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Laser Scanning Microscopic Investigations of the Decontamination of Soot Nanoparticles from the Skin

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Keywords
Skin · Nanoparticles · Decontamination · Ex vivo testing · Laser scanning microscopy

Abstract
Background/Aims: Airborne pollutants, such as nano-sized soot particles, are increasingly being released into the environment as a result of growing population densities and industrialization. They can absorb organic and metal compounds with potential biological activity, such as polycyclic aromatic hydrocarbons and airborne pollen allergens. Local and systemic toxicities may be induced in the skin if the particulates release their harmful components upon dermal contact. ***Methods:*** In the present study, skin pretreatments with serum and/or shield as barrier formulations prior to exposure and washing with a cleanser subsequent to exposure were evaluated as a protection and decontamination strategy using laser scanning microscopy. ***Results:*** The results indicate that while the application of serum and a cleanser was insufficient for decontamination, the pretreatment with shield prior to nanoparticle exposure followed by washing led to the removal of a considerable amount of the carbon black particles. The combined application of serum and shield before the administration of carbon black particles and subsequent washing led to their elimination from the skin samples. ***Conclusion:*** The application of barrier-enhancing formulations in combination with a cleanser may reduce the penetration of harmful airborne particulates by preventing their adhesion to the skin and facilitating their removal by subsequent washing with the cleanser.

Introduction

In the present study, the protective efficacy of different topically applied skin products, i.e., serum, shield, and a cleanser, against the permanent deposition of potentially noxious particulate pollutants (<1 μm), such as traffic-related soot [1] on ex vivo porcine ear and human skin samples was investigated using laser scanning microscopy. Due to their small size and large surface per unit mass, these nanoparticles are highly reactive toward biological surfaces and structures [2]. Although particles deposited on the skin tend not to penetrate beyond the upper layers

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of the stratum corneum [3], they have been reported to readily accumulate in the follicular ducts [4], from where active substances may be released and translocated to deeper skin layers and into tissues surrounding the hair follicles and cells. Particulate contaminants such as soot have been shown to be associated with a higher risk for cancer [5, 6], pulmonary and cardiovascular diseases [6, 7], and harmful effects to human skin [2, 8, 9]. They can absorb organic chemicals such as polycyclic aromatic hydrocarbons and metals, which are capable of localizing in mitochondria and generating reactive oxygen species [8], and penetrate cellular targets in the lung and systemic circulation [10]. In particular, polycyclic aromatic hydrocarbons appear to be key compounds in the toxicity of ambient nanoparticles [2]. Pollen allergens have been shown to exacerbate skin conditions in atopic eczema patients and pass through a disrupted skin barrier and can be taken up by Langerhans cells in vitro [11]. Air pollution has also been significantly correlated with extrinsic skin aging, in particular to pigment spots and wrinkles [2]. Textile covering of the skin can provide protection against the detrimental effect of these particles. As some skin areas, such as the face and hands, remain continuously exposed, specific protective strategies should be developed, particularly for areas with high concentrations of airborne nanosized pollutants. Decontamination of skin exposed to such potentially harmful particulates is therefore critical for reducing the risk of local and systemic toxicity. Currently, the most widely available cleansing strategy is washing with soap and water [12]. However, this strategy presents several limitations for persons exposed to high concentrations of potentially harmful particles (i.e., radioactive dust) or the population in extremely dust-polluted areas including metropolises such as Beijing, and may even result in the enhanced permeation and systemic absorption of the harmful substances [13, 14]. The administration of barrier shields that seal the skin surface while guaranteeing an adequate moisture regulation between the skin and the environment prior to exposure and washing of the skin after exposure provides a more suitable option for persons subject to high-risk exposure [15]. The present study aimed at investigating whether skin care products can prevent the penetration of potentially harmful particles into the skin and contribute to their removal from the skin surface. Pretreatments of the skin with serum and/or shield and washing with a cleanser after contamination with model particulate contaminants, i.e., carbon black particles, were therefore evaluated as an alternative protection and decontamination strategy using laser scanning microscopy (LSM).

Material and Methods

Skin Samples

The experiments were performed ex vivo on skin obtained from 6 pairs of porcine ears provided by a local butcher and 2 human skin samples excised from the abdomen of patients who had undergone cosmetic reduction surgery at the Charité – Universitätsmedizin Berlin. All experiments were conducted within 24 h of excision. Visibly diseased, injured, or scarred skin was not included in the study. Prior to the treatments, the excised ears and the human skin samples were rinsed with cold water and dabbed dry using paper towels so as not to injure the skin surface, and subsequently maintained at an ambient temperature and humidity. Four test areas sized 4 × 4 cm were marked on the dorsal side of each ear belonging to the same animal with silicone barriers, providing a total of 8 test areas per pair of ears. Eight test areas were marked in the same manner on each human skin sample. The allocation of the test areas was randomized. Approval for the experiments on porcine ear skin was obtained from the Veterinary Board of Control (Veterinäramt Treptow-Köpenick, Berlin, Germany). Approval for the investigations using excised human skin samples were obtained from the Ethics Committee of the Charité – Universitätsmedizin Berlin.

Test Formulations and Model Contaminants

The serum (ref. 5189002000), shield (Crème Solaire ref. 5240001000), and cleanser (ref. 4949002000) implemented in the investigations were provided by Coty Lancaster SAM (International R & D Center, Monaco, Monaco). The serum was comprised of siloxane derivatives and xanthan gum associated with alkyl acrylate cross-polymers and alcohol. It had a light emulsion-gel texture which resulted in a soft and smooth film on the skin surface. The shield was a light fluid oil-in-water emulsion including a hydroxyethyl acrylate/sodium acryloyldimethyltaurate copolymer gel and 2 surfactant tribehenin polyethylene glycol (PEG)-20 esters combined with PEG-100 stearate and glyceryl stearate. It also included UV-A and UV-B filters with a 1:3 ratio. The cleanser was a white lightweight creamy foam which consisted of C_{12} to C_{16} fatty acids combined with a mild sodium cocoyl glycinate tenside. 1% fluorescein (Fluoreszein SE Thilo, Alcon Pharma GmbH, Freiburg, Germany) was mixed homogenously into the serum and shield prior to their use to enable visualization of their distribution on the skin surface by LSM. Fluorescein was administered to the test regions of the controls so as to similarly visualize their surfaces using the confocal microscope. Carbon black particles (150 nm), implemented as model contaminants, were obtained from the Leibniz Research Institute for Environmental Medicine (Düsseldorf, Germany).

Application Protocols

For each application protocol, 2 test areas were allocated. These were either dusted with carbon black particles and used as controls, or pretreated with serum, shield, or both and then dusted with carbon black particles. After application of the model contaminants with a penetration time of 5 min, 1 of the test areas allocated to each application protocol was washed using the cleanser. A pea-sized amount of cleanser was frothed up with 1 mL water prior to being dabbed onto the skin, after which it was washed off under cool running water until all traces of foam were removed from the skin. The serum was allowed to dry on the skin complete-

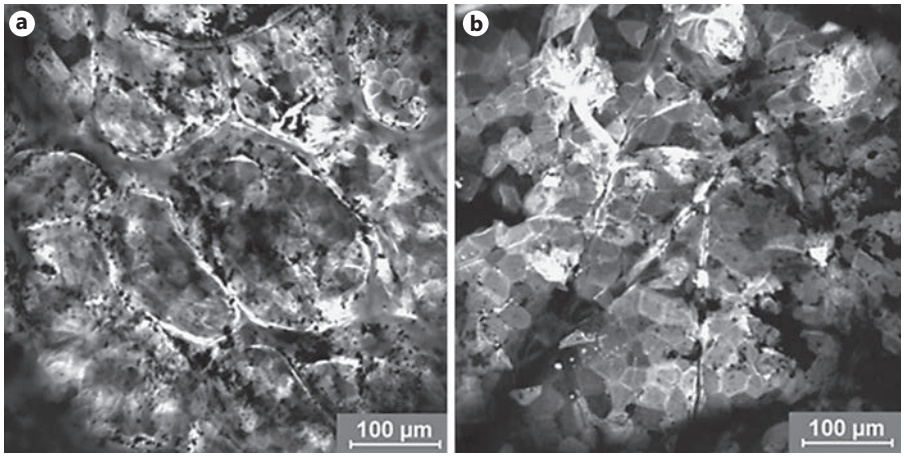

Fig. 1. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) after topical application.

ly for 10 min while the shield required 30 min to dry. All experiments were conducted at room temperature (19°C ± 4°C).

Control

One drop of fluorescein was administered to the control test areas and allowed to penetrate for 2 min. Carbon black particles (0.1 mg/cm^2) were lightly dusted onto the test regions. One of the test areas was subsequently washed with a small amount of cleanser under running water.

Serum

Serum mixed with 1% fluorescein (2 mg/cm^2) was applied topically to the serum test areas and dispersed homogenously using a gloved finger. After 10 min, carbon black particles (0.1 mg/cm^2) were lightly dusted onto the treated and dried test areas, and 1 of the test areas was washed using the cleanser under running water.

Shield

Shield mixed with 1% fluorescein (2 mg/cm^2) was administered homogenously to the test areas using a gloved finger. Carbon black particles (0.1 mg/cm^2) were lightly dusted onto both dried test regions after a contact time of 30 min and subsequently washed off with the cleanser under running water from 1 of the test regions.

Combination Serum/Shield

Serum mixed with fluorescein (2 mg/cm^2) was topically applied to the test areas and allowed to dry for 10 min, after which shield mixed with fluorescein (2 mg/cm^2) was administered. This was also allowed to dry. After 30 min, carbon black particles (0.1 mg/cm^2) were applied to the skin and washed off from 1 of the test areas using the cleanser and running water.

Laser Scanning Microscopy

A reflectance confocal LSM (VivaScope® 1500, MAVIG GmbH, Munich, Germany) was used to conduct the present investigations. The VivaScope® implements a laser beam at a wavelength of 445 nm to visualize horizontal skin sections noninvasively. The single test field of view is 500 × 500 µm^2. The optical resolution is <1.25 µm in the center of the image field horizontally and <5 µm in the center of the image field vertically. Cellular details can be depicted at high spatial resolution and good contrast without necessitating a biopsy and further histological processing or staining. The technique is described in detail in Meyer and Lademann [16]. In the present study, single confocal images of horizontal sections of the skin were obtained. The laser intensity was adjusted manually to achieve images with a suitable contrast of the dermal structures. LSM images of all test samples were taken subsequently to the specific pretreatments with the serum, shield, and particle contaminants and prior to washing, as well as after washing of the skin samples, i.e., porcine ear skin and human ex vivo skin, using the cleanser.

Results and Discussion

Unwanted and noxious airborne particles found in the environment are formed during combustion or industrial production processes, and can cause a wide range of health problems [17]. Occupationally exposed persons or persons living in highly polluted regions are particularly at risk. Although particles deposited on the skin mostly remain in the uppermost layers of the stratum corneum [3], they may accumulate in susceptible skin appendages, such as the hair follicles [4], from where diffusion of the noxious substances from the particulates could result in their translocation into deeper skin layers and the tissues surrounding the hair follicles and cells, including the skin vasculature. In the present study, carbon black particles, implemented as model contaminants, were administered to porcine ear skin and ex vivo human skin subsequent to pretreatments with serum and/or shield, after which a cleansing agent was utilized for washing of the treated skin. LSM investigations were conducted to evaluate the efficacy of the different protection and decontamination procedures by examining the test regions for the presence of carbon black particles.

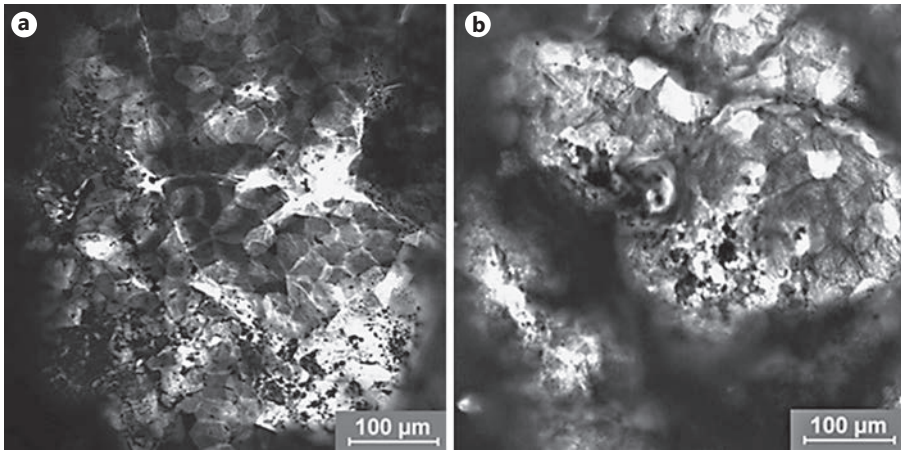

Fig. 2. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) after washing.

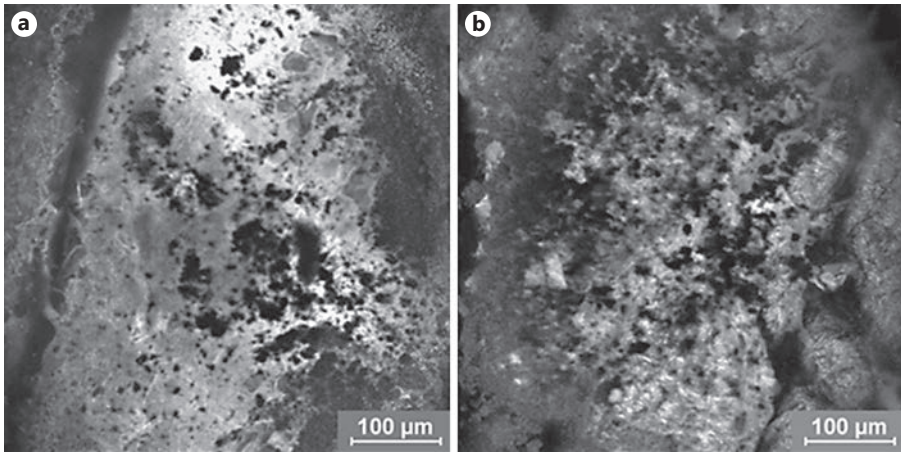

Fig. 3. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with serum.

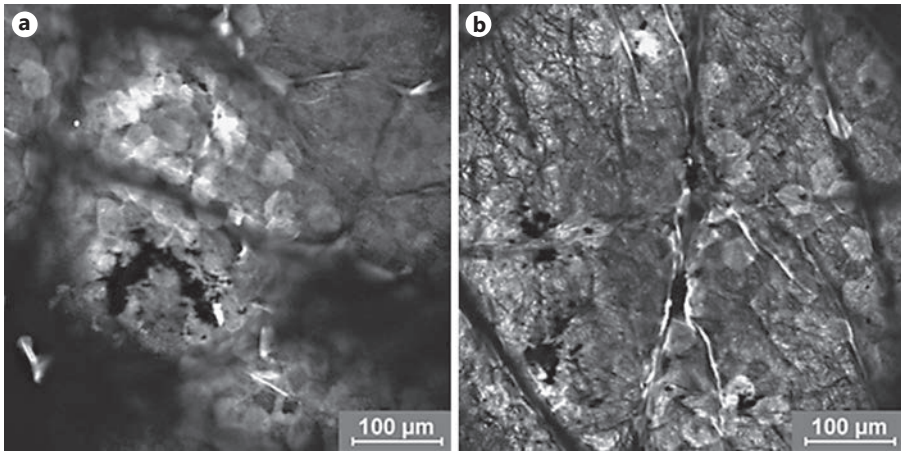

Fig. 4. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with serum after washing.

Control

Figure 1a and b represents LSM images of the distribution of carbon black particles on the skin surface of porcine ear skin and human ex vivo skin subsequent to their topical application, respectively.

In both porcine (Fig. 2a) and human ex vivo skin (Fig. 2b), washing using the cleanser resulted in only a slight reduction of the carbon black particles on the skin surface. Black spots were still visible in the LSM images.

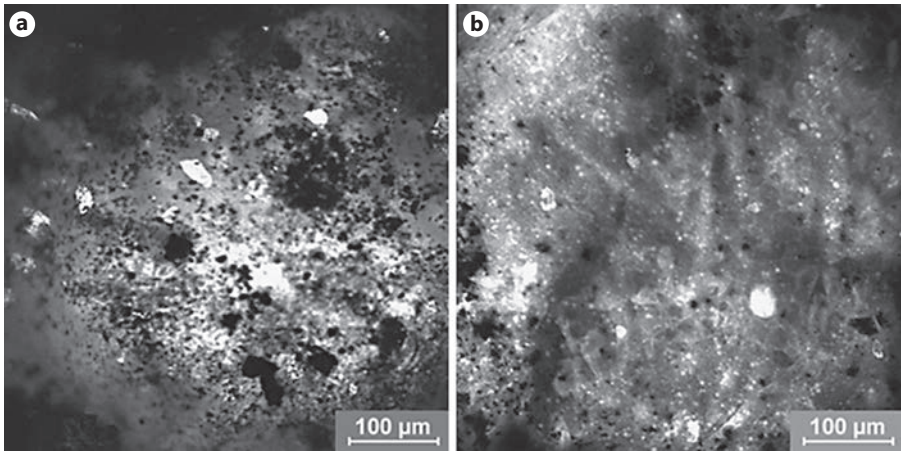

Fig. 5. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with shield.

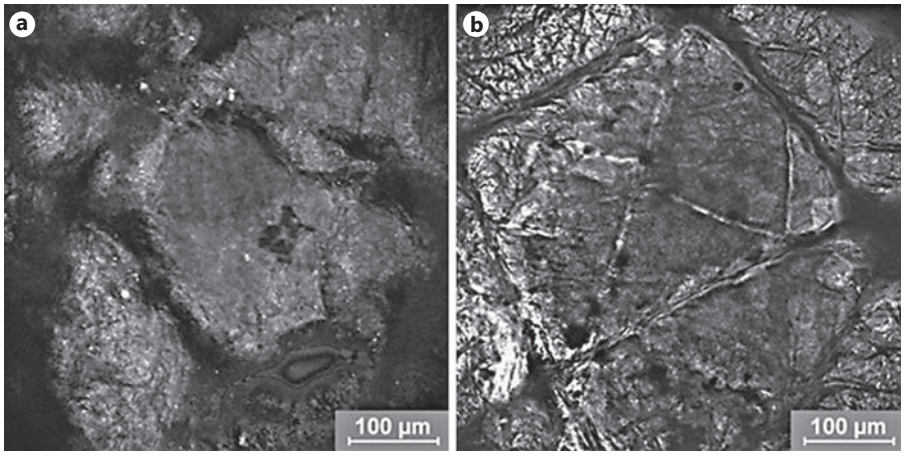

Fig. 6. a, **b** Only traces of carbon black particles (black spots) are detectable on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with shield after washing.

Serum

Pretreatment of the porcine ear skin and human ex vivo skin with serum resulted in the formation of a confluent serum film on the stratum corneum. The distribution of the carbon black particles, which were applied topically subsequently to the serum, are shown in Figure 3a and b for porcine ear skin and human ex vivo skin, respectively.

After washing, a small amount of residual carbon black particles was still detectable on the porcine (Fig. 4a) and human ex vivo skin (Fig. 4b) samples.

Shield

As in the case of serum application, the administration of shield resulted in the formation of a confluent shield film on the surface of the porcine ear skin samples. The distribution of the carbon black particles on pig skin and human skin pretreated with shield is shown in Figure 5a and b, respectively.

Washing of skin pretreated with shield using the cleanser resulted in a more pronounced reduction of the carbon black particles from the skin surface in porcine ear skin (Fig. 6a) and human ex vivo skin (Fig. 6b) compared to the pretreatment with serum.

Combination Serum/Shield

Serum and shield were consecutively applied onto the porcine ear skin samples and human ex vivo skin, which also resulted in the formation of a confluent film on the horny layer. The distribution of the deposited carbon black particles on the skin pretreated with both shield and serum is shown in Figure 7a and b, respectively.

Subsequent washing with the cleanser resulted in the elimination of the confluent film and the particulate contaminants from the skin surface, which were no longer detectable using LSM in both types of skin samples (Fig. 8a, b).

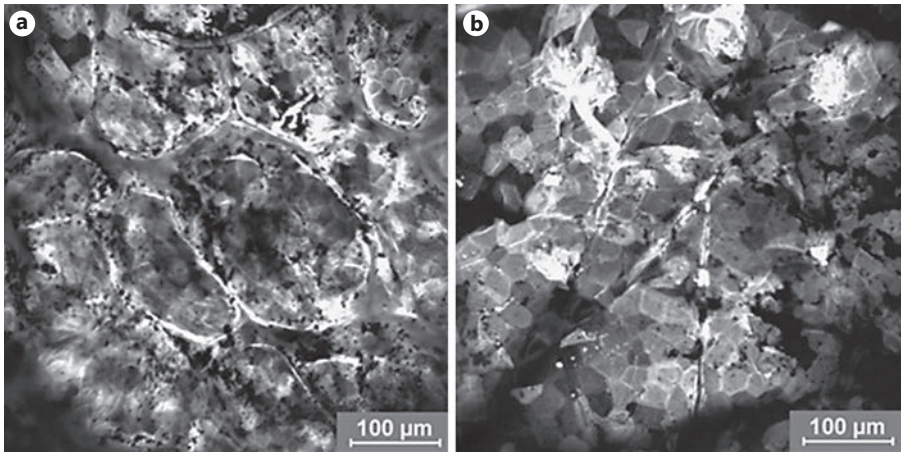

Fig. 7. a, **b** Distribution of carbon black particles (black spots) on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with the combination of serum and shield.

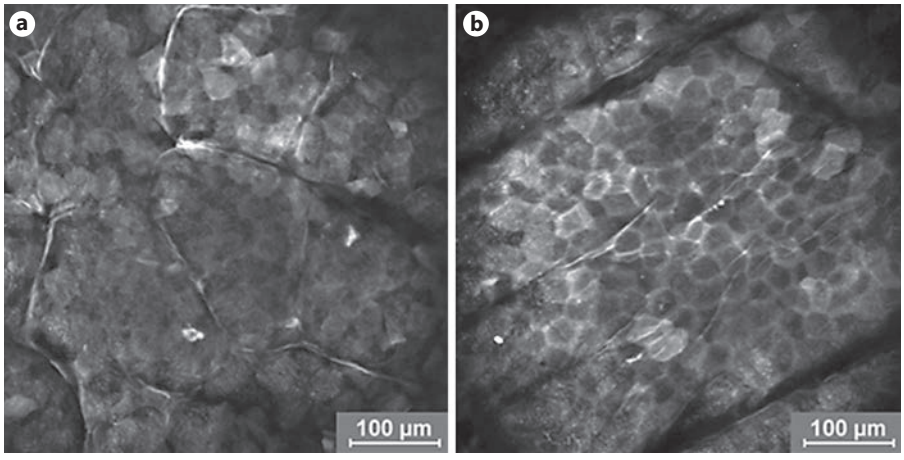

Fig. 8. a, **b** The confluent film and the carbon black particles (black spots) were no longer detectable on the skin surface of porcine ear skin (**a**) and human ex vivo skin (**b**) pretreated with the combination of serum and shield after washing.

Skin barrier formulations are designed to reduce or prevent penetration and absorption of irritants and allergens into the skin of highly exposed persons by presenting a physical barrier, i.e., a thin film, between the skin and the contaminant [18]. Many in vivo and in vitro investigations have been performed using animal and human skin to test the efficacy of barrier formulations as pre-exposure skin protectors [15, 19–21]. The commonly quoted general rule is that water-in-oil emulsions are effective against aqueous solutions of contaminants and oil-in-water emulsions are effective against lipophilic substances [22, 23], with few exceptions [21, 24]. The protective film may interfere with absorption and penetration by trapping the contaminants, e.g. previous investigations have shown that the penetration of pollen allergens into hair follicles was significantly reduced when the skin had been treated with commercial care products [25]. The present work illustrated that while topical pretreatments of exposed skin with serum or shield followed by washing subsequently to contamination facilitated the removal of a large portion of the deposited particulate contaminants from the skin, the combined use of the serum and shield with a cleanser resulted in the most efficient barrier effect in porcine and human skin samples. The serum and shield were applied before the skin was exposed to the carbon black particles, therefore acting as barrier-enhancing formulations. The topical application of the serum and the shield resulted in a confluent film on the surface of the skin samples, covering the furrows and wrinkles. This effect was more pronounced for the shield and facilitated the removal of the carbon black particles from the skin surfaces when the serum was used in combination with the shield. Highly exposed persons could implement this protection strategy to successfully minimize exposure to harmful particulates. In a previous study, the silicone glycol polymer PEG-12 dimethicone and an absorbent textile material based on nanofibers were used to remove topically

applied fluorescing hydroxyethyl starch nanocapsules implemented as model contaminants from porcine ear skin [26]. While the absorbent textile materials alone did not result in sufficient decontamination, PEG-12 dimethicone acted as a wetting agent, transferring the nanocapsules into a liquid phase which was subsequently taken up by the absorbent textile material. Similarly, the carbon black particles implemented in the present study deposited on the film comprised of the serum and shield, which facilitated their removal from the skin surface upon washing [23]. In the present study, LSM investigations were conducted to visualize the effect of barrier formulations in facilitating the removal of carbon black particles from the skin surface using a washing agent. The combined use of serum and shield provided a more complete barrier function than the individual applications of the serum or the shield, whereby the shield was comparably more effective than the serum. To ensure that no penetration of the particulates occurred, i.e., into the hair follicle openings, which has been described previously [27], further histological investigations would be necessary. Through this pathway, ambient nanoparticles may be able to reach deeper skin layers, releasing surface-bound compounds such as polycyclic aromatic hydrocarbons, directly affecting the function of skin cells [2] and gaining access to the circulatory system.

Conclusions

Removal of particulate contaminants such as soot particles from the skin is essential for preventing further permeation of harmful compounds, i.e., absorbed organic or metal compounds. In the present study, the efficacies of different application procedures using serum, shield, and a cleanser for protecting against and removing topically applied carbon black particles from the skin surface were evaluated using LSM. Similar results were obtained for porcine ear and human ex vivo skin. The findings indicated that while the application of serum and a cleanser was insufficient, and a large amount of particles could still be found on the skin, the pretreatment with shield and subsequent washing led to the removal of a considerable amount of the carbon black particles. The combined application of serum and shield, followed by washing, led to the complete elimination of the carbon black particles from the skin samples. Persons exposed to high amounts of pollutants could thereby benefit not only from textile covering, etc., but also from the application of effective barrier-enhancing formulations in combination with a cleanser to reduce the invasion of harmful particulates into the skin and improve the skin barrier.

Acknowledgments

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Statement of Ethics

The study was approved by the Ethic Commission of the Charité-Universitätsmedizin Berlin (EA1/281/16) and by the Agency of Consumer Protection and Agriculture of the administrative district Dahme-Spreewald.

Disclosure Statement

The authors have no conflict of interest to declare.

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