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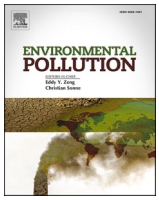

Evaluating the role of protective creams on the cutaneous penetration of Ni nanoparticles☆

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ABSTRACT

There is an increase of application of Nickel in the form of nanoparticles (NiNPs) in several fields including modern metallurgy, bioengineering, and medicine. Such growth of the areas of application is actually accompanied with an increase of exposure to Nickel, thus an intensification of the negative effects, the most frequent being the allergic contact dermatitis. Indeed, due to their smaller size, and therefore their higher surface area, NiNPs can release more Ni ions compared to bulk material, that can penetrate and permeate through the skin. To reduce the Ni cutaneous penetration, barrier creams (BC) are applied on the skin surface. There is little information, however, on the efficiency of such commercial protective creams on decreasing Ni cutaneous penetration. For this reason, the objective of the current study was to investigate the protective role of one commercially available formulation for Ni (Nik-L-Block™ containing a chelating agent) and one moisturizing cream (Ceramol 311 basic cream without chelating agent), following exposure to NiNPs, using *in vitro* Franz cells, as well as the cytotoxicity of NiNPs in primary human dermal fibroblasts was studied. Our results demonstrated that although both tested formulations can decrease Ni accumulation in the skin (4.13 ± 1.74 μg/cm^2 for Nik-L-Block™ and 7.14 ± 1.46 μg/cm^2 for Ceramol 311 basic cream); there are significant differences between the two creams ($p = 0.004$). Based on the experimental evidence, we therefore conclude that the composition of such formulations has an imperative role for dermal uptake of Ni. Finally, NiNPs showed no cytotoxic effect on cultured human dermal fibroblasts after 24 and 72 h.

1. Introduction

NiNPs have increasingly attracted researchers' attention for their unique physical and chemical properties such as small size effect, surface effect, quantum size effect, and macroscopic quantum tunneling effect of nanomaterials (Flauraud et al., 2017). Owing to these characteristics, they are widely used in high magnetic tapes, conducting pastes, chemical catalysis, adjuvant for enhancing immune responses to protein-based vaccines, microfilters, gas sensing equipment, combustion promotion, supercapacitor electrode material, and light absorbance (Alonso et al., 2010; Bhattacharjee et al., 2018; Wu et al., 2012; Lei et al., 2016; Bajpai et al., 2012; Patel et al., 2007; Wu et al., 2018). The broad application of NiNPs represents a new type of environmental (Lippmann et al., 2006) and occupational exposure. Unintended exposure to human skin may occur through the manufacture and removal of used NPs, mainly in an occupational setting. Notably, it is known that the study of NPs trafficking through the skin barrier represents a crucial step for basic and applied research (Carton and Malatesta, 2022). As nanoparticles NiNPs, may easily permeate and penetrate through the skin compared to bulk material leading to various health problems. In fact, Wang and co-authors have recently published an interesting review article on the most recent developments to the field, giving a detailed understanding of the risks and toxicity following the topical application of different metallic nanoparticles (Wang et al., 2018). It is well reported in literature that metals easily penetrate through the skin in their ionized form (Franken et al., 2015; Larese et al., 2007; Larese Filon et al., 2009)

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and their percutaneous penetration 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). The study of Crosera et al. (Crosera et al., 2016) showed that NiNPs were retained in the human skin layers, but metal ions were observed in the receptor compartment. The ionized metal represents the content of Ni able to permeate through the skin. Similarly, it was experimentally shown that NiNPs dissolved in artificial sweat, produce ions that may penetrate through the skin and directly induce an immune response (Stefaniak et al., 2014). Moreover. Tanojo et al. (2001) demonstrated that, water solution of nickel salts can pass through the stratum corneum, in in-vitro experiments. Recently, Magnano et al. (Magnano et al., 2022b) demonstrated higher permeation of Ni ions through intact skin compared to other metal ions, after exposure to road dust containing multiple metals at relatively low dose, which may represent a potential health hazard. Then, following exposure to metallic NPs, the metallic ions released by NPs may cause sensitization and irritation. Notably, percutaneous permeation of metals may activate localized immune responses in allergic individuals, ultimately resulting in allergic contact dermatitis (ACD) and systemic intoxication owing to the potential metal diffusion into the circulatory system. Additionally, it has been reported a case report of a 26-years-old female chemist that developed an important skin sensitization while handling nickel nanopowder in absence of any protective tools (Journeay and Goldman, 2014). Further, it is important to consider that an exposure (or accidental contamination) to NiNPs can bring to a potential local and/or systemic health risk. According to the International Agency for Research on Cancer (IARC) Ni compounds are classified as carcinogenic to humans (Group 1) and metallic nickel is classified as possibly carcinogenic to humans (Group 2B) (IARC (The International Agency for Research on Cancer) 2012). NPs can exhibit various toxic effects, such as oxidative stress, cell apoptosis, mitochondrial dysfunction, and cell membrane and DNA damage, once infiltrate through the skin (Sufian et al., 2017). However, it is important to note that a detailed description of the diffusion mechanisms and the knowledge of all possible adverse effects following exposure to NiNPs are still a matter of investigation. Some *in vitro* data have demonstrated the cytotoxic and apoptosis effects of NiNPs in mouse epidermal cell line (JB6 cells) (Gu et al., 2016). Similarly, the study of Alarifi et al. (Alarifi et al., (2014) explored the cytotoxicity of NiNPs and found that NiNPs at concentrations of 2, 4, 8 and 20 μg/mL could induce apoptosis and DNA damage with a dose–time response relationship in human skin epidermal cells (A431). Specific measures of protective equipment such as gloves, moisturizing and barrier creams have been developed to reduce the penetration of nickel through the skin. It is reported in the literature that nickel is soluble in rubber gloves, resulting in a less resistance of the gloves, which may promote Ni penetration and cause dermatitis (Wall, 1980; Estlander et al., 1996; Mathias, 1990). Further, due to the fact that the hands are continuously sealed inside a glove, the continuous glove-wearing can lead to maceration of the skin and inhibit skin barrier function (Wiggeralberti, 1998). For such reason barrier creams (BC) are preferred compared to gloves. Barrier creams are targeted as the common measures to limit skin contact, having several advantages: (i) they are able to add a lipid mixture to the skin surface (occlusion) provoking an immediate effect on the epidermis, (ii) the addition of the lipid mixture to the intercellular spaces leads to an intermediate effect, and (iii) they are able to provide lipids to the epidermal cells (Kucharekova et al., 2002), promoting the restoration of the natural barrier function of the skin (Kresken and Klotz, 2003), inducing a delayed effect on the epidermis. However, barrier creams may reduce dermal absorption and possible penetration of irritants into the skin due to their ability to build up a physical barrier, as a thin film, between the skin surface and the agent (Mühlen et al., 2004; Kresken and Klotz, 2003; Zhai and Maibach, 1996). Due to these features two commercially available creams are selected as protective tools to reduce skin penetration of Ni for our study. However, the evaluation of the effectiveness of barrier creams is a topic still discussed at the regulatory and clinical level (Mostosi and Simonart, 2016; Kresken and Klotz, 2003; Kütting and Drexler, 2003; Alvarez et al., 2001). Additionally, it is also important to mention that there are not official methods for testing the efficacy of barrier creams (Chilcott et al., 2005; Chilcott et al., 2007). The aim of this work therefore was to evaluate the protective capacity of one commercially available formulation to be used to protect from Ni (Nik-L-Block™) compared to a moisturizing cream (Ceramol 311 basic cream) on Ni human skin penetration following exposure to NiNPs, as well as to investigate the response of primary human dermal fibroblast cells (NHDF) to NiNPs, in order to correlate the cytotoxicity of the particles and the nickel uptake.

2. Material and methods

2.1. Literature search

Information and data related to the skin permeation of NiNPs were performed primarily in the PubMed database using the search term "NiNPs and skin" (2000 to present) with supplemental information from other relevant databases as Google scholar, Scopus. Additional studies were retrieved through reviewing the references cited in the publications identified through the original search.

2.2. Materials

All chemicals were of analytical grade: urea, sodium chloride, sodium hydrogenphosphate, potassium dihydrogenphosphate were purchased from Carlo Erba (Milan, Italy); ammonium hydroxide (25% w/v) was provided from J. T. Baker (Deventer, Holland); lactic acid (90% v/v) from Acros Organics (Geel, Belgium); and nitric acid (67–69% v/v, Normatom) from VWR (Milan, Italy). Ni nanopowder (CAS 7440-02-0) came from Sigma Aldrich (Milan, Italy), APS (Average Particle Size) < 100 nm, purity $\geq$99.9% (metals basis). Nik-L-Blok™ was provided from Chemotechnique Cosmeceuticals (Sweden) and Ceramol 311 basic cream from Unifarco Biomedical (Italy). Primary human dermal fibroblast cell line (NHDF) was purchased from Lonza, (Milan, Italy) and grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (0.1 mg/mL), amphotericin (0.25 μg/mL), and L-Glutamine (2 Mm) (all products from Euroclone, Milan, Italy). Water reagent grade was produced with 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.

2.3. Formulation

The two tested commercially available creams are Ceramol 311, Nik-

Table 1
Composition of the tested formulations.

Formulation	Ingredients
CERAMOL 311 BASIC CREAM	Butyrospermum parkii butter, Ceramide 3, Palmitamide MEA, Hydrogeneted Polydecene, Glycerin, Ammonium acryloyldimethyltaurate/VP copolymer, Cholesterol, Stearic acid, Squalane, Caprylyl Glycol, Hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, o-cymen-5-ol, Polysorbate 60, Citric Acid, Xanthan Gum, Water
NIK-L-BLOCK™	Diethylenetriamine pentaacetic acid (DTPA) 7.5%, Steareth-2, Steareth-21, Cetostearyl alcohol, Chitosan, Glycerol, Light liquid paraffin, Methylparaben, Propylparaben, Sodium hydroxide, Tinogard TT, Water

L-Block™. According to the manufacturers instructions, the composition of each cream was reported in Table 1.

2.4. Rheological properties

The two creams were analyzed for rheology. The viscosities of the formulations were determined using stress controlled rotational rheometer (Haake Mars Rheometer, 379-0200 Thermo Electron GmbH, Karlsruhe, Germany) equipped with plate-plate geometry (PP35, diameter = 35 mm) at 37 °C. By applying an increasing sequence of constant stress segments to the samples and measuring the corresponding shear rate (γ') the steady flow behavior was determined. The stress was kept constant until the relative variation of the shear rate satisfied the following constraint, $(\Delta\ \gamma'/\gamma')/\Delta t \leq 0.05$, or the segment duration (Δt) was no longer than the cut off value of 90 s. Viscosity assays were performed for Ceramol 311 and Nik-L-Block™. Briefly, 1.0 mL of the sample was loaded into the rheometer for each viscosity measurement, and the viscosity was determined at shear stress from 10^{-1} to 10^{2} Pa at 37 °C.

2.5. Preparation of ninps in synthetic sweat solution

The donor phase solution was freshly prepared by weighing 80 mg of Ni nanopowder in 80 mL of synthetic sweat at pH 4.5. Before application in the donor chamber, the suspension was sonicated in an ultrasonic bath for 10 min in order to disperse the powder as homogeneously as possible. The total nickel concentration (1.0 g L^{-1}) of the donor solution has been confirmed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) analyses.

2.6. Skin samples preparation

2.6.1. Human skin membranes

Full thickness human abdominal skin was obtained as surgical waste and approved by the Trieste Hospital Ethical Committee n° 236/2007. The two donors were men and women with ages ranging from 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 with a micrometric caliper (Mitutoyo, Roissy en France, France). 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., 2022a): the average TEWL values of the skin samples were found to be below 10 g $m^{-2}{\cdot}h^{-1}$ (Guth et al., 2015).

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

Skin absorption studies were performed in static diffusion cells according to the OECD guidelines (OECD, 2004). The protocol for testing the skin permeation of Ni after exposure to NiNPs was derived from our previous works (Crosera et al., 2016; Magnano et al., 2022b). 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 fluid (RF) 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 RF. Mounted Franz cells were maintained at 32 ± 1 °C by means of circulation of thermostated water in the jacket surrounding the cell.

2.7.1. Application of creams

Skin pre-treatment consisted of 25 mg/cm^2 of cream applied as homogenously as possible using a cotton swab with a gloved finger prior to NiNPs exposure. This quantity was chosen according to the protocol of Bignon et al., (2017). After a complete drying of the deposit (~3 min) cells were closed. The formulations used were Ceramol 311 basic cream (moisturizing cream) and Nik-L-Block® (barrier cream for Ni).

2.7.2. Sampling

The skin absorption experiments were carried out as follows:

Exp. 1 skin pre-treated with formulations: Briefly, infinite doses of 0.6 mL of pure freshly made suspension of NiNPs in synthetic sweat at pH 4.5 was applied to the skin surface pre-treated with the two creams. This resulted in a theoretical applied dose of Q_0 = 0.63 mg/cm^2. The choice of 0.6 mL in the donor compartment was dictated to be in agreement with the protocol of the European Project EDETOX 2000. 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 Ni remaining and permeating through the skin. At selected time points (3, 6, 9, 18, 21, and 24h) 0.5 mL of each receptor sample was collected and analyzed. An equal volume of fresh receptor fluid was immediately replaced in each sample. All experiments were conducted on 6 independent biological replicates. Skin from two donors was tested.

Exp. 2 skin without pre-treatment: Experiments were performed following the same procedure described above (Exp. 1), but the skin was not pre-treated with creams. All experiments were conducted on 6 independent biological replicates. Skin from two donors was tested.

Blanks: A skin sample with no NiNPs applied to the skin surface and without pre-treatment with creams was used as a blank in each run. In the donor chamber 600 μL of synthetic sweat (pH = 4.5) were added, and the experiment was performed following the procedure described in Exp. 1. All experiments were conducted on 4 independent biological replicates. Skin from two donors was tested.

The amounts of Ni in the RF as well as in each skin layer after 24 h were quantified by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS), method described below, paragraph 2.9.2.

In an infinite dose experiment, the permeant concentration is constant over a long enough experimental duration to establish a steady state characterized by flux J reaching a constant (i.e., steady state) value, designated J_{ss}. The ratio of J_{ss} (i.e., the slope of the linear portion of M(t)) to the external concentration difference ΔC_{ext}) is defined as the permeability coefficient, K_p. $K_p = J_{ss}/\Delta C_{ext}$ The intercept of the linear (i.e., steady state) portion of M(t) with the time axis is defined as the time lag, t_L (Hopf et al., 2020).

2.8. Collection and treatment of samples

After 24 h of exposure, the cells were dismantled. All the receptor fluid was removed, and frozen for subsequent analysis. 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 membranes (4 cm^2) were cut in a "little circle" in order to get only the exposed "exposed area" (0.95 cm^2). From exposed skin area (0.95 cm^2), 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 2.9). The receptor fluid was diluted 1:10 in MilliQ water acidified with 1% nitric acid before the ICP-MS analysis.

2.9. 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.

2.10. Analytical measurements

2.10.1. Quantification of nickel by icp-oes

The total Ni concentrations in the donor solutions were determined by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP – OES) using an Optima 8000 ICP – OES Spectrometer (PerkinElmer, USA) equipped with an S10 Autosampler (PerkinElmer, USA). The donor solutions were collected at the end of the experiment, ultrafiltrated in centrifuged (2400 g, 10 min, 20 °C) using the Amicon Ultra-4 centrifugal filters (10 K MWCO), in order to evaluate the percentage of ionized metal in the donor phase, according to our previous work (Crosera et al., 2016). The filtered solutions were recovered, diluted 1:10 in MilliQ water and analyzed using ICP-OES at the operative wavelength of 231.604 nm. The analyses were conducted using a calibration curve obtained by dilution (range: 0–10 mg L^{-1}) of a multistandard solution (10 mg L^{-1}) for ICP analyses (Periodic Table MIX 1, Sigma-Aldrich). The calibration curve was linear from 0.1 to 10 mg L^{-1} ($R^2 = 0.9999$) and five calibration points from 0 to 10 mg L^{-1} (0; 0.1; 1; 5; 10) were carried out. The limit of detection (LOD) was 0.020 mg L^{-1} by ICP-OES. The precision of the measurements as relative standard deviation (RSD%) for the analysis was always less than 5%.

2.10.2. Quantification of nickel by icp – ms

The Ni concentration of controls and exposed skin samples, together with receptor solutions, were evaluated by Inductively Coupled Plasma – Mass Spectrometry (ICP – MS) using a NexION 350X 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 ($R^2 = 0.999$ Ni; ion mass selected: 60 u.m.a.) in the concentration range of 0.1–100 μg L^{-1} according to the dilution of a multistandard solution of 10 mg L^{-1} for ICP analysis (Periodic Table MIX 1, TraceCERT Sigma-Aldrich). Six calibration points from 0 to 100 μg L^{-1} (0; 0.1; 1; 5; 10; 100) were used. 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 0.025 μg L^{-1} for Ni. The coefficient of variation of repeatability (RSD %) was <3%. Moreover, the analysis was performed using Sc (45 u.m.a.; spike of 100 μg L^{-1}, prepared by dilution from a standard solution at 1000 mg L^{-1}, Scandium Standard for ICP, TraceCERT Sigma-Aldrich) as an internal standard to minimize the potential matrix effects. An additional quality control was performed by the analysis of laboratory-fortified samples prepared by spiking 1 or 5 μg L^{-1} (depending on Ni concentrations in the investigated samples) of Ni into actual samples to calculate the recovery percentage. These laboratory-fortified samples were prepared for each matrix (solutions from skin digestion, and receptor fluid) to obtain a robust method for the analysis. Acceptable recoveries from spiked samples were obtained (ranging between 90 and 110%).

2.11. In vitro cytocompatibility

2.11.1. Cell culture and exposure to ninps

Primary human dermal fibroblast cells (NHDF) were used to determine the cytotoxicity of NiNPs and other chemical forms of nickel ($NiCl_2$). Cells were cultured in 75-cm^2 flasks in Dulbecco's modified Eagle medium (DMEM) with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 μg/mL amphotericin, and 2 mM L-Glutamine at 37 °C in a 5% CO_2 humidified atmosphere. When subconfluent (about 80%), cells were harvested by using 0.25% trypsin and seeded into 48 multiwell plastic microplates and allowed to adhere to the surface for 24 h before treatments. Following, medium was renewed and supplemented with 1, 0.1, 0.01, and 0.001 mg/mL of NiNPs and $NiCl_2$. Then, cells were cultured for 24 and 72 h. Before treatment, the suspension of NiNPs was sonicated for 10 min in an ultrasonic bath in order to disperse the powder as homogeneously as possible. After sonication, NiNPs were vortexed at room temperature for 1 min and then tested on the cells. At the end of each incubation time, cells were processed as described below. Untreated cells were used as control. The experiments were repeated in triplicate.

2.11.2. Mtt assay

Cell viability was analyzed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT assay. MTT assay was used to investigate mitochondrial function as described by Mossman (Mosmann, 1983). Briefly, 8×10^4 cells/well were seeded in 48-well plates and exposed to different concentrations (1, 0.1, 0.01, and 0.001 mg/mL) of NiNPs or $NiCl_2$ for 24 and 72 h. After each incubation time, culture media was replaced with 100 μL of 0.5 mg/mL MTT and incubated at 37 °C for 4 h. Then, MTT solution was removed and the formazan crystals were dissolved in 100 μL of dimethyl sulfoxide (DMSO), that were placed in a 96 multiwell microplates. Additionally, in order to avoid interference with micro traces of Ni, the different NiNPs and $NiCl_2$ concentrations were solubilized in medium and used as blank. The plates were kept on a shaker for 10 min at room temperature and then analyzed at 570 nm using a multiwell microplate reader (2030 Multilabel Reader Victor YMX4, PerkinElmer, Milan, Italy).

2.11.3. Cell morphology

The morphology of NHDF cells was observed at 24 and 72 h after exposure to different concentrations (1, 0.1, 0.01, and 0.001 mg/mL) of NiNPs and $NiCl_2$. Representative images were taken at 20X magnification for each condition directly from 48 multiwell plate using IM-3 OPTIKA inverted microscope equipped with a digital microscope camera (Leica DFC320, Wetzlar, Germany).

2.12. Statistical analysis

The results are expressed as the quantity penetrated per skin surface unit (μg·cm^{-2}) or as the quantity permeated per skin surface unit (ng/cm^2). Data from skin absorption experiments were expressed as mean ± standard deviation (SD). Data from skin permeation experiments were expressed as mean ± standard error of the mean (SEM). Statistical analysis of differences between two groups were analyzed by Student's t-test and 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). Regarding cell viability, data were expressed as mean ± standard deviation (SD). Student's t-test was used for evaluating the significance of the results compared to the controls. The considered statistical significance was $p < 0.05$ (*$p < 0.05$, **$p < 0.01$).

3. Results

3.1. Characterization of the creams: rheological properties

In the present study, the viscosity of the formulations was measured by using an increasing shear stress. It was observed that by increasing the shear stress, the viscosity of the formulations significantly declined, and vice versa. The viscosity of creams is significant in their application, since it is inversely correlated to the spreading: on the one hand a highly viscous cream shows low spreadability, while on the other hand low viscosity eases the application. The viscosity behavior of both formulations (Ceramol 311 basic cream and Nik-L-Block™) was very similar. Viscosity drops down at the same deformation (Jurić Simčić et al., 2023), suggesting that creams present same internal structure, hence a

comparable resistance versus nickel particles.

3.2. Ni quantification in donor solutions

The concentration of solubilized Ni in donor solutions (DS) after 24 h of exposure was quantified using an ICP – OES after the removal of NiNPs by ultrafiltration. The analysis revealed that the effective dose of Ni ions, expressed in mg/cm^2, was 0.017 mg/cm^2. The ionized metal represents the effective dose (Ni ions able to cross the skin barrier and reach the receiving phase) (Crosera et al., 2016; Larese Filon et al., 2015). This result was further used in the permeability coefficient (K_p) calculation as effective dose (see section 3.4 and see Table 4 and Fig. 1).

3.3. Human skin permeation of NiNPS

The Ni concentrations in the receptor fluid expressed in ng/cm^2 are represented in Fig. 2. In the experimental condition, the mean amounts of Ni observed in RF in exposed skin samples increase over time. Specifically, the amount of Ni ions that has permeated through skin samples was relatively higher for skin samples pre–treated with the two tested formulations (543 ± 81.8 ng/cm^2 for Ceramol 311 basic cream and 714 ± 308 ng/cm^2 for Nik-L-Block™) compared to the samples without pre-treatment (351 ± 85.2 ng/cm^2), respectively, at 24 h. As expected the total amount of Ni was 0.95 ± 0.05 ng/cm^2 in blank samples. However, no statistically significant differences between not pretreated samples and pre-treated skin samples with creams were found.

3.4. In vitro skin content of NiNPs

The amount of Ni retained by the different skin layers was quantified and presented in Table 2 and Fig. 3. The results reported in Fig. 3, clearly show that both samples pre-treated with the two creams showed a lower skin penetration of Ni following NiNPs exposure, compared to control samples (not pretreated), indicating the protective effect of the tested products. As it can be seen in Fig. 3, the lowest total amounts of Ni penetrated in human skin layers, epidermis and dermis (E + D) observed for samples pre-treated with Nik-L-Block™ were closed to samples pre-treated with Ceramol 311 basic cream, reaching 4.13 ± 1.74 μg/cm^2 and 7.14 ± 1.46 μg/cm^2 respectively. Moreover, accumulation of Ni in the viable epidermis for both samples pre-treated with Ceramol 311 basic cream and Nik-L-Block™ ranged around 1.41 ± 1.00 μg/cm^2. Further, these two formulations exhibited higher reduction of Ni compared to not pretreated samples (36.0 ± 25.0 μg/cm^2). Importantly, the different dermis and total skin (E + D) data of Ni obtained by the two formulations are statistically different (dermis p = 0.008; total skin p = 0.004). As expected, the total quantity of Ni retained into (E + D) was detectable

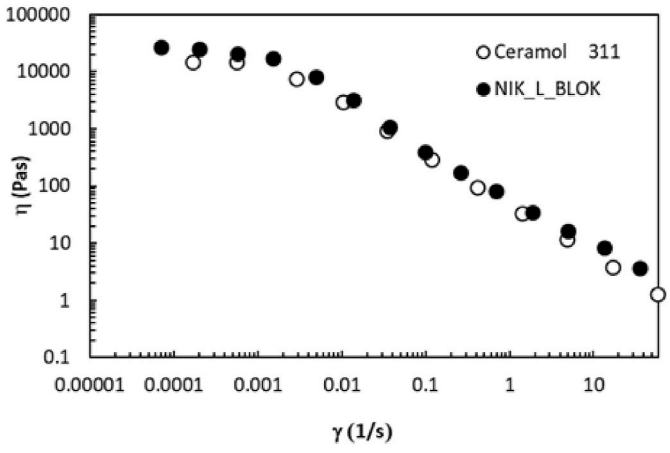

Fig. 1. The viscosity versus deformation rate of Ceramol 311 basic cream (white circles) and Nik-L-Block™ (black circles).

at very low level (0.72 ± 0.29 μg/cm^2) in blank samples, without NiNPs exposure. A statistically significant difference between blanks and exposed skin samples to NiNPs were found.

Further, the full absorbed recovered amount (Q_{abs}) was calculated utilizing Eq. (1).

$$Q_{abs} = E + D + RF \quad (1)$$

where E is the sum of the recovered amounts of Ni in the epidermis, D is the sum of the recovered amounts of Ni in the dermis, RF is the sum of the recovered amounts of Ni in the receptor fluid.

Q_{abs} of Ni values obtained from samples pre-treated with Nik-L-Block™ and samples pre-treated with Ceramol 311 basic cream reached values of 4.84 ± 1.83 μg/cm^2 and 7.68 ± 1.35 μg/cm^2, respectively. On the contrary, Q_{abs} of Ni values obtained from not pretreated samples was 36.4 ± 25.3 μg/cm^2. A statistically significant difference was found between the two creams (p = 0.007).

Finally, considering only the Ni permeated in the receiving solutions, using the Ni ion concentration in the donor solutions as effective dose, the permeability coefficient K_p (cm/h) of Ni from each cell was determined by dividing the flux at the steady state by the concentration of Ni ions in each donor solution. The flux has been calculated as the slope of the linear range of the permeation profiles: this range has been identified as 3–9 h of exposure in some cells and 3–12 h in other cells. Beyond this range permeation decreased and, in some cases, stabilized in a plateau (see Fig. 2). The lowest K_p was observed for not pretreated samples ranging around $1.4*10^{-3}$, while the highest K_p of Ni was obtained for samples pre-treated with Nik-L-Block™ with a values measured in the range of $3.5*10^{-3}$. The values were summarized in Table 4.

3.5. Cytotoxicity of NiNPs towards primary human dermal fibroblasts

The in vitro cell viability assay is showed in Fig. 4. Results shown a dose-dependent cellular toxicity for both compound tested: NiNPs and $NiCl_2$. Notably, after 24 h a significant reduction of cells viability was observed at the highest concentrations (1 and 0.1 mg/mL) for both the compounds tested, with the lowest cells viability for HNDF exposed to 1 mg/mL of $NiCl_2$ (6.4 ± 0.14%). After 72 h, a significant decrease of cell viability was observed for the cells treated with NiNPs and $NiCl_2$ up to 0.01 mg/mL. Interestingly after 72 h, at the highest concentration (1 and 0.1 mg/mL), $NiCl_2$ appears to be more toxic than NiNPs; this situation is reversed at low concentrations (0.01 and 0.001 mg/mL).

3.6. Morphological evaluation of primary human dermal fibroblast cells

Morphological examination revealed that at the higher concentrations (1 and 0.1 mg/mL) the cells growth was significantly inhibited, and some of them became irregularly shaped, dead and floating (arrows in Fig. 5). Moreover, even after 72 h exposure, cells exposed to 0.01 and 0.001 mg/mL (lower concentrations) were elongated, flat, with a spindle morphology, representing the typical phenotypes of human fibroblasts (Fig. 5).

4. Discussion

In the rapidly expanding field of nanotechnology, various metal nanoparticles have been developed for many fields such as catalysts, energy technology, biomedicine (Lei et al., 2016; Wu et al., 2018) and in some case adopted as the most efficient way to reduce intoxication by dermal contamination (Magnano and Rui, 2021). Notably, the advantages of using NiNPs in biomedical applications have been stressed in the literature (Jaji et al., 2020). It was reported that NiNPs have a potential role in the suppression of *Staphylococcus aureus* and *Escherichia coli* (microbial pathogens) (Helan et al., 2016), as well as they represent a good drug carrier in the transmission of lymphatic filariasis (Angajala et al., 2014). Then, NiNPs are able for targeting the cytotoxicity of

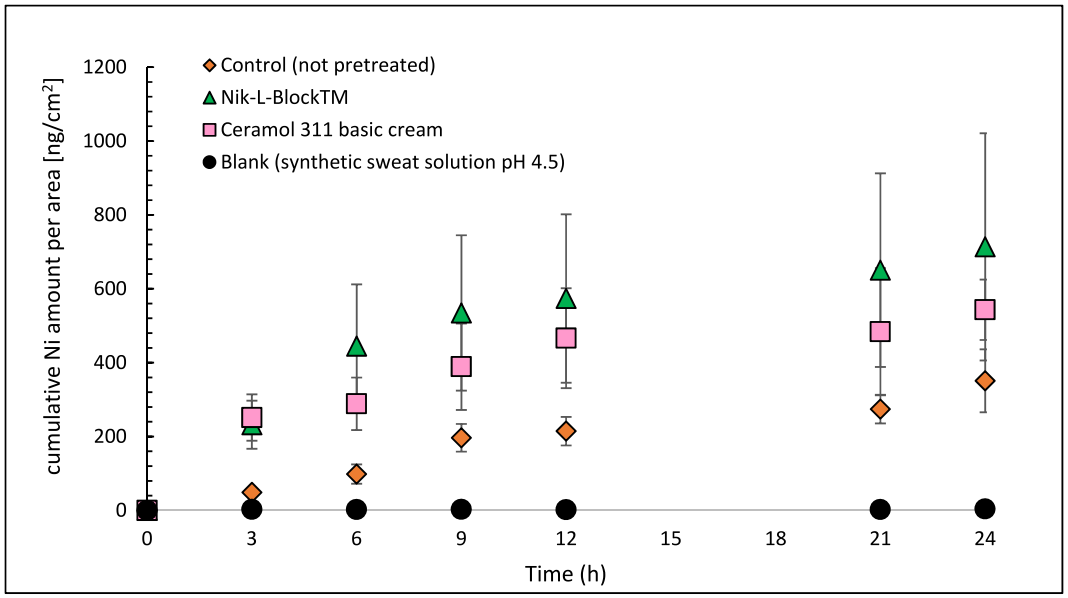

Fig. 2. Ni concentration that permeated into the receptor fluid at specific extraction times. Values are expressed as mean ± standard error of the mean (SEM) (n = 6 exposed skin samples; n = 4 blank samples).

Table 2
Ni amount found in skin layers in blanks and exposed skin after 24 h exposure of NiNPs. Applied dose was 0.63 mg/cm^2. Data are given as mean ± SD. Asterisk (*) indicates statistically significant difference obtained between blanks and exposed skin samples to NiNPs in the Mann-Whitney test ($p < 0.05$). (¥) show the statistically significant differences obtained between samples pre-treated with Nik-L-Block™ and skin samples pre-treated with Ceramol 311 basic cream in the Student's t-test ($p < 0.05$).

	Epidermis (E) (μg/cm^2)	Dermis (D) (μg/cm^2)	Total Skin (E + D) (μg/cm^2)
Blanks	0.04 ± 0.00	0.69 ± 0.17	0.72 ± 0.17
Control (not pretreated)	19.8 ± 14.5*	16.2 ± 10.8*	36.0 ± 25.3*
Ceramol 311 basic cream	1.58 ± 0.59*	5.56 ± 1.88*¥	7.14 ± 1.46*¥
Nik-L-Block™	1.41 ± 1.00*	2.72 ± 0.75*¥	4.13 ± 1.74*¥

leukaemia cancer cells (Guo et al., 2008) and the study of Sudhasree et al., (2014) showed that green Ni nanoparticles are good antimicrobial agents. Similarly, green AgNPs can be used effectively as antimicrobial and anticancer agents in the food industry and medical applications as reported by the study of Baran et al., (2022). Although the biomedical effects of NiNPs, the widely use of such nanoparticles may represent a new type of occupational exposure. It is reported that NPs can penetrate through skin cells, (transcellular pathway) and through hair follicles (follicular penetration pathway) (Nangia and Sureshkumar, 2012). Theoretically, the smaller the size of the particles, the higher the rate of penetration (Yokota and Kyotani, 2018). Two commercially available products were applied on the skin one to prevent the dermal absorption of Ni and the other as moisturizing cream, following exposure to NiNPs. Moreover, excised human skin, which is the gold standard for permeation studies compared to animal skin, or artificial membranes (Khan et al., 2005; Přborský and Mühlbachová, 2011; Godin et al., 2007) was chosen as a biological barrier to perform the experiments. In the current study, the skin was pre-treated with two formulations and then exposed to NiNPs solubilized in synthetic sweat at pH 4.5. Since NiNPs tend to aggregate in water dispersion and considering that the tested NPs have a size around 80 nm, it seems that they are too big to be absorbed through the skin (Larese Filon et al., 2015). Thus it can be hypothesized that the permeation is most likely due to the ionized metal released from the NiNPs in synthetic sweat. Moreover, metallic ions 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. However, it is important to note that, generally, a protective cream is marked as cosmetic products or locally-applied medical devices to immobilize the potential allergens or irritants, in order to prevent the skin penetration and permeation of such agents (Zhai and Maibach, 2002). As it can be noticed from our results (Fig. 2), permeation of Ni after 24 h was higher for samples pre-treated with creams to that observed for controls (not pretreated samples), but no statistically significant differences between the groups were found. Interestingly, there have been examples reporting an increase of skin penetration of toxic molecules following application of skin barrier creams (SBCs) compared to untreated samples. The study of Korinth (Korinth et al., 2008) showed that after the application of SBC higher skin permeations of Aniline (ANI) and the human carcinogen o-toluidine (OT) were registered. This effect may be attributed to different reasons: ethoxylated emulsifiers, substances acting as penetration enhancer (glycols, ethers, etc) which may make the upper layers of stratum corneum more permeable, affecting the percutaneous uptake of the substances (Marjukka Suhonen et al., 1999). Concerning our results, the higher permeation of Ni observed by the application of Nik-L-Block™, may be explained by the presence of the two emulsifiers such as Steareth-2, Steareth-21, which act as transdermal enhancers affecting the permeation pathway (Otto et al., 2009). On the other hand, Ceramol 311 basic cream is an oil-in-water (o/w) emulsion containing a polymeric surfactant such as Hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, which does not interfere with the skin structure (Opatha et al. 2022). It was generally presumed that the penetration of ingredients in a o/w emulsion, is higher when they are dissolved in the continuous phase of the emulsion (Wiechers, 2005). In case of the o/w emulsion, the ingredients are mainly distributed into the water continuous phase of the emulsion (Otto et al., 2009); hence considering that in our case the substance is hydrophilic, it maybe hypothesized that the affinity of Ni for some ingredients of Ceramol 311 basic cream in the continuous phase of the emulsion, may apparently

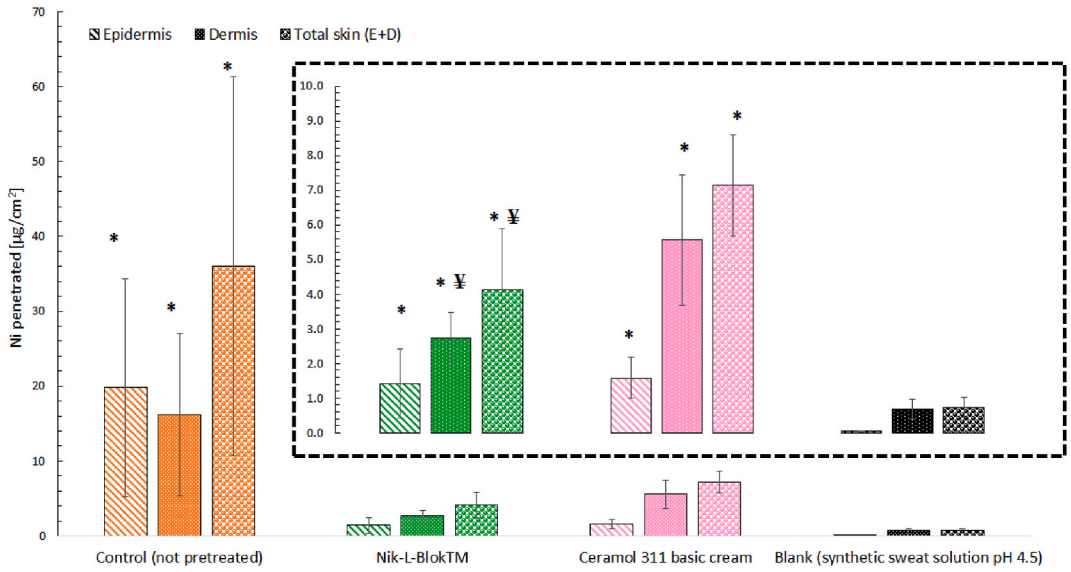

Fig. 3. Ni concentration found in skin layers (E and D) after 24 h exposure of NiNPs. Applied dose was 0.63 mg/cm^2. Data is given as mean ± SD (n = 6 exposed skin samples; n = 4 blank samples). (*) show the statistically significant differences obtained between blanks and exposed skin samples to NiNPs in the Mann-Whitney test ($p < 0.05$). (¥) show the statistically significant differences obtained between samples pre-treated with Nik-L-Block™ and skin samples pre-treated with Ceramol 311 basic cream in the Student's t-test ($p < 0.05$).

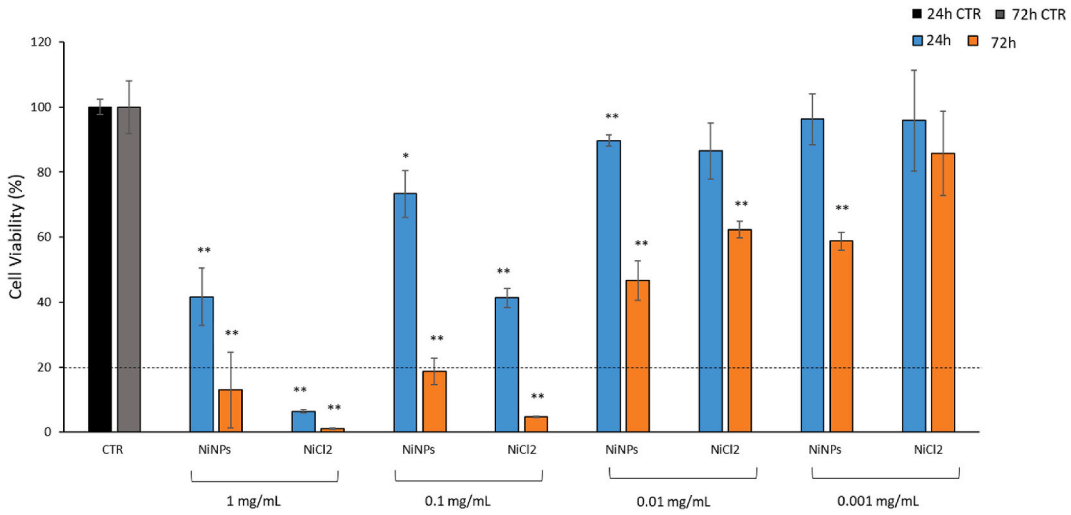

Fig. 4. Effect of different concentrations (1 mg/mL – 0.001 mg/mL) of NiNPs and $NiCl_2$ on NHDF viability measured by MTT assay after 24 and 72h exposure. Data are given as mean value ± S.D. Stars show the statistically significant differences obtained between treated samples and controls (24h and 72h) in the Student's t-test *$p < 0.05$; **$p < 0.01$.

facilitate the permeation. Furthermore, occlusion, large amounts in the donor solutions, as well as the water act as penetration enhancers (Otto et al., 2009; Williams and Barry, 2012). The increased skin hydration results in an increase in transdermal delivery of both hydrophilic and lipophilic permeants (Williams and Barry, 2012). In addition, the distribution of Ni in the various skin layers was also assessed post-exposure. Concerning the samples pre-treated with creams, the total skin amounts of Ni were totally lower compared to those quantified in the untreated samples, confirming the ability of these two formulations to prevent the Ni skin accumulation, acting as an "invisible glove" (Zhai and Maibach, 2002). Further, it is important to point that the degree of protection of the two tested products was able to reduce the Ni penetration through the skin layers, with a high efficacy for Nik-L-Block™ (Table 2 and Fig. 3). This positive effect is attributed to the presence of the active ingredient such as the chelator DTPA, intended to complex the metal ions and to block them from penetrating the skin (Blanusa et al., 2005). Similarly, Ceramol 311 basic cream which is an o/w emulsion without chelating agents, showed a good protection for dermal uptake of Ni. This can be attributed to the presence of some ingredients such as ceramide 3, cholesterol, hydrogenated polydecene and fatty acids which are able to form a more resistant lipid film, playing the major role to reduce cutaneous penetration of Ni. Such formulation is a repairing and protective cream for the epidermal barrier containing a balanced mixture of fundamental lipids of the epidermis (ceramide 3, cholesterol, and fatty acids) able to restore the skin barrier function (Kahraman et al., 2019; Mao-Qiang et al., 1996) and palmitamide MEA with soothing activity on irritation and itching (Corazza et al., 2014). However, this cream exhibited a slightly higher percutaneous penetration of Ni compared to Nik-L-Block™, due to the fact that the ingredients are mainly distributed into the aqueous continuous phase of the emulsion (Otto et al., 2009) and thus not allow easy diffusion through the stratum corneum, resulting in a less efficacy of this formulation. Furthermore, the total

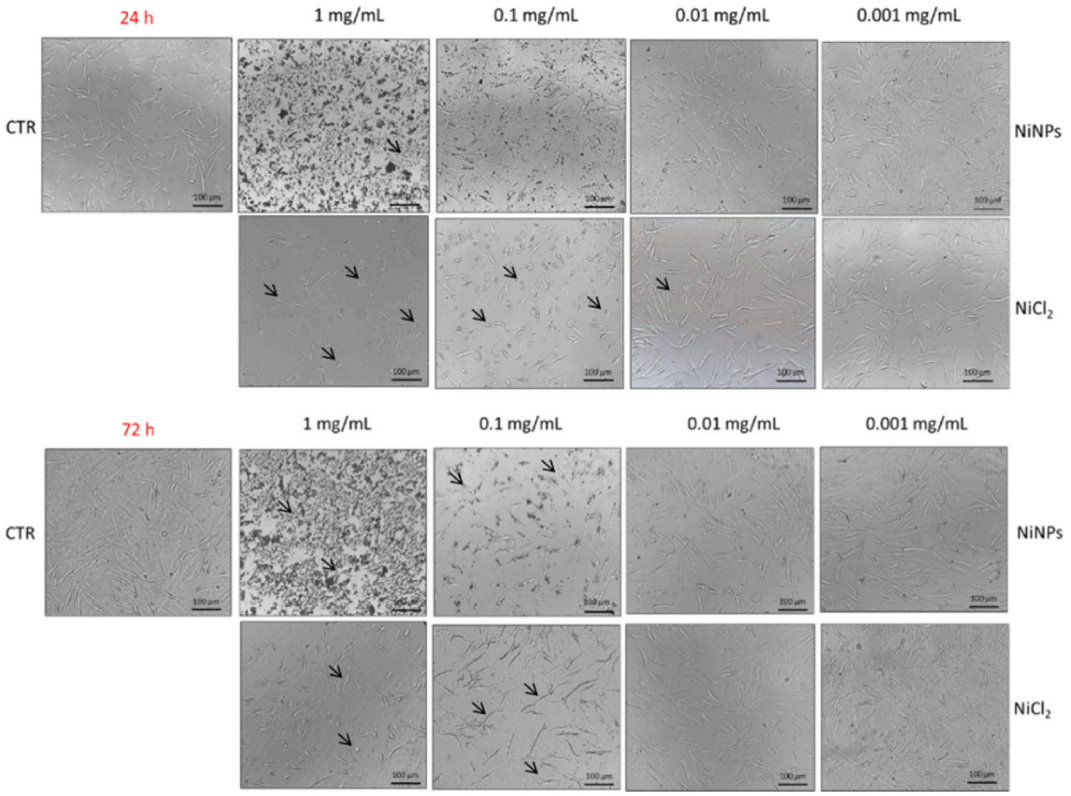

Fig. 5. Morphological evaluation of NHDF cells after 24h and 72h exposure to different concentrations of NiNPs and $NiCl_2$ (1 mg/mL - 0.001 mg/mL). Representative bright field images were collected at 20X magnification.

absorbed amounts (Q_{abs}) of Ni for samples pre-treated with the two creams were statistically different, while the highest total skin absorption of Ni was measured for not pretreated samples (Table 3), which was expected based to the absence of the treatment with the creams. Further, the K_p of Ni from each sample was determined to compare the percutaneous kinetics. It can be noticed from our data that the highest K_p was obtained for samples pre-treated with Nik-L-Block™, following Ni exposure (Table 4). Furthermore, it is important to point out that both creams are able to reduce Ni concentrations in epidermis and dermis layers, resulting in a lower risk of allergic reactions. However, this study confirms the capability of nickel ions to permeate and accumulate through the skin and it is in line with the study of (Hagvall et al., 2021). Notably, Hagvall (Hagvall et al., 2021) showed that nickel ions were found to penetrate in the skin mainly in the stratum corneum and to some extent in the upper parts of the epidermis, while in our tests Ni ions were more retained in dermis layer than epidermis layers for samples pre-treated with creams. Then, from an exposure point of view, our data demonstrated that a skin contact with small amount of NiNPs, compared to bulk materials, may lead to a relevant absorption of nickel through the skin, but the application of a protective cream is able to reduce the Ni uptake. Although metal-based NPs represent an important component of engineered NPs, multiple mechanisms contribute to cellular toxicity. Indeed, it is well known that following cellular uptake, metal-based NPs can generate oxidative stress either by releasing metal ions directly or by causing mitochondrial dysfunction. The excessive ROS generation has a considerable impact on several cell signaling pathways leading to cell apoptosis and necrosis (Zhang et al., 2022). For this reason, in this work the cytotoxic effect of different concentration of NiNPs was detected thorough MTT assay, a reliable test that measure the mitochondria activity. The cytotoxic effects of NiNPs and $NiCl_2$ were investigated using primary human dermal fibroblast (NHDF) as cell model. The analysis was performed with NiNPs and Ni ion solutions having concentrations similar to those found in the skin layers (0.01–0.001 mg/mL). Our data revealed that both NiNPs and $NiCl_2$ induced cytotoxicity in a dose-and time-dependent manner. After 24 and 72 h, concerning 1 mg/mL and 0.1 mg/mL concentrations, a lower cell viability was observed when dermal fibroblasts were incubated with $NiCl_2$ compared to NiNPs (Fig. 4). This different behavior between $NiCl_2$ and NiNPs can be ascribed to Ni^{2+}, the most common oxidation state in aqueous solutions, which combines readily with elements found in rows IV–VII of the periodic table, oxygen-, nitrogen-, and sulfur-containing ligands. While in solution, it can easily form complexes, including hydroxides, carbonates, carboxylic acids, phosphates, amines, and mercaptans. Then, it is well reported that Ni for ionic forms can enter the cell via calcium channels or by diffusion (Refsvik and Andreassen, 1995; Davidson et al.,

Table 3
Full absorbed recovered amount (Q_{abs}) of Ni after 24 h exposure of NiNPs. Applied dose was 0.63 mg/cm^2. Data are given as mean ± SD. Asterisk (*) indicates statistically significant difference obtained between samples pre-treated with Nik-L-Block™ and skin samples pre-treated with Ceramol 311 basic cream in the Student's t-test ($p < 0.05$).

	Total Skin (E + D) (μg/cm^2)	Receptor Fluid (RF) (μg/cm^2)	Q_{abs} (E + D + RF) (μg/cm^2)
Control (not pretreated)	36.0 ± 25.3	0.35 ± 0.15	36.35 ± 25.3
Ceramol 311 basic cream	7.14 ± 1.46*	0.54 ± 0.14	7.68 ± 1.35*
Nik-L-Block™	4.13 ± 1.74*	0.71 ± 0.53	4.84 ± 1.83*

Table 4
Ni flux and skin permeability coefficients (K_ps) for each treatment.

Ni	Flux (ng/cm^2/h)	Permeability Coefficient K_p (cm/h)
Control (not pretreated)	22.0 ± 11.4	$1.4 \times 10^{-3} \pm 0.7 \times 10^{-3}$
Ceramol 311 basic cream	34.5 ± 25.7	$2.3 \times 10^{-3} \pm 1.7 \times 10^{-3}$
Nik-L-Block™	57.9 ± 38.7	$3.5 \times 10^{-3} \pm 2.3 \times 10^{-3}$

2005; Lu et al., 2005); while Ni for particulate forms via endocytosis and generate Ni ions (Ni^{2+}) (Costa et al., 1980; Costa et al., 2005; Beyersmann and Hartwig, 2008). The Ni distribution within the cell is regulated by the relative concentrations and affinities of intracellular and extracellular ligands to nickel (Coogan et al., 1989). However, it is important to known that at the concentration (0.001 mg/mL), both $NiCl_2$ and NiNPs do not affect cell viability after 24 h. Interestingly after 72 h exposure, the lowest concentration of nickel chloride salt (0.001 mg/mL) showed a cell viability comparable to the control (Fig. 4). However, despite the reduction of cell viability after 72 h in the samples treated with 0.001 mg/mL of NiNPs, the cell viability remains over 50% suggesting an adequate biocompatibility. These observations were also confirmed by the morphological analyses in which alterations of cellular morphology were mainly observed at the highest concentrations while the typical spindle shaped morphology was preserved at 0.01 and 0.001 mg/mL for both $NiCl_2$ and NiNPs.

Our study is the first, on the best of our knowledge, that investigated the efficacy of the application of a barrier cream containing a chelating agents for nickel and a moisturizing cream containing a balanced mixture of fundamental lipids of the epidermis (ceramide 3, cholesterol, and fatty acids) to stop the permeation of NiNPs through the skin with the aim to prevent NiNPs skin absorption and related toxicity. Due to their bigger size (around 80 nm), NiNPs cannot permeate and penetrate through the skin as they are, but they can release more Ni ions than bulk material. Therefore, Ni ions can easily penetrate and permeate through the cutaneous barrier, leading to higher penetration of Ni (Ni inside the skin) and higher Ni permeation (Ni amount that reaches the receiving solution in vitro and the general circulation in vivo) (Larese Filon et al., 2015). Finally, it is important to point out that our conditions do not reflect those recommended for the use of the product: for example, the application of a barrier cream (Nik-L-Block™) should be reapplied after contact with water or in case of heavy sweating. Moreover, the current study has some limitations: firstly, the percutaneous absorption was investigated in static Franz cells, an in vitro method, which may not reproduce the 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. Secondly, to mimic sweat, the stratum corneum was exposed for 24 h, but the excessive hydration may promote the transcutaneous passage of many compounds.

5. Conclusion

Our findings demonstrated that both tested formulations are able to reduce the Ni accumulation through the skin layers compared to the not pretreated samples with a high efficacy for Nik-L-Block™. This positive effect is attributed to the presence of the active ingredient such as the chelator DTPA, intended to complex the metal ions and to block them from penetrating the skin. Similarly, Ceramol 311 basic cream which is an o/w emulsion without chelating agents showed a good protection for dermal uptake of Ni, due to the capacity of some ingredients such as ceramide 3, cholesterol, hydrogenated polydecene and fatty acids, to form a more resistant lipid film able reducing the cutaneous penetration of the metal. However, such creams are statistically different. Finally, our data on cultured human dermal fibroblasts suggest that after 24 and 72 h no cytotoxic effect was observed for NiNPs and $NiCl_2$ at the lowest concentrations used (0.01 and 0.001 mg/mL), indicating that at this level NiNPs and Ni^{2+} are not toxic.

Author contributions

Greta Camilla Magnano: Conceptualization, Data Curation, Investigation, Writing - original draft. **Flavia Carton**: Data Curation, Investigation, Writing - original draft. **Francesca Boccafoschi**: Supervision, Resources, Writing - review & editing. **Giovanna Marussi**: Investigation. **Elisa Cocetta**: Investigation. **Matteo Crosera**: Supervision. **Gianpiero Adami**: Supervision. **Dario Voinovich**: Supervision, Writing - review & editing. **Francesca Larese Filon**: Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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