent grade water was obtained using a Millipore purification pack system (MilliQ water). The physiological solution used as the receptor fluid was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4 and 9 g of NaCl into 1 l of MilliQ water (final pH = 7.35). The synthetic sweat solution used as the donor fluid consisted of 0.5% *w/v* sodium chloride, 0.1% w/v urea and 0.1% w/v lactic acid in MilliQ water; and the pH was adjusted with ammonium hydroxide (1 N) to pH 4.5.

2.2. Preparation of cerium solutions in synthetic sweat solution

Solutions of cerium (1000 ppm) in MilliQ water were prepared. Each Ce aqueous solution was designed such that the final concentration was 50 mg of Ce in 50 ml of water. The solutions were left overnight at room temperature until equilibrium was reached. Then, Ce aqueous solutions were diluted 1:20 in synthetic sweat at pH 4.5, before application in the donor chamber.

2.3. Skin samples preparation

2.3.1. Human skin membranes

Full thickness human abdominal flank skin was obtained as surgical waste and approved by the Trieste Hospital Ethical Committee n° 236/2007. The donors were men and women with ages rangingfrom 45 to 71 years. Prior to storage in a freezer (−25 °C), subcutaneous fat was removed using a scalpel blade, and the hair was shaved from the epidermis. Skin samples were stored in a freezer at −25 °C for up to 4 months. Skin samples were prepared to a final thickness of 1.05 ± 0.02 mm (Micrometer Mitutoyo). On the day of the experiment, skin samples were thawed in a physiological solution at room temperature and the skin samples were cut into 4 cm^2 square sections. Skin integrity was checked by measuring the Trans Epidermal Water Loss (TEWL) (Delfin Vapometer, Delfin Technologies, Sweden), which was used in our previous work (Magnano et al., 2022): the average TEWL values of the skin samples were found to be below 10 $g{\cdot}m^{-2}{\cdot}h^{-1}$ (Guth et al., 2015).

2.4. In vitro permeation and Ce distribution in skin layers after 24 h exposure

Skin absorption studies were performed in static diffusion cells according to the OECD guidelines (OECD, 2004). The skin pieces were mounted between the donor and receptor chamber of Franz-type static diffusion cells with the *stratum corneum* facing the donor chamber. The effective skin area for diffusion was 0.95 cm^2. The receptor medium (RM) was composed of a physiological solution that was continuously stirred using a Teflon coated magnetic stirrer. The concentration of the salt in the receptor fluid is approximately the same as that found in the blood. The receptor compartment had a mean volume of 4.5 ml filled with RM. Mounted Franz cells were maintained at 32 ± 1 °C by means of circulation of thermostated water in the jacket surrounding the cell. The skin absorption experiments were carried out as follows:

Exp. 1: Briefly, infinite doses (1.0 ml of each freshly made solution of Ce in synthetic sweat at pH 4.5) was applied on the skin surface. This resulted in a theoretical applied dose of $Q_0 = 52.6$ μg/cm^2. The donor compartment was closed with parafilm during the experiment. The permeation study was then carried out for 24 h to determine the permeation profile of Ce remaining and permeating through the skin. At selected time points (2 h, 4 h, 6 h, 8 h, 10 h, 20 h, 24 h) 0.5 ml of each receptor sample was collected and analyzed. An equal volume of fresh receptor medium was immediately replaced in each sample. In each run, 3 cells were used. The experiment was performed twice with 6 independent cells for the Ce salts investigated (a total of 18 cells). Skin samples from at least 2 donors were tested.

Controls: A skin sample with no cerium solution applied to the skin surface was used as control in each run. The donor chamber was filled with synthetic sweat at pH 4.5 and the manipulation was performed as described for skin absorption studies (Exp.1). The experiment was performed twice with a total of 4 independent cells. Skin samples from at least 2 donors were tested.

The amounts of Ce in the RM as well as inside the skin layer after 24 h were quantified by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS), method described below, paragraph 2.7.1.

2.5. Collection and treatment of samples

After 24 h of exposure, the cells were dismantled. All receptor fluids were removed, and frozen for subsequent analyses. The non-absorbed fraction was removed from the skin surface by washing the donor chamber thrice with 1.0 ml of MilliQ water for 20 s and gently wiped with a cotton swab. The skin layers were separated as follows: the viable epidermis (VE) was separated from the dermis (D) by heat treatment (1 min in water at 60 °C) before digestion of the tissue (see section 2.6). The receptor medium was diluted 1:10 in MilliQ water acidified with 1% nitric acid before the ICP-MS analysis.

2.6. Skin digestion after the experiment

At the time of the analysis, the skin membranes were thawed, and the exposed area was weighted placed in Teflon based sealed beaker with 2.0 ml of HNO_3 69% *v*/v; 0.5 ml of H_2O_2; 1.0 ml of MilliQ water. Subsequently, the reaction mixture was heated in a microwave oven (Multiwave-PRO, Anton Paar) at 180 °C for 25 min. After the digestion treatment, the solutions were diluted 1:10 in MilliQ water for the ICP – MS analysis.

2.7. Anali analytical measurements

2.7.1. Quantification of cerium by ICP – MS

The Ce of controls and exposed skin samples, together with receptor and donor solutions, were evaluated by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS) using a NexION 350× Spectrometer (PerkinElmer, USA) equipped with an ESI SC Autosampler. The analysis

was performed in KED mode (Kinetic Energy Discrimination) using ultra-high purity helium (flow rate of 4.8 ml min^{-1}) to control and minimize cell-formed polyatomic ion interference. The ICP-MS calibration curve was linear (R^2 = 0.999 Ce) in the concentration range of 1–100 µg l^{-1} according to the dilution of a multistandard solution of 10 mg l^{-1} for ICP analysis (Periodic Table MIX 3, TraceCERT Sigma-Aldrich). Measurements of the samples were performed using the calibration curve method obtained by analyzing standard solutions for instrumental calibration. The limit of detection (LOD) was 0.0003 µg l^{-1} for Ce. The coefficient of variation of repeatability (RSD %) was <3%.

2.8. Statistical analysis

The results are expressed as the quantity penetrated per skin surface unit (µg·cm^{-2}). Data from skin absorption experiments are expressed as mean ± standard deviation (SD). Differences between independent data were evaluated using the nonparametric Mann-Whitney test. The significance level was set at $p < 0.05$. Data were treated and analyzed using Excel for Windows (release 2010) and Stata Software (version 11.0; StataCorp LP, College Station, TX, USA).

3. Results

3.1. Ce quantification in donor solutions

Skin absorption of the selected Ce salts was investigated. The amount of Ce in donor phases after 24 h of exposure was quantified using an ICP – MS. The analysis revealed that the effective dose of Ce in each donor solution (DS), expressed in µg/cm^2, were respectively 67.9 µg/cm^2 for $Ce(NO_3)_3$; 62.7 µg/cm^2 for $CeCl_3$; and 44.3 µg/cm^2 for $Ce(NH_4)_2(NO_3)_6$. These results were further used for the permeability coefficient (K_p) calculation (see section 3.3).

3.2. Skin permeation of Ce

The Ce concentrations in the receptor medium expressed in ng/cm^2 are represented in Fig. 1. In the experimental condition, the mean amounts of Ce observed in RM in blanks samples were closed to exposed skin samples reaching 1.71 ± 0.35 ng/cm^2 and 1.97 ± 0.50 ng/cm^2 respectively at the end of the contact time (24 h). This can be explained by the minimal release of Ce in the skin. Notably, for all the Ce salt samples, the Ce concentrations were similar with values of 1.82 ± 0.55 ng/cm^2 for $Ce(NO_3)_3$; 1.98 ± 0.41 ng/cm^2 for $CeCl_3$ and 2.10 ± 0.54 ng/cm^2 for $Ce(NH_4)_2(NO_3)_6$. No significant differences were observed in any of the tested samples.

3.3. In vitro skin distribution of Ce

Post-exposure, the amount of Ce retained in the skin was quantified and presented in Table 1 and Fig. 2. The results clearly show that the total Ce quantity penetrated the exposed skin samples was higher than that in the blank samples. As it can be seen in Fig. 2, apparently the highest total amounts of metal penetrated in exposed human skin were observed for $Ce(NO_3)_3$: 0.53 ± 0.45 µg/cm^2 and $CeCl_3$: 0.36 ± 0.19 µg/cm^2, while the lowest penetration was measured for $Ce(NH_4)_2(NO_3)_6$: 0.30 ± 0.12 µg/cm^2 after 24 h of contact, but no significant differences were found among the three Ce salts. As expected, the total quantity of Ce retained into the epidermis and dermis (E + D) was detectable at very low level (0.07 ± 0.04 µg/cm^2) in blank samples. A statistically significant difference between the blank samples and skin samples exposed to Ce was found.

Finally, considering the effective dose, the permeability coefficient K_p (cm/h) of each Ce salt was determined by dividing the effective absorption rate by the equilibrium concentration of each salt in the donor solution. Apparently, the highest K_p was observed for $Ce(NO_3)_3$ ranging around $2.97*10^{-4}$, while $CeCl_3$ was the less permeable salt with K_p measured in the range of $2.03*10^{-4}$. The obtained values were summarized in Table 2.

4. Discussion

Dermal absorption of Ce after exposure to inorganic Ce salts ($CeCl_3$; $Ce(NO_3)_3$ and $Ce(NH_4)_2(NO_3)_6$) was assessed using Franz static diffusion cells. This method allows *in vitro* to define penetration (applied substance into the skin) and permeation (applied substance that crosses the skin and arrives at the receiving solution) of a substance applied on the skin. Using these cells, the absorption is monitored by sampling the stirred receptor chamber solution. Although the flow-through diffusion cell (Bronaugh and Stewart, 1985) offers various advantages over the static cell design such as automatic sampling, it is well reported that both types of cells are similar in their ability to measure the percutaneous absorption of water soluble penetrants (Clowes et al., 1994). Therefore, a good agreement in the permeability coefficients for the absorption profiles of water, cortisone, benzoic acid, and mannitol was

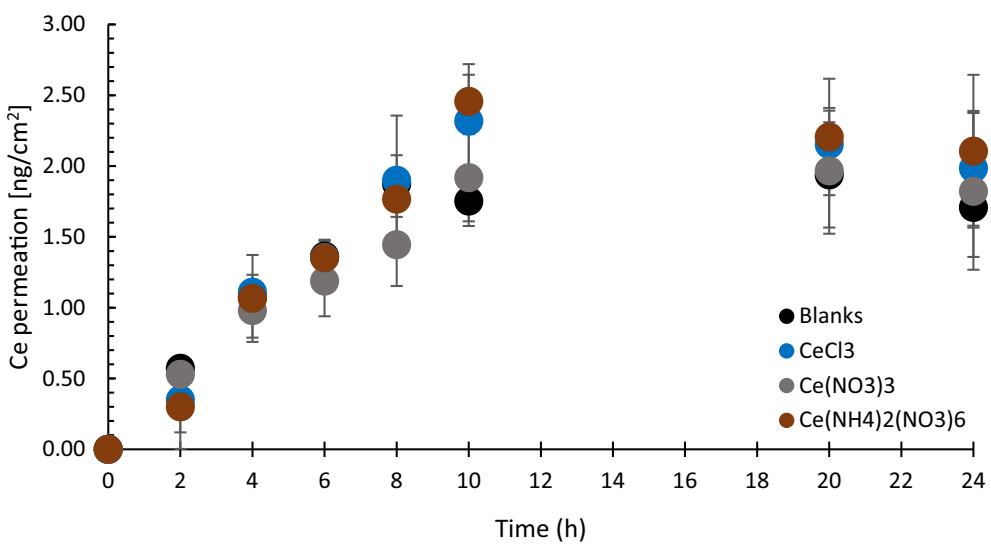

Fig. 1. Ce concentrations (ng/cm^2) that permeated in the receptor medium at specific extraction times in blanks and exposed skin samples. Values are expressed as mean ± SD.

Table 1
Ce amount found in skin layers in blanks and exposed skin after 24 h exposure. Applied dose was 52.6 μg/cm^2. Data are given as mean ± SD. Asterisk (*) indicates statistically significant difference obtained between blanks and exposed skin samples to Ce in the Mann-Whitney test ($p < 0.05$).

	Epidermis (E) (μg/cm^2)	Dermis (D) (μg/cm^2)	Total skin (E + D) (μg/cm^2)
Blanks	0.02 ± 0.01	0.05 ± 0.02	0.07 ± 0.04
$CeCl_3$	0.27 ± 0.21*	0.09 ± 0.03	0.36 ± 0.19*
$Ce(NO_3)_3$	0.41 ± 0.38*	0.12 ± 0.07	0.53 ± 0.45*
$Ce(NH_4)_2(NO_3)_6$	0.19 ± 0.11*	0.11 ± 0.02*	0.30 ± 0.12*

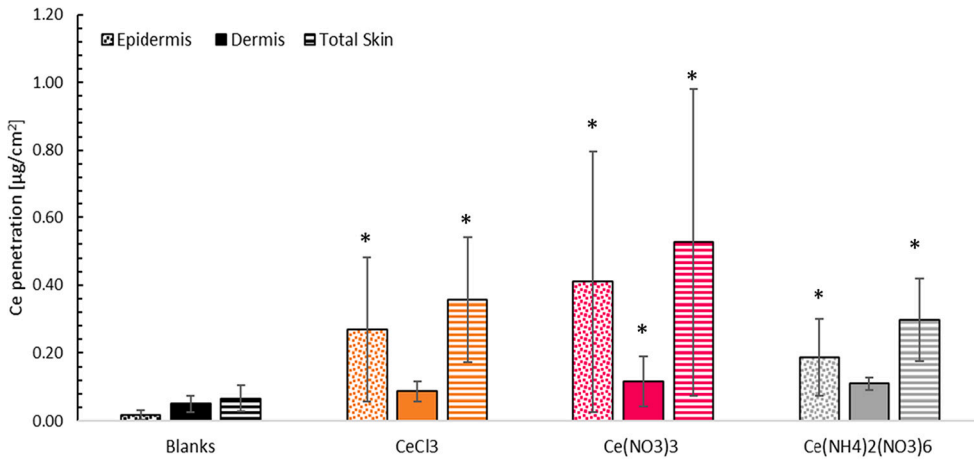

Fig. 2. Ce concentration found in skin layers (epidermis and dermis) after 24 h exposure in blanks and exposed skin samples. Applied dose was 52.6 μg/cm^2. Data are given as mean ± SD. Stars show the statistically significant differences obtained between blanks and exposed skin samples to Ce in the Mann-Whitney test ($p < 0.05$).

Table 2
Skin permeability coefficients (K_ps) for each Ce salt.

Ce salt	Permeability coefficient K_p (cm/h)
$CeCl_3$	$2.03*10^{-4}$
$Ce(NO_3)_3$	$2.97*10^{-4}$
$Ce(NH_4)_2(NO_3)_6$	$2.27*10^{-4}$

found between the flow-through cells and static diffusion cells (Clowes et al., 1994; Bronaugh and Stewart, 1985). Based on this knowledge and the laboratory equipment, our permeation tests were performed using Franz static diffusion cells. Moreover, *in vitro* percutaneous absorption experiments are commonly carried out using water or buffered saline solutions as the receiving phase; however it is well known that aqueous solutions are usually replaced by bovine serum albumin (BSA) to measure the *in vitro* absorption of poorly water-soluble lipophilic compounds (Cross et al., 2003). In our experiments, the receptor fluid was composed of saline solution without BSA because our study aimed to investigate the skin permeation of Ce (a metal compound) which is not lipophilic. Furthermore, we used human skin, which is the gold standard for studying skin permeation compared to animal skin, or artificial membranes (Khan et al., 2005; Příborský and Mühlbachová, 2011; Godin and Touitou, 2007). Then, it is well known that metals in their ionized form readily penetrate through the skin (Franken et al., 2014). In the current study the skin was exposed to three inorganic Ce salts solubilized in synthetic sweat at pH 4.5. Metallic ions (*e.g.*, nickel, cobalt, chromium, and lead) may influence percutaneous absorption, because they can diffuse through this cutaneous membrane. Most elements increase their ionized form as acidity increases; in some cases it becomes approximately 10 to 100-fold higher at each one pH unit decreases (Zlotogorski, 1987). For this reason, our experiments were performed using a synthetic sweat solution at pH 4.5 in order to reproduce the typical pH of the skin around 4 to 5.5 and to increase metal release. Our results demonstrated that the concentrations of Ce found in the receptor medium were comparable to those observed in the blank samples. Specifically, the values obtained at 24 h were respectively: 1.82 ng/cm^2 for $Ce(NO_3)_3$; 1.98 ng/cm^2 for $CeCl_3$; 2.10 ng/cm^2 for $Ce(NH_4)_2(NO_3)_6$ and 1.71 ng/cm^2 for blanks. Thus, the permeation of these Ce salts through the skin was negligible. However, the total amounts of Ce found in whole skin in the blank samples were lower compared to exposed skin samples, which was an expected result. The Ce quantity that penetrated the exposed skin samples was relatively equal for all Ce salts, with a higher extent for $Ce(NO_3)_3$ (up to 0.53 ± 0.45 μg/cm^2). This limited difference in skin penetration of Ce salts could be attributed to metal binding to skin components such as glycolipids, phospholipids, carboxylate, phosphate groups and carbonyl groups of sn-2 phospholipid chains. This binding may occur in all layers of the skin with the formation of a depot in the *stratum corneum*, which can reduce metal diffusion (Samitz and Katz, 1976; Hostynek, 2003; Franken et al., 2015). Notably, trivalent ions such as Cr (III) ions show a strong affinity for skin tissue, forming more stable complexes than divalent ions such as Ni (II) and Co (II) (Filon et al., 2009). Therefore, Ce seems to follow the same behavior as Cr because of its ability to bind to skin components without reaching the receiving phase. Additionally, these results are also in line with our previous study where CeO_2NPs were used as the donor phase (Mauro et al., 2019). It was visible that nanoparticles were retained in whole intact skin (3.64 ± 0.15 μg/cm^2), but no Ce amount was detected in the receptor medium (Mauro et al., 2019). Further, the K_p for each Ce salt was determined to compare the percutaneous kinetics. This parameter describes the membrane penetration and as can be noticed from our data $Ce(NO_3)_3$ presented the higher value. Several analyses have demonstrated the bacteriostatic and bactericidal activities of Ce $(NO_3)_3$, $CeCl_3$ and $Ce(SO_4)_2$ against a wide spectrum of bacteria. In a study by Burkes et al. (Burkes and Mccleskey, 1947) $Ce(NO_3)_3$ was an effective pH dependent bacteriostatic agent against a panel of 39 bacterial species across 16 genera, including *Staphylococcus aureus* and *Pseudomonas aeruginosa*, showing the greatest efficacy at slightly acidic pH values. Apart from these antibacterial properties, $Ce(NO_3)_3$ also possesses specific effects in burn settings, owing to its ability to help the

prevention of sepsis in burn patients. It was demonstrated that in humans, $Ce(NO_3)_3$ seems to preserve normal T-cell functions such as the production of interleukin 2 (IL-2) and IL-2 receptor expression due to the neutralizing action on the immunosuppressive burn toxins formed at the wound site (Sparkes, 1993; Jakupec et al., 2005). There is limited information regarding cell response in cell types when cerium is applied, for example, in the form of cerium chloride ($CeCl_3$). Different cell types may also react differently to cerium exposure, presenting diverse cytotoxicity effects (Feyerabend et al., 2010). However, it was reported by Preeta and Nair (Preeta and Nair, 1999) that low levels of $CeCl_3$ (0.5 mM) stimulated a mitogenic response in cardiac fibroblasts, whereas pulmonary fibroblasts were not stimulated by the metal. It was also observed a positive $CeCl_3$ effect on the proliferation, differentiation, adipocytic transdifferentiation and mineralization function of primary mouse osteoblasts depending on the concentration and cell culture time (Zhang et al., 2010). The effect of $CeCl_3$ on cell migration and gene expression of human foreskin fibroblasts (HFF) was investigated by (Ramenzoni et al., 2017). The authors demonstrated that following a long exposure to $CeCl_3$, the element did not negatively influence the cells in terms of survival or activity. In this study the gene expression of cyclins CCNB1, CCND1 and CCNE1 as markers of cell proliferation and the cell cycle was also studied. The data reported cyclin B1, D1 and E1 upregulation, as a result of increased cell proliferation. Moreover, the same authors also confirmed a positive $CeCl_3$ effect on the proliferation of osteoblasts and human foreskin fibroblasts after short time periods (10 s) (Schmidlin et al., 2012). Thus, $CeCl_3$ may be beneficial as a cell-stimulating agent. Further Kumar et al. showed that low doses of $CeCl_3$ stimulated collagen and non-collagen protein synthesis in cardiac fibroblasts *in vivo* (Kumar et al., 1995). Finally, it is important to point out that the current study has some limitations. First, it is performed in static Franz cells, an *in vitro* method, which may not reproduce a real scenario. The obtained results can underestimate the *in vivo* conditions, because only passive diffusion is evaluated, while *in vivo* skin absorption can be enhanced by active mechanisms. Second, to mimic sweat the *stratum corneum* was exposed for 24 h, but the excessive hydration can promote the absorption of many compounds.

5. Conclusion

The current study aimed to investigate the human skin absorption of inorganic Ce salts, which are usually adopted for the synthesis of CeO_2. Our experimental findings demonstrated that Ce skin permeation of the exposed samples was negligible because the Ce content observed in the receptor compartment was close to that in the blank samples. Regarding the distribution of Ce into skin layers, the highest penetration profile was measured for $Ce(NO_3)_3$ compared to $CeCl_3$ and $Ce(NH_4)_2(NO_3)_6$, probably due to its stronger skin protein binding capacity. In conclusion, these data suggest that there is no potential risk of Ce to reach the systemic circulation, representing an encouraging result for Ce salt applications involving direct skin exposure. It is important to consider that pure Ce salts can cause corrosion and irritate the skin, but data concerning the potential sensitization of this metal have not been explored to date. Further experiments should be conducted with human skin to evaluate the safety of long-term exposure to Ce. Therefore, the use of personal protective equipment such as gloves, and gowns is recommended for exposed workers.

CRediT authorship contribution statement

Greta Camilla Magnano: Investigation, Data curation, Writing – original draft. **Giovanna Marussi:** Investigation. **Francesca Larese Filon:** Conceptualization, Supervision, Writing – review & editing. **Matteo Crosera:** Supervision, Writing – review & editing. **Massimo Bovenzi:** Funding acquisition, Supervision. **Gianpiero Adami:** Conceptualization, Supervision.

Declaration of Competing Interest

The authors have no conflict of interests to disclose.

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