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Laser scanning microscopy for control of skin decontamination efficacy from airborne particulates using highly absorbent textile nanofiber material in combination with PEG-12 dimethicone

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Abstract

Background: The decontamination of the skin is indispensable if airborne particulate contaminants deposit on the skin surface. Skin washing can have adverse effects as by skin rubbing the particles can be transferred deeply into the hair follicles, where they can be entrapped for a period of more than 10 days. Thus, alternative skin decontamination strategies are necessary.

Materials and Methods: For imaging the contaminants in the skin, sodium fluorescein-labeled soot particles of submicron size (≈600 nm) were visualized using laser scanning microscopy.

Results: In the present ex vivo pilot study on porcine ear skin, it was shown that sodium fluorescein-labeled soot particles of submicron size (≈600 nm) could be efficiently removed from the skin with highly absorbent textile nanofiber material, whose efficacy could be further increased by spraying the contaminated skin area with the viscous fluid PEG-12 dimethicone before textile application.

Conclusion: In case of skin contamination with particulates, the contact washing should be avoided due to rubbing particles deeply into the hair follicles, where they can accumulate for a long time and induce negative consequences. Efficient skin decontamination could include pretreatment of skin surface with the viscous fluid PEG-12 dimethicone and subsequent application of highly absorbent textile nanofiber material.

KEYWORDS
contamination, desquamation, fluorescence spectroscopy, hair follicles, pollutants, skin protection, washing

1 | INTRODUCTION

Increasing industrialization and traffic is strongly correlated with increased air pollution[1-3] leading to a severe impairment of the population of many big cities.[4-6] Many factors contribute to air pollution, whereby particulate matters of nanometer size and submicron size, which are released into the air with exhaust gases, play a very critical role.[7,8] Especially hydrocarbons, which are important components of particulate matters, can act as activated carbon and absorb further environmental pollutants. If these particulate matters get in contact with the skin, the absorbed pollutants can be transferred to the skin and might induce skin damage or aggravation of preexisting skin diseases.[9,10]

So far, skin antipollution strategies have mainly been based on washing the skin.[11,12] However, data of different studies demonstrate that skin rubbing rather enhances skin penetration of particulate substances by pressing them into the hair follicles.[13,14] Once penetrated, the particulate substances can remain entrapped within the hair follicles for a period of more than 10 days.[15] During this storage time, pollutants can be released from their particulate transporters and can eventually overcome the follicular barrier and enter the viable epidermis or dermis. The upper part of the hair follicle provides a barrier, which is similar to that of the skin surface, whereas in the lower hair follicle, mainly tight junctions are responsible for the barrier properties.[16,17] In previous studies, it was shown that particulate substances of approx. 600 nm in size exhibit the deepest follicular penetration.[18] Moreover, many investigations could demonstrate that particulate substances of ≥40 nm are unable to pass either the stratum corneum or the follicular barrier into healthy and intact skin.[19,20] On the contrary, for non-particulate substances, the hair follicle is a very fast and efficient penetration pathway. Lademann et al[21] and Abd et al[22] could demonstrate, for example, for caffeine and minoxidil, an efficient transfollicular penetration. The hair follicle is surrounded by a dense network of blood capillaries and immune cells; therefore, transfollicularly penetrating substances are easily absorbed by the blood circulation and are able to provoke immune reactions.

For non-particulate substances, it could already be shown that highly absorbent textile materials are well suited to efficiently remove substances from the skin[23] by pressing them gently onto the skin without rubbing in order not to influence the integrity of the stratum corneum[24] or to accelerate follicular penetration.[18] In a previous study, it was shown that the textile material was able to absorb a non-particulate test substance together with sebum from the skin surface.[25]

It was hypothesized that a skin decontamination from particulate substances might be more challenging as the skin surface is not homogeneous but characterized by the presence of wrinkles, furrows, follicular orifices, and sweat glands, which serve as an appropriated reservoir for topically applied particulate substances.[26] Therefore, a viscous fluid will be applied to the skin, which extracts these particulate substances from the structures of the skin surface, allowing a facilitated removal by the highly absorbent textile material afterward.[27] The aim of the present pilot study is to investigate whether the highly absorbent textile material is likewise able to remove particulate test substances from the skin. The efficiency of this process was investigated in the present pilot study for soot particles of submicron size ≈600 nm, which have shown the highest penetration depth into the hair follicles.

Different non-invasive optical methods are used for analyzing the penetration depth of topically applied substances into the skin,[28] such as confocal Raman microscopy[29-32] including surface-enhanced Raman spectroscopy of silver nanoparticles,[20] two-photon tomography[33,34] also in combination with fluorescence lifetime imaging analysis[20] and laser scanning microscopy.[35,36] Among existing methods, laser scanning microscopy is an imaging method, which provides the practical advantage of being able to quickly scan large skin areas (up to 5 × 5 mm) and to visualize the follicular penetration.[21] Moreover, the sodium fluorescein-labeled soot particulates could be efficiently analyzed using laser scanning microscopy.[37]

2 | MATERIALS AND METHODS

2.1 | Skin models

The investigations were carried out ex vivo on porcine ear skin, which is widely used as a model for human skin.[38,39] Six porcine ears were freshly obtained from a local butcher and used in the study within 24 hours after slaugthering. Prior approval of the competent Veterinary Board of Dahme-Spreewald had been obtained.

2.2 | Test substance

In the present study, soot particles of ≈600 ± 50 nm in size (IUF - Leibniz Research Institute for Environmental Medicine), as generated under standard traffic conditions, were used. The soot particles (powder) were labeled with the fluorescent dye sodium fluorescein allowing them to be detected by laser scanning microscopy.

2.3 | Highly absorbent textile nanofiber material

The investigations were carried out using a highly absorbent textile nanofiber material comprised of three layers (SNS Nano Fiber Technology LLC). The top and bottom layers consisted of polyurethane nanofibers produced using the electrospinning process. The center layer contained the same electrospun polyurethane nanofibers along with entrapped superabsorbent particles. The majority of the nanofibers had diameters in the range of 400-800 nm. The absorbent textile nanofiber material was hydrophilic, soft, and elastic.

2.4 | Laser scanning microscopy

The skin surface of the porcine ears was analyzed using a Vivascope 1500 (Mavig GmbH) laser scanning microscope. For these experiments, a laser wavelength of 488 nm was applied for the excitation of the fluorescent dye sodium fluorescein of the labeled soot particles on the skin surface at low intensity (≤1 mW) not able to excite skin autofluorescence. The size of the sole images was limited to 500 × 500 μm.[40] About 100 sole images were stacked together in the entire image of 5 × 5 mm size, making a quick measurement of hair follicle possible, which were visualized at depths ≈30-70 μm under ≤5 mW laser intensity.

2.5 | Study design

Before starting the experiments, the porcine ears were carefully washed with cold water and then dabbed with paper towels. Three skin areas (4 × 4 cm^2 each) were marked on every porcine ear. Subsequently, approx. 1 mg/cm^2 of the sodium fluorescein-labeled particulate soot powder was applied on the skin for 30 minutes passive penetration. Afterward, the distribution on the skin surface was determined by laser scanning microscopy. In the first experiment, the porcine ears were washed and subsequently scrubbed with a brush under running water. In the second experiment, an attempt to remove the particulates from one of the skin areas was made by gently pressing the textile material onto the respective skin area for 15 seconds. Thereafter, the textile material was removed. Onto the third skin area, a small amount of PEG-12 dimethicone (Schill + Seilacher)—a proved cosmetic silicone glycol polymer substance, was sprayed evenly. Subsequently, the textile material was applied, then gently pressed onto for 15 seconds and removed from the respective skin area. Afterward, all skin areas were investigated by laser scanning microscopy again.

3 | RESULTS

The laser scanning microscopy image demonstrated in Figure 1A shows a typical example of the distribution of the soot particles on the skin surface directly after application. Soot particles are distributed almost homogeneously, saturating the superficial depths of the stratum corneum (maximum 6 μm) and the entire volume of furrows and wrinkles (depths up to 30 μm). An image presented in Figure 1B shows the soot particles-treated skin after washing and scrubbing under running water. In this case, the amount of soot particles on the surface was substantially reduced, but a strong fluorescent signal within the hair follicles could be detected at depth ≈50 μm. Even when a highly absorbent textile nanofiber material was pressed onto the skin surface after washing and scrubbing, the particulates could be efficiently removed out of furrows and wrinkles but not out of the hair follicles (data not shown).

When the soot particles were applied without subsequent washing and scrubbing and the highly absorbent textile nanofiber material was pressed onto the skin and then removed, a clear reduction in fluorescent signal was observed on the skin areas treated with this procedure. However, quite a number of soot particles were still detectable on the skin surface, especially in the furrows and wrinkles (Figure 1C), whereas no fluorescence was detectable in the hair follicles (image not shown). The other skin area was treated with the PEG-12 dimethicone spray before the highly absorbent textile nanofiber material was applied to the skin. This textile material was also gently pressed onto and then removed from the skin. As can be seen from Figure 1D, the soot particles were removed from the skin completely, no fluorescent signal was detected in the superficial layers of the stratum corneum and in the furrows and wrinkles. No soot particles were found in the hair follicles (image not shown). It should be mentioned that washing of treated skin without scrubbing does not efficiently reduce amount of soot particles from the superficial layers of stratum corneum, as well as from furrows and wrinkles (data not shown, images look similar as shown in Figure 1C).

The same measurement protocol was applied on six porcine ears, and the results were strongly reproducible.

4 | DISCUSSION

An efficient decontamination of the skin presents a general challenge, especially at work outdoors[41,42] and, in daily life, as air pollutants are regularly deposited on the human skin, especially as a consequence of exhaust traffic gases.[43] Ideally, these pollutants must be removed before they are able to induce any harm.

The results of the present pilot study have shown that laser scanning microscopy enables a highly sensitive detection of fluorescence-labeled soot particles on the skin. As can be seen from Figure 1A, the soot particles tend to aggregate in the superficial layers of the stratum corneum.

Normally, the stratum corneum serves as an efficient barrier against the penetration of xenobiotics.[44,45] Therefore, under passive penetration, the investigated soot particles of submicron size were not able to pass the stratum corneum barrier and were only located in the superficial layers. It has to be concluded that efficient skin decontamination strategies must exclude any mechanical stimulations as these can induce follicular penetration and follicular accumulation, as represented in Figure 1B. Long-term storage, again, can lead to a release of the pollutant from its transporting particulate substance followed by transfollicular translocation into the viable epidermis or dermis with corresponding negative consequences.

Therefore, follicular accumulation represents an increased risk of adverse effects for the organism in comparison to a distribution of the soot particulates merely on the skin surface. Due to physiological skin desquamation and textile contact, approximately one cell layer of corneocytes is desquamated every day,[46] so that the stratum corneum reservoir is normally rapidly depleted from particulate substances.

The pollutants absorbed to airborne particulate substances provide an additional risk.[47] These pollutants can be released from their

FIGURE 1 Typical images showing distribution of sodium fluorescein-labeled soot particulates (white spots) on the porcine ear skin surface measured ex vivo using laser scanning microscopy (fluorescence mode, excitation at 488 nm, power ≤1 mW for (A), (C), and (D) and power ≤5 mW for (B)). A, After 30 min passive penetration; B, after washing and scrubbing; C, After contact with highly absorbent textile nanofiber material; D, after combined application of the PEG-12 dimethicone spray treatment and contact with highly absorbent textile nanofiber material
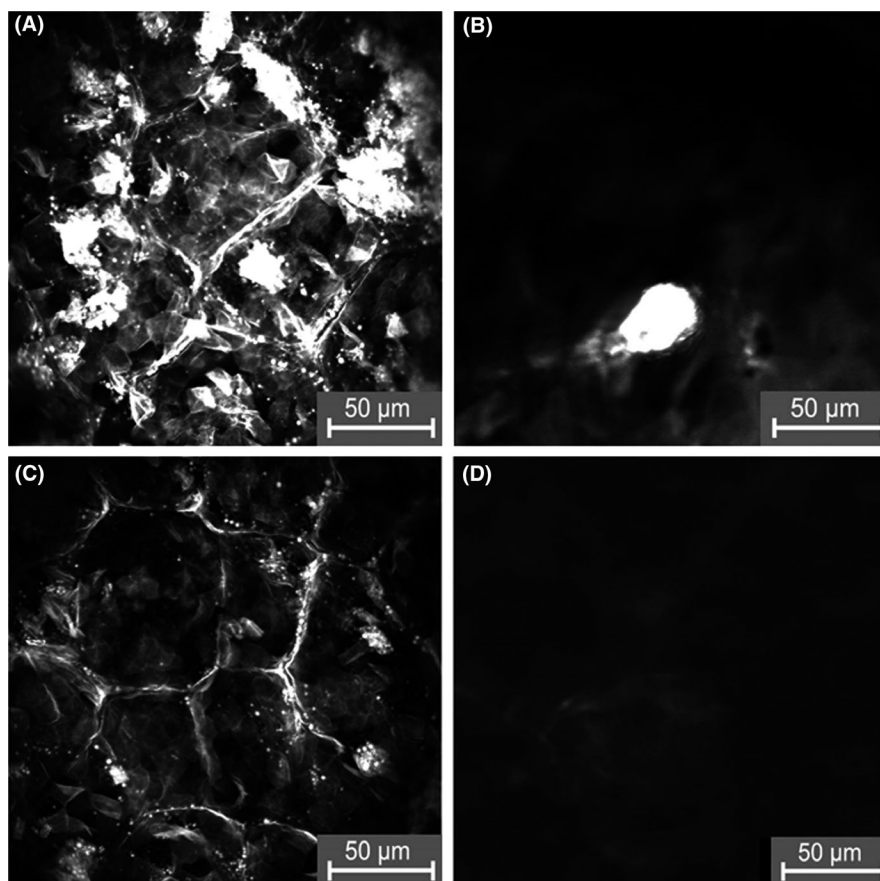
particulate transporters by contact with sweat and sebum and can damage the skin. A rapid and total removal of the particles and their absorbed pollutants is, therefore, indispensable. If the soot particles were removed by the highly absorbent textile nanofiber material, the fluorescence was clearly reduced, but the wrinkles, furrows, and desquamated corneocytes presented a persistent reservoir for the soot particles. They could not be removed completely by pressing only the textile material onto the skin. However, when PEG-12 dimethicone was sprayed onto the skin surface so that the skin was slightly moisturized, and probably due to viscosity, the soot particles could be detached from the skin structures and subsequently removed by pressing the textile material onto the skin. A follicular penetration was not detected in this combination as no skin rubbing was performed.

Thus, a novel decontamination method has been established permitting the complete removal of soot particles of submicron size (≈600 nm) from the skin surface, which considerably decreases the risk of skin damage.

In general, different skin decontamination strategies can be discussed. For instance, preventive creams cover the skin surface with a film that absorbs the skin pollutants and prevents or at least reduces the penetration of the particulates into the wrinkles and furrows of the skin, the hair follicles, and into the superficial corneocyte layers. In this case, it can be hypothesized that a highly absorbent textile nanofiber material might be efficient to remove the prevention cream together with the skin pollutants.

5 | CONCLUSIONS

The present pilot study using non-invasive laser scanning microscopic skin imaging shows that soot particles of submicron size (≈600 nm) could be efficiently removed from the skin surface/furrows/wrinkles by spraying the contaminated skin area with PEG-12 dimethicone and subsequent application of highly absorbent textile nanofiber material. In order to exclude rubbing of soot particles deeply into the hair follicles, where they can accumulate for a long time and induce negative consequences, the contact washing should be avoided. Laser scanning microscopy could be an appropriate imaging method to investigate decontamination efficacy non-invasively and in vivo in human skin.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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