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Analysis of the Penetration of a Caffeine Containing Shampoo into the Hair Follicles by in vivo Laser Scanning Microscopy[1]

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Abstract—In previous in vitro investigations, it was demonstrated that caffeine is able to stimulate the hair growth. Therefore, a penetration of caffeine into the hair follicle is necessary. In the present study, in vivo laser scanning microscopy (LSM) was used to investigate the penetration and storage of a caffeine containing shampoo into the hair follicles. It was shown that a 2-min contact time of the shampoo with the skin was enough to accumulate significant parts of the shampoo in the hair follicles. A penetration of the shampoo up to a depth of approx. 200 μm could be detected, which represents the detection limit of the LSM. At this depth, the close network of the blood capillaries surrounding the hair follicles commences. Even after 24 h, the substance was still detectable in the hair follicles. This demonstrates the long-term reservoir function of the hair follicles for topically applied substances such as caffeine.

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INTRODUCTION

In the past, it was assumed that topically applied substances penetrate exclusively via the intercellular route, i.e., inside the lipid layers around the corneocytes, through the skin barrier [1–4]. Recently, it was demonstrated that also the hair follicles represent efficient penetration pathways [5–10].

Moreover, the hair follicles represent an important target for topically applied substances, because they are surrounded by a close network of blood capillaries [11–13]. Otberg et al. [14] demonstrated that the reservoir of the hair follicles is comparable with the reservoir of the stratum corneum (SC) [15–17] on some body sites. In contrast to the SC, the hair follicles represent a long-term reservoir where topically applied substances can be stored for a number of days [18–20].

The penetration pathways of caffeine have been well investigated [7, 21, 22]. Caffeine is an antioxidant [23–25] which can protect cellular systems against the destructive action of free radicals, usually produced by UV sun irradiation in the skin [26, 27]. Additionally, it was demonstrated under in vitro conditions that caffeine can stimulate hair growth [28]. Therefore, caffeine is an important addition to shampoo formulations.

Otberg et al. [5] and Teichmann et al. [7] demonstrated that caffeine added to a shampoo formulation penetrates efficiently via the hair follicles into the blood under in vivo conditions on human skin. Otberg et al. [5, 6] compared the penetration of caffeine formulations through normal skin and skin with selectively blocked hair follicles.

The caffeine concentrations in the blood of both groups of volunteers were measured before and at different time points after topical application of the caffeine-containing formulation. In the case of open hair follicles [29], the caffeine could be detected in the blood already after 5 min. In the case of the closed hair follicles, caffeine was detectable in the blood samples only after 15–20 min. This was considered as primary evidence that the caffeine in the formulations reached the living cells through the hair follicles.

Nevertheless, the detection of caffeine in the blood represents an invasive, time-consuming and expensive method to investigate follicular penetration of caffeine.

In the present study, the penetration and storage of a caffeine-containing shampoo into the hair follicles was investigated, non-invasively, using in vivo laser scanning microscopy [30–35]. The test shampoo has a different composition from the former one [5, 7]. There was an additional cosmetic claim, to give white, yellowish hair a bright, silver-like sheen by a complementary dyestuff.

MATERIALS AND METHODS

Volunteers

The investigations were carried out on the scalp of 10 healthy female volunteers, aged between 45 and 60 years. The terminal and vellus hairs of the skin area

[1] The article is published in the original.

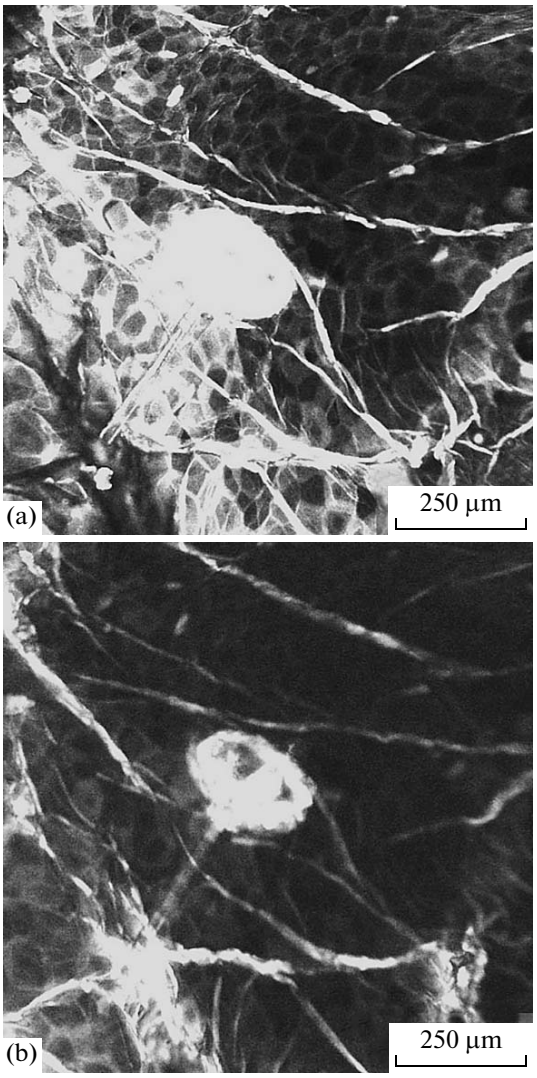

Fig. 1. Typical distribution of the dye containing shampoo on the skin surface (a) and in the hair follicle (b) of one volunteer (5 min after washing).

under investigation (occipital region, 2×2 cm^2) were cut. Approval for the investigation had been obtained from the Charité Ethics Committee. The volunteers had given their informed written consent.

Shampoo

The shampoo formulation contained 1.0% caffeine. The formula consisted of an adapted blend of surfactants and a complementary dyestuff. Instead of a typical pH-value of 5.5, the new formula had a neutral pH-value of 7.0. For the visualization of the shampoo on the scalp skin surface and in the hair follicles with laser spectroscopic measurements, 0.1% of the fluorescent dye fluorescin was added to the formulation [36, 37]. In preliminary experiments, it was shown that the penetration properties of the fluorescent dye are determined by the penetration properties of the formulation, in which it was applied [38].

Application Protocol

The shampoo was applied at a concentration of 2 mg/cm^2 onto the scalp skin. After a penetration time of two minutes, the shampoo was removed by a standard washing procedure, followed by drying the skin surface with a soft tissue.

Laser Scanning Microscopy

The laser scanning microscopic measurements were immediately commenced after washing and drying the shampoo treated skin. The distribution of the dye on the skin surface was analyzed by using the in vivo laser scanning microscope STRATUM with a field of vision of 200×200 μm^2 (OptiScan Ltd., Melbourne, Australia) [39–41]. The laser excitation wavelength was 488 nm. The penetration depth of the dye in the single hair follicles was determined by moving the laser focus from the skin surface into the hair follicles up to the depth where the fluorescent signal disappeared. The limit of detection of the LSM is ~200 μm [31]. The distance to which the focus plane was moved corresponds to the penetration depth of the shampoo. The laser scanning microscopic measurements were repeated 24 h after removal of the shampoo from the skin by washing.

RESULTS

Analyzing the distribution of the dye containing shampoo, it was found that the highest concentration of the shampoo was located on the skin surface and in the orifices of the hair follicles. Typical images are presented in Fig. 1. The penetration and distribution behaviour of the dye was investigated for terminal as well as for vellus hairs.

In Fig. 2, the distribution of the shampoo formulation in different depths of a vellus hair follicle is demonstrated. For a terminal hair follicle this situation is demonstrated in Fig. 3. Some of the orifices of the hair follicles contained amounts of water left from the washing process. This water is seen as a dark spot covering the orifices of the hair follicles (Fig. 2a). Moving the laser focus deeper into the tissue, the fluorescent signal can only be seen in the hair follicles.

For each volunteer, 10 hair follicles were investigated. The average penetration depth was determined at 185 ± 27 μm.

Additionally, the distribution of the fluorescent dye in the hair follicles was analyzed 24 h after application of the shampoo. Significant amounts of the fluores-

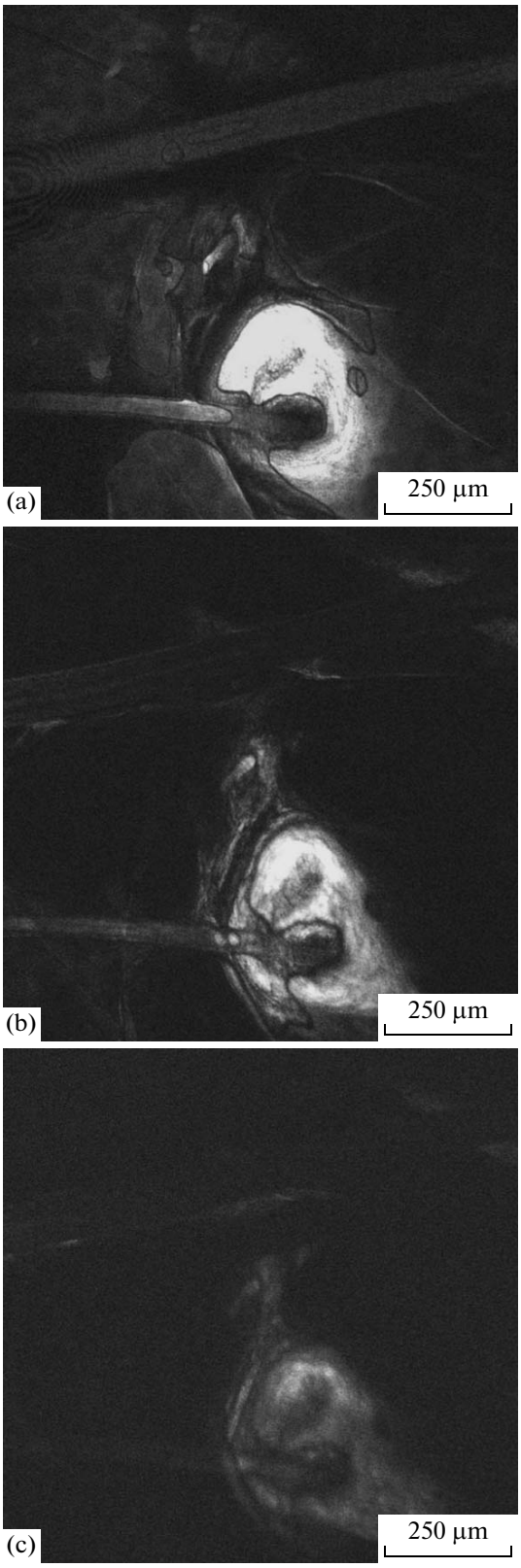

Fig. 2. Distribution of the dye containing shampoo in different depths of a vellus hair follicle (5 min after washing); (penetration depth: (a) skin surface, (b) 100 μm, (c) 200 μm).

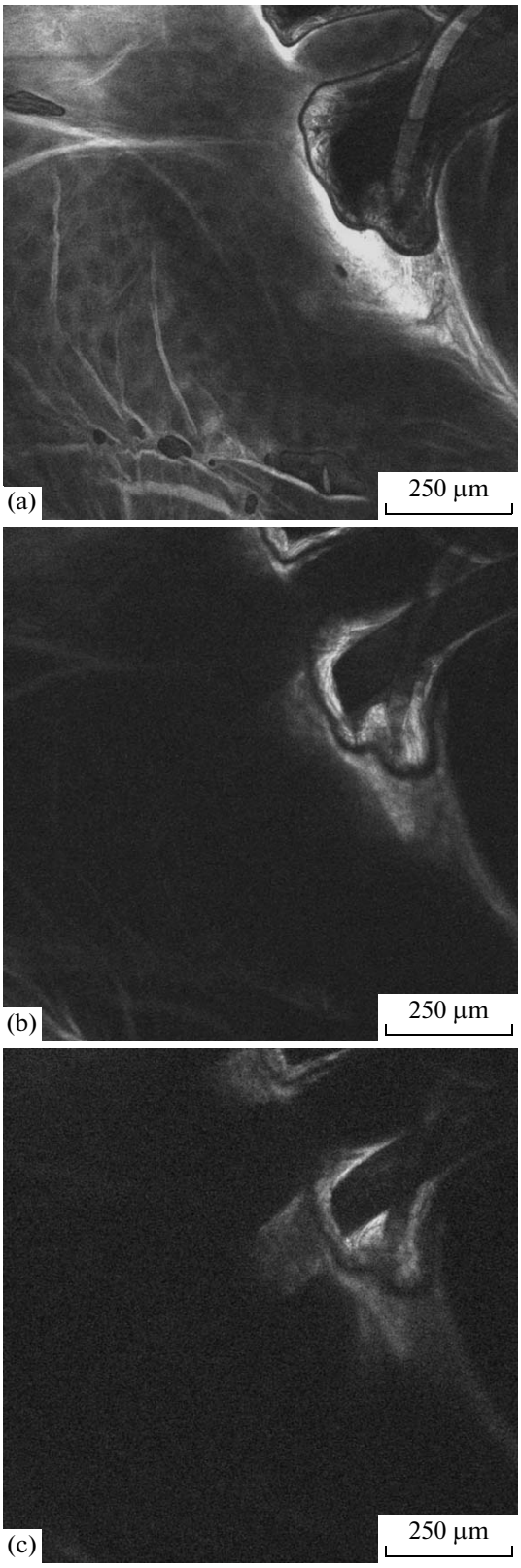

Fig. 3. Distribution of the dye containing shampoo in different depths of a terminal hair follicle (5 min after washing); (penetration depth: (a) skin surface, (b) 100 μm, (c) 200 μm).

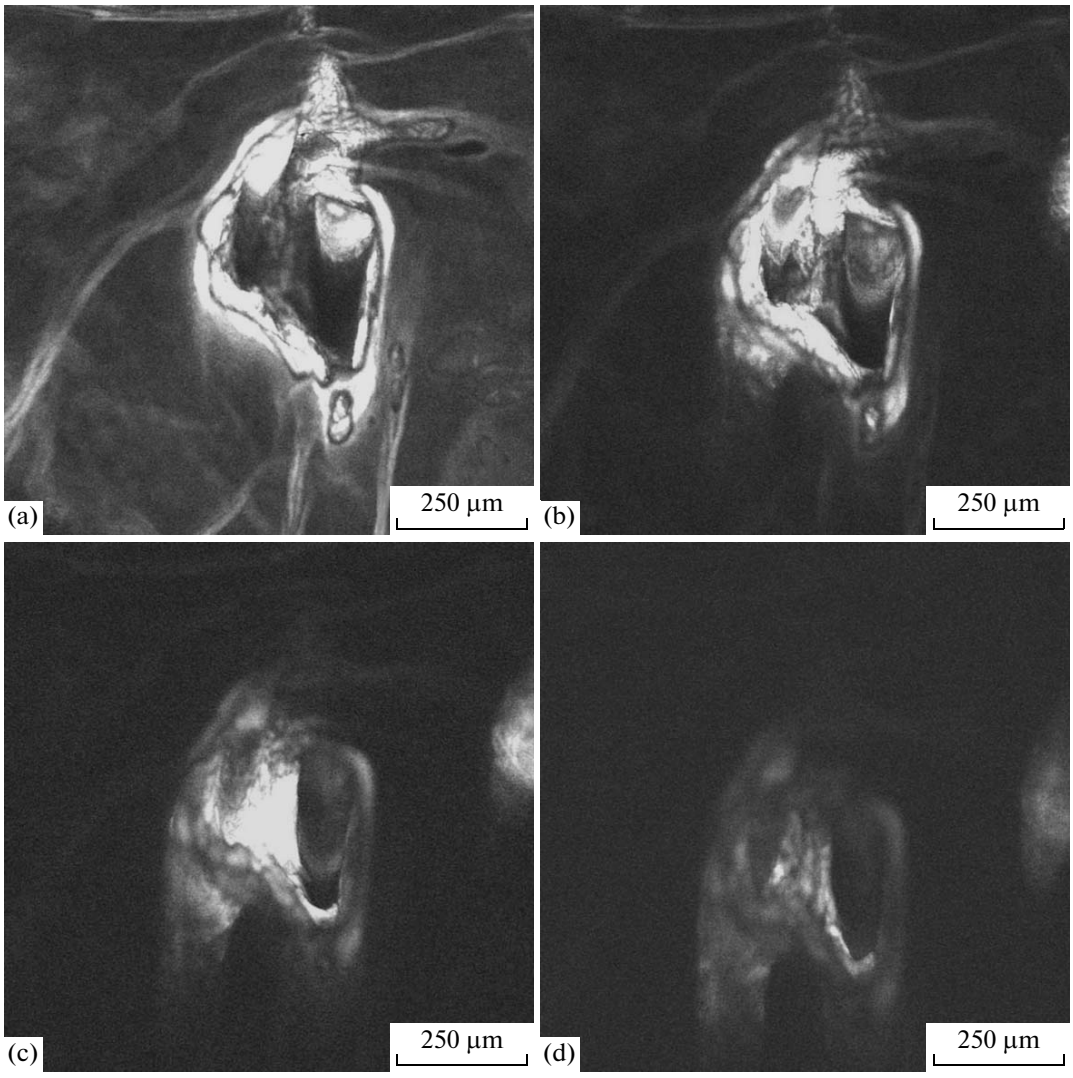

Fig. 4. Distribution of the dye containing shampoo in different depths of a vellus hair follicle (24 h after washing); (penetration depth: (a) skin surface, (b) 50, (c) 200, and (d) 150 μm).

cent dye could still be detected in the hair follicles, while almost no shampoo was left on the skin surface. A typical example is given in Fig. 4.

DISCUSSION

In vivo studies using cultured hair follicles, it was demonstrated that caffeine stimulates the hair growth [28].

In order to achieve this effect under in vivo conditions, it is essential that the caffeine-containing shampoo penetrates into the hair follicles, where it is stored and can pass through the barrier of the hair follicles into the blood flow system, as demonstrated by Otberg et al. [5, 6].

In the present study, the fluorescent dye fluorescein was added to the caffeine-containing shampoo, which was applied onto the scalp of volunteers. Using in vivo laser scanning microscopy, the distribution and storage of the shampoo in the hair follicles was investigated. The laser scanning microscope STRATUM is well suited to analyze the distribution of fluorescent dyes on the skin surface and in deeper layers of the skin [42, 43]. After washing, only small amounts of the fluorescent dye remained on the skin surface, while higher amounts could be detected in the orifices of the hair follicles.

From the analysis of the optical properties of the skin it is known that laser irradiation at 488 nm penetrates approximately 200 μm into the tissue. In the present study, this was also the penetration depth up to which the fluorescent dye of the shampoo could be detected (penetration depth $d = 185 \pm 27$ μm). Identical results were obtained for terminal and vellus hair follicles. This means that the fluorescent dye penetrated deep enough into the hair follicles to reach the

close network of blood capillaries, which surround the hair follicles. In contrast to the SC, the hair follicles represent a long-term reservoir for the shampoo formulation, as demonstrated in this study. Even after 24 h, a strong fluorescent signal could still be detected in the hair follicles.

The results obtained in this study demonstrate that the caffeine formulation penetrated efficiently into the hair follicles of human scalp skin. It is necessary however to conduct in vivo experiments regarding the effect of caffeine on hair growth in further studies. Summarizing the results, it can be established that the hair follicles represent an efficient reservoir for topically applied substances. In the present study, the fluorescent dye containing shampoo formulation could be detected in the hair follicles for a period of 24 h. A penetration of the dye into the hair follicles could be detected up to a depth of ~200 μm, which also represents the detection limit of the in vivo laser scanning microscopic system. A penetration of the shampoo even in deeper parts of the hair follicles can be expected. Taking into consideration the results of Otberg et al. [5, 6], who also showed the penetration of caffeine via the follicular route into the blood flow system and the results obtained in the present study, it can be expected that the caffeine of the shampoo formulation is able to pass through the hair follicles into the blood flow system.

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