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Effectiveness of Hand Washing on the Removal of Iron Oxide Nanoparticles from Human Skin Ex Vivo

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ABSTRACT

In this study, the effectiveness of washing with soap and water in removing nanoparticles from exposed skin was investigated. Dry, nanoscale hematite (α-Fe_2O_3) or maghemite (γ-Fe_2O_3) powder, with primary particle diameters between 20-30 nm, were applied to two samples each of fresh and frozen *ex vivo* human skin in two independent experiments. The permeation of nanoparticles through skin, and the removal of nanoparticles after washing with soap and water were investigated. Bare iron oxide nanoparticles remained primarily on the surface of the skin, without penetrating beyond the stratum corneum. Skin exposed to iron oxide nanoparticles for 1 and 20 hours resulted in removal of 85% and 90%, respectively, of the original dose after washing. In the event of dermal exposure to chemicals, removal is essential to avoid potential local irritation or permeation across skin. Although manufactured at an industrial scale and used extensively in laboratory experiments, limited data are available on the removal of engineered nanoparticles after skin contact. Our finding raises questions about the potential consequences of nanoparticles remaining on the skin and whether alternative washing methods should be proposed. Further studies on skin decontamination beyond use of soap and water are needed to improve the understanding of the potential health consequences of dermal exposure to nanoparticles.

INTRODUCTION

Guidelines for handling engineered nanoparticles (NPs) have focused on engineering controls and personal protective equipment to reduce occupational exposures and avoid potential health effects. In addition to preventing exposure, it is also important to evaluate the effectiveness of first aid measures recommended for NPs in the event of mishandling or an accident. Evidence of health effects, including skin reactions, have been reported for a worker weighing dry, nanoscale, nickel powder on an open lab bench instead of using a fume hood or ventilated enclosure.[1] Based on the description, the onset of symptoms were not immediate, suggesting repetitive exposure and adherence of the aerosolized powder to the skin. Our research focuses on iron oxide NPs for which only two studies report skin penetration testing data.[2, 3]

Public concern about the incorporation of TiO_2 and ZnO NPs in sunscreens is reflected in the large number of dermal studies using TiO_2 and ZnO compared to other core compositions of NPs.[4] The *ex vivo* and *in vivo* human skin studies focused on determining the extent of skin permeation and penetration of NPs after application in suspension or emulsion form. There is little evidence in these human studies that demonstrates NPs cross the stratum corneum into the epidermis and dermis.[5-7] This lack of penetration is supported as most, if not all, of the NPs that were experimentally tested had particle diameters greater than 5 nm, for which the predicted permeability coefficient is zero.[8]

Although NPs may not penetrate to living skin tissue, few studies investigate the removal of NPs from the skin surface.[9-11] The adherence of spilled or deposited solid nanopowder onto uncovered skin is the likely exposure scenario for workers handling dry NPs. For example, the possibility of dermal exposure from handling NPs or touching contaminated surfaces are present in our laboratory. The aim of this study was to determine whether the procedure of washing skin with soap and water would effectively remove NPs from exposed skin. Qualitative microscopy observations and quantitative metal analysis results from *ex vivo* human skin samples that were washed with soap and water after exposure to dry, nanoscale, hematite (α-Fe_2O_3) and maghemite (γ-Fe_2O_3) powder are presented.

METHODS

Commercial nanoscale iron oxide powders, α-Fe_2O_3 (3310DX) and γ- Fe_2O_3 (3315DX), were examined (Sky Spring Nanomaterials, Houston, TX, USA). The manufacturer reported for both powders 99% purity, 20-40 nm particle size, and 40-60 m^2/g surface area. The particle size was confirmed by transmission electron microscopy (TEM, Phillips CM-20 operating at 200 kV).

Human abdominal skin was obtained as surgical waste from the Department of Plastic and Reconstructive Surgery at the University Hospital of Lausanne (DAL biobank, CHUV, Lausanne, Switzerland). Full consent was obtained by the surgeon from the three anonymized donors, who consisted of men and women aged 35-56 years. Immediately following surgery, the skin was dermatomed (Acculan®II, B. Braun/Aesculap, Sempach, Switzerland) to a thickness of 0.8 mm, and

mounted on static diffusion cells (PermaGear, SES Analytical System, Bechenheim, Germany). Frozen human skin samples from four other donors (stored at -20°C for < 2 years) were also used in this study. In each experiment, skin samples from a single donor were used in triplicate per test condition. Skin integrity was verified before starting the experiment by measuring the trans-epidermal water loss (VapoMeter wireless, Delfin Technologies Ltd., Kuopio, Finland).

Iron oxide NPs (~1 mg), were applied to the top of the skin samples pre-mounted on static diffusion cells[12] using a spatula spoon; weighed before and after removing the iron oxide nanopowder from the stock bottle. To reduce loss due to electrostatic charge, a polonium 210 static neutralizer was used during the weighing process. The total surface area of skin exposed to powder was 1.77 cm^2, yielding a concentration of approximately 0.56 mg NPs/cm^2.

Skin permeation of the iron oxide NPs was tested following the OECD Test Guidelines 28 and 428.[13] Briefly, skin samples were maintained at 32±1°C for the 20-hour duration of exposure. Each static diffusion cell consisted of a donor and receptor chamber, which were separated by the skin sample placed epidermal side up. The receptor chamber contained 12 mL of saline water (0.9% NaCl (aq)) which was continuously stirred. The total volume of receptor fluid was collected for each sample after 1 hour (n=4) or 20-hour (n=4) exposure duration.

Following 1 or 20-hour exposure durations, the exposed skin samples were cleaned using non-medicated soft soap (Softaskin, B. Braun Melsungen AG, Melsungen, Germany) following a modified protocol described by Messager *et al.*[14] which was adapted from the European hand washing method EN 1449. Using a micropipette, 50 µL of soft soap was added to the skin followed by 50 µL of MilliQ water. The skin was lathered using a glass rod for 1 minute. Afterwards, 1 mL of MilliQ water was added to the donor chamber, and using a glass Pasteur pipette, the solution was pipetted up and down and then transferred to a collection vial. This was repeated with another 1 mL of MilliQ water and then the Pasteur pipette was rinsed by adding 1 mL of MilliQ water, which resulted in a total rinse solution volume of 3.1 mL. This hand washing procedure was repeated one additional time. The washed skin sample was then removed from the diffusion cell and either processed immediately or flash frozen in liquid nitrogen before it was placed in an Eppendorf tube and stored at -80°C until further processing. All collected fluid samples were stored at 4°C until further processing.

The amount of iron oxide NPs present in the washed skin samples, receptor fluids, and rinse fluids were first measured by UV/Vis spectrophotometry (Infinite M200, Tecan Group Ltd., Mannedorf, Switzerland) at an absorbance wavelength of 550 nm. For some samples, the skin was either tape stripped thirty times or cryo-sectioned perpendicular to the skin surface to qualitatively determine the depth of NPs permeation. The remaining samples were digested (aqua regia), and iron ions quantified using inductively coupled plasma-optical emission spectroscopy (ICP-OES, ICPE-9000, Shimadzu Europe GmbH, Duisburg, Germany) with a limit of detection (LOD) of 0.1 ppm (0.1 µg Fe/mL). γ-Fe_2O_3 NPs were also measured before digestion by magnetic susceptibility (MagS, MS3 Magnetic Susceptibility System, Bartington Instruments Ltd., Oxford, England) following a method developed by Maurizi *et al.*[15] with a LOD of 1.5 µg Fe.

RESULTS

Measured by TEM (Figure 1), the average diameters were 29 ± 6 nm and 32 ± 4 nm for α-Fe_2O_3 and γ-Fe_2O_3 NPs respectively. The concentration of NPs in the receptor liquid was below the LOD for all experiments and all three analytical methods used (UV/Vis, ICP-OES, and MagS). Testing the removal of iron oxide NPs by soap and water, less than 15% of the recovered dose or less than 10% of the initial dose were measured on the skin after two wash steps with soap and water (Table 1). These values were determined by comparing the amount measured on the skin to the total mass measured in the skin and rinse liquids using UV/Vis and ICP-OES or to the initial mass added to the skin, respectively. Processing the washed skin by tape stripping and cryotome sectioning revealed that the iron oxide NPs remain adsorbed to the stratum corneum. Although NPs were observed in all thirty tape strips (Figure 2), which removed between 6-30 μm of skin, images of the cryo-sectioned skin revealed wrinkles or pores in which the powder was contained.[16]

DISCUSSION

To our knowledge, there are no previous studies examining the interactions of dry powder, iron oxide NPs on human skin. Baroli *et al.* [2] tested suspensions of γ-Fe_2O_3 NPs coated with tetramethylammonium hydroxide (quarternary ammonium salt) and reported skin permeation across the stratum corneum into the epidermis. We suspect that this permeation was facilitated by the surface coating as it is known that strong bases damage the stratum corneum, thus can facilitate the absorption of NPs; and also influenced by the method of exposure, particles in suspension versus dry powder. Raphael *et al.* [11] reported a human volunteer study where, after two hours of exposure, 85% of the applied ZnO nanoparticles were removed with soapy water from both intact and tape stripped skin. This is consistent with our findings, 85-90% was removed after two wash steps, despite differences in exposure duration (2 hr vs. 1 and 20 hr), soap washing time (5 s vs. 1 min.), amount of water (210 mL vs. 6.2 mL), and washing implement (unspecified vs. glass rod) used. The tape stripping and tissue sectioning data together suggest that the iron oxide NPs remaining on the skin do not permeate through skin when applied in powder form for exposure durations < 24 hours. Although the influence of skin damage was not assessed in our study, dermal absorption depends mostly on the barrier function of the outermost layer of the epidermis (stratum corneum), and is modulated by a number of factors including skin integrity. Published studies[17] comparing intact versus damaged skin report higher concentrations of nanoparticles in viable skin (epidermis, dermis). Raphael et al.[11] did not report significant differences in nanoparticle removal between intact and tape stripped skin. Skin decontamination guidelines[18] recommend washing damaged skin with saline alone. This could result in less nanoparticle removal compared to soap and water. Prolonged skin contact due to ineffective removal of NPs from the skin surface could result in potential biological effects, such as metal ion toxicity due to particle dissolution or oxidative stress due to particle reactivity with other chemicals or UV light. NPs in cream were completely removed out of the furrows and hair follicles of the skin using a new absorbent textile.[10] These studies suggest that

alternative decontamination strategies could be more effective than soap and water at removing NPs from exposed skin.

CONCLUSION

Many Safety Data Sheets for metal oxide nanopowders advise washing skin with soap and water in case of skin contact. The results of this study raise questions about the impact of NPs residue remaining on the skin after washing with soap and water and whether alternative methods would be more effective. Further studies on skin decontamination beyond use of soap and water are needed to improve the understanding of the potential health consequences of dermal exposure to NPs.

RECOMMENDATION

Although NPs do not generally penetrate skin, their prolonged skin contact could result in potential biological effects, such as metal ion toxicity due to particle dissolution or oxidative stress due to particle reactivity with other chemicals or UV light. These effects are generally due to ineffective removal of NPs from the skin surface. We therefore recommend removing NPs from skin by washing their hands with soap and water immediately after spills or clean-ups, before eating, and at the end of the work day.

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Table 1. Results from *ex vivo* hand washing experiments. Values are averaged across α-Fe_2O_3 and γ-Fe2O3 NPs test conditions (* indicates values are for γ-Fe2O3 NPs only). Results from each individual *ex vivo* hand washing experiment are provided in Table S1.

Skin type	Measurement method	Exposure duration (hr)	% remaining on skin	% recovered in rinse
Frozen	ICP-OES	1	12±6%	-
		20	4±5%	-
	UV/Vis	1	-	64±24%
		20	-	47±6%
Fresh	ICP-OES	1	10±4%	25±12%
		20	14±9%	33±16%
	UV/Vis*	1	-	49±16%
		20	-	44±13%
	MagS*	1	5±2%	53±18%
		20	7±3%	58±33%

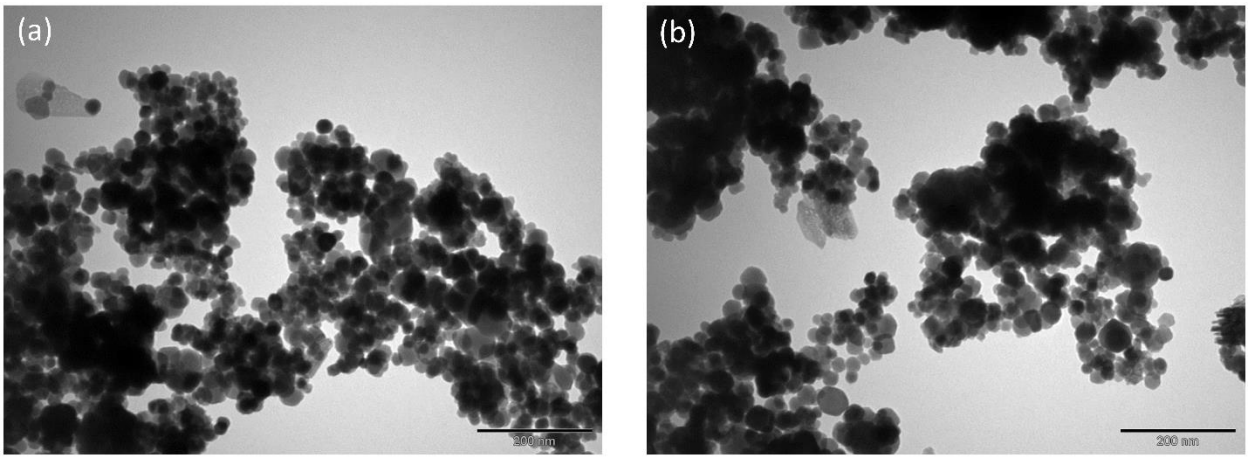

Figure 1. TEM images of α-Fe_2O_3 (a) and γ-Fe_2O_3 (b) nanoparticles. Scale = 200 nm.

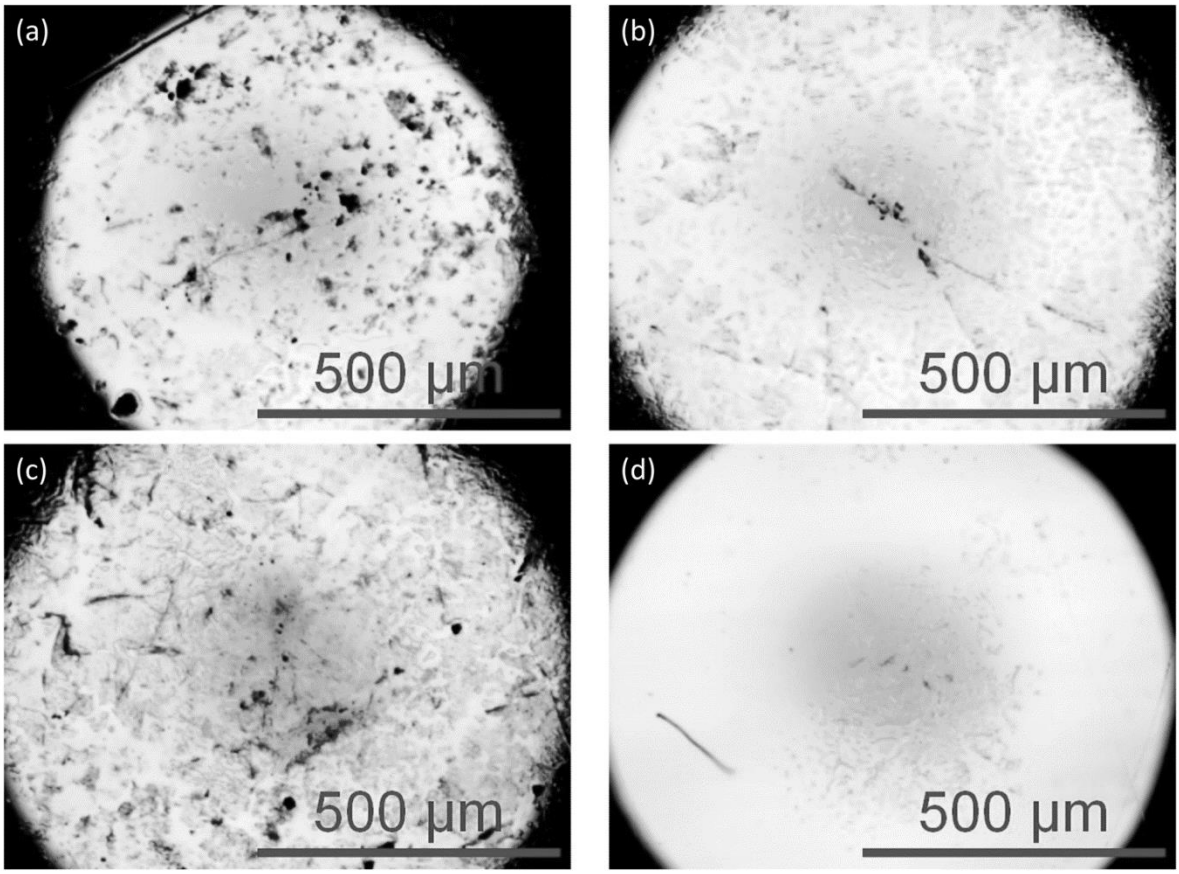

Figure 2. Brightfield images of tape strips immediately after washing (a & c), after 10 tape strips (b), and after 30 tape strips (d).

