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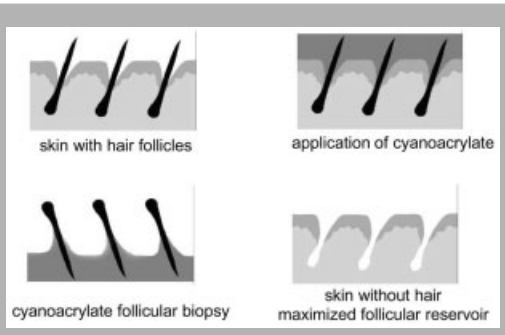
Abstract: Hair follicles have been assigned an increasingly significant role in dermatopharmacology. Previous studies have shown that the penetration of substances into the follicle depends on its activity. Telogen follicles that were not producing sebum were closed for follicular penetration. The present study uses optical coherence tomography (OCT) to show that the orifices of inactive follicles are blocked by plugs formed of corneocytes. The follicular penetration of a fluorescent dye was analyzed by cyanoacrylate surface biopsies in combination with laser scanning microscopy. Surface exfoliation and cyanoacrylate surface peels were used as a pre-treatment to remove the corneocyte plugs from the follicular orifices and therefore to open up closed follicles. It was shown that both procedures could increase the number of open hair follicles significantly. The present experiments show that OCT in combination with laser scanning microscopy is an excellent non-invasive in vivo method for the investigation of hair follicle properties and follicular penetration processes.



Maximization of the follicular reservoir by Cyanoacrylate peel

Laser spectroscopic methods for the characterization of open and closed follicles

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1. Introduction

The role of hair follicles as possible shunt routes for the absorption of topically applied drugs [1–5] and as a target for gene therapy of hair diseases [6–8] has been a focus of interest in recent years.

Moreover, hair follicles are considered to form a suitable reservoir for topically applied substances, depending on the skin area and the follicular properties [9]. Previous studies have shown that the follicular pathway is not always open to topically applied substances; therefore, open and closed hair follicles must be distinguished. In vivo experiments were performed using tape stripping, in combination with spectroscopic measurement, for the investigation of the penetration of coated titanium dioxide microparticles into the stratum corneum. Small amounts of titanium dioxide were found on tapes taken from deeper parts of the stratum corneum. These amounts were clearly located in the area of the follicular orifices, although the microparticles were not found in every hair follicle [10].

In continuing studies, laser-scanning microscopy in combination with cyanoacrylate skin surface biopsy was used to observe follicular penetration of topically applied curcumin emulsion into the hair follicle. The dye was found in most of the hair follicles, although some follicles were obviously closed for penetration. Reasons for

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this phenomenon are found in the specific follicular properties. A combination of various tape stripping and staining methods made it possible to measure hair growth and sebum excretion of every single hair follicle in the defined skin area. A correlation between the penetration properties, hair growth activity and sebum production was found. Telogen hair follicles that were not excreting sebum were closed for the penetration process [11–13].

Our aim was to find the reasons why inactive follicles are closed for penetration and therefore also for transfollicular absorption.

First it was necessary to measure the activity of every single hair follicle within the test area. After classifying each follicle, optical coherence tomography (OCT) was used to measure the follicle orifices. The non-invasive OCT method recently became popular for skin diagnostics because it provides optical cross sections through the skin with few micron resolutions and because it has been shown that hair follicles and sweat glands can be well detected and analyzed in-vivo by this method. [14].

Moreover, since the recent discovery that hair follicles and sweat glands form important pathways through the skin barrier [14–18], our aim was to increase the number of open follicles for the penetration process by different pretreatments. For the detection of open and closed hair follicles a combination of cyanoacrylate surface biopsies with laser scanning microscopy was used, as described in previous studies [11–13].

2. Methods and Materials

The experiments were performed on the forearms of 6 healthy male volunteers from 28-45 years of age with normal body mass indices (22-24). None of the volunteers suffered from any kind of skin disease or adipositas. All test subjects were thoroughly informed about the reason for and possible side effects of the test and provided their signatures on the informed consent forms. The Commission on Ethics of the Charité University Clinic approved the conducting of the experiment.

An area of skin 1×1 cm in size was marked on the lower arm. The hair within this area was trimmed to approximately 1 mm in length and the region was photographed using a macrophotographic system. This system is composed of a Canon® EOS 50/50 camera, a macro lens (MP-E 65 mm F 2.8 1-5X) and a ring flash (ML-3). The resulting photos were scanned and the software program Photoshop® (Adobe® Photoshop® 5.5.) was used to determine the locations of the follicles. Using another software program (analySIS®, Soft Imaging System GmbH SIS, Münster) the precise length of the hairs was measured. Five days later, new images were made of the same skin regions, and once again the follicle locations were determined and the hair length measured. Using this method it was possible to determine which hairs within the test region were in the anagen phase, i.e., were growing during the time frame of the experiment, and which were in the resting phase (telogen). In preparation for the first experiment this information was entered on a follicle map [11–13].

Sebum production was measured by Sebutape® (Cuderm, Dallas, Texas, USA), which is an adhesive polymorphic film containing innumerable air-filled microcavities. The film was applied to the test areas for 20 minutes. During the collecting period, the sebum issuing from every single follicle replaces the air in the tape with lipids, which registers as a transparent spot. The sebum-excreting follicles were marked on the map.

After completing the follicular map, the hair follicles within the test areas were measured with an OCT system (”SkinDex 300” from ISIS optronics, Mannheim, Germany), which achieves a depth and lateral resolution in tissue of approximately 5 μm and 3 μm. The OCT image is presented almost online on the monitor.

The penetration experiments were performed on three skin areas on the upper arm (regio deltoidea) of the same 6 volunteers.

Curcumin ($C_{21}H_{20}O_6$) in an O/W formulation (2% oil in water emulsion) was used as a model for a non-particular substance. Curcumin has distinct fluorescent properties and can easily be detected by laser scanning microscopy with argon laser radiation. It shows a strong fluorescence signal after excitation at 488 nm in the spectral region of 550 nm. The fluorescence signal can be detected at 590 nm, which is distinct from the background fluorescence caused by protein bands and cyanoacrylate.

Skin surface biopsies and follicular biopsies were performed by applying one drop of cyanoacrylate glue (UHU® GmbH, Brühl, Germany) onto the skin and covering it with a glass slide under light pressure. After polymerization, which occurs in one minute, the glass slide was gently lifted and removed. The surface biopsy contains the vellus hairs and a cast of the follicle infundibula.

Three skin areas of 2.25 cm were marked on the upper arm. The test regions were located close to each other to achieve a similar follicular density. The curcumin emulsion was applied to one of the three selected areas on the upper arm of the volunteers. After an application time of half an hour, a cyanoacrylate surface biopsy was taken from the test area and measured using laser-scanning microscopy. The second skin area was pretreated with a mechanical exfoliant (First Beauty, AOK Schwarzkopf & Henkel®) that is rubbed into the skin for 3 minutes and then removed with a paper towel. After this pretreatment the same curcumin application was carried out as on the untreated skin, and after half an hour a cyanoacrylate biopsy was taken once again and the presence of curcumin fluorescence in the follicle infundibula measured. From the third area, a cyanoacrylate peel was performed prior to the start of the penetration experiment. Afterwards the application of the curcumin emulsion and the fluorescence measurements were carried out as with the other two test regions.

Statistical analysis was performed by using SPSS software (SPSS, Chicago, IL).

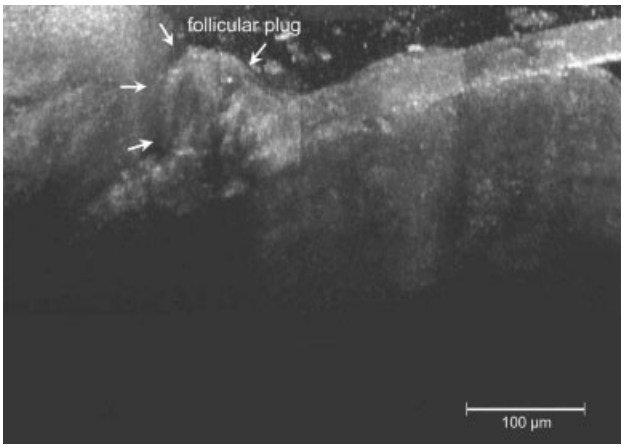

Figure 1 OCT image with inactive hair follicle

3. Results

Results of the OCT measurement:

With the help of the follicular map the follicles within the investigated skin region could be relocated using optical coherence tomography (OCT). The resting hair follicles, which showed neither sebum excretion nor hair growth, a structure with a diameter of ca. 100 μm, which partially protruded over the skin surface, was present in the region of the follicle opening. These structures were not found in the active follicles. Figure 1 shows an OCT image of a telogen follicle that was not showing signs of sebum production. In the area of the follicle orifice a distinct structure whose thickness roughly corresponds to that of the stratum corneum is visible.

Results of the penetration experiment:

The fluorescence measurements of the cyanoacrylate biopsies, which were taken from the untreated skin (skin area 1) after the application of curcumin emulsion, showed a mean follicular density of 23 follicles/cm^2 on the upper arm. 74% of the follicles displayed a fluorescence signal during laser-scan microscopy. The cyanoacrylate biopsies of the skin pretreated with exfoliant (skin region 2) showed a mean follicular density of 20 follicles/cm^2. 100% of the follicles displayed a fluorescence signal.

A cyanoacrylate peel was performed on skin area 3 prior to the application of the curcumin emulsion. The cyanoacrylate biopsies from this skin region further taken after the penetration of the curcumin displayed a mean follicular density of 27 follicles/cm^2 100% of the follicles displayed a fluorescence signal.

Figure 2 shows a follicular biopsy that displays a clear fluorescence signal during laser scanning microscopy. The curcumin emulsion has obviously penetrated into the follicular infundibulum, therefore we are dealing here with an open follicle.

Figure 3 shows the mean follicular density and the number of open follicles for all three skin regions.

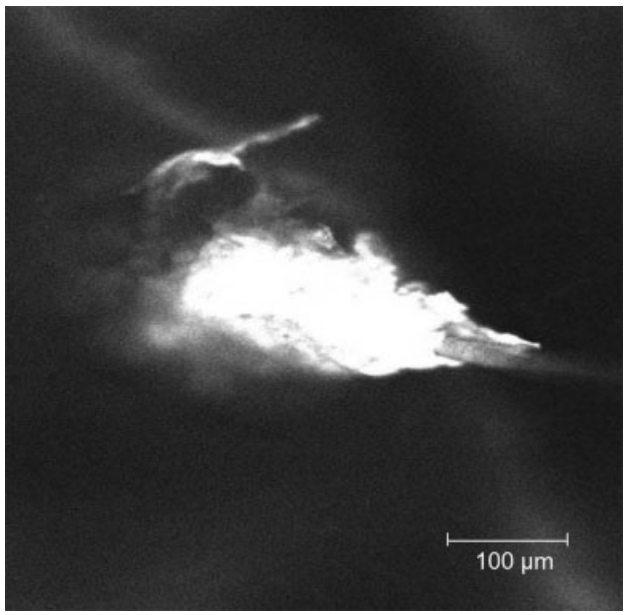

Figure 2 Follicular biopsy showing a curcumin fluorescence signal

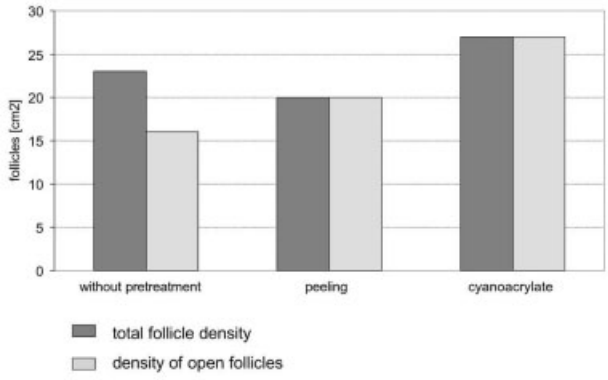

Figure 3 Follicular density on the upper arm and number of open follicles after different pretreatments

4. Discussion

Using optical coherence tomography (OCT), it was possible to depict the hair follicle openings and the follicle infundibula in cross section. In correlation with the results of Lademann et al. [11–13, 19] it could be demonstrated that the orifices of inactive hair follicles, i.e., follicles that evidence neither hair growth nor sebum excretion, were filled with a plug. This plug demonstrated morphological characteristics to those of the stratum corneum, had a thickness of approximately 100 μm and protruded from the surface of the skin. It seems likely that this structure consists of shedded corneocytes, which are pushed out of the orifices by growing hairs or emerging sebum, thereby opening the

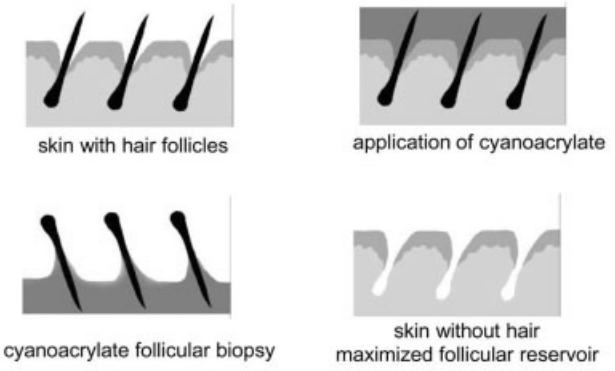

Figure 4 Maximization of the follicular reservoir by Cyanoacrylate peel

follicles for penetration of topically applied substances. The penetration experiments prove once again the phenomenon of open and closed hair follicles. Only 74% of the hair follicles on the upper arm were open for the penetration of the curcumin emulsion. After pretreatment with the exfoliant the percentage of open follicles was raised to 100%. The mechanical peeling usually removes the upper layer of the stratum corneum, and the results show that such a pretreatment can also dislodge the follicular plugs and improve follicular penetration. After the pretreatment with cyanoacrylate, 100% of the follicles were open for penetration by the curcumin emulsion. Cyanoacrylat removes not only the plugs blocking the orifices of the closed vellus hair follicles, but also pulls the contents of the follicular infundibula and the vellus hair from the skin. In this way penetration into the follicle can be optimized and a larger follicular reservoir achieved. Figure 4 schematically depicts pretreatment with cyanoacrylate and the maximizing of the follicular reservoir.

According to the preceding findings, the optimal pretreatment for follicular drug delivery is a cyanoacrylate peel. Enhanced penetration of substances into the hair follicle, as well as their remaining in the skin over a longer period, is essential for the treatment of follicle-related skin diseases. Pretreatment with cyanoacrylate is however impractical for larger skin regions. These results demonstrate that even simple mechanical pretreatments, such as with the exfoliant used in the present tests, can significantly improve follicular penetration. The tests carried out in vivo with optical coherence tomography (OCT), together with the documentation of follicular physiology, allow an explanation for the phenomenon of closed follicles for the first time. As a result of these findings, further development and optimization of methods for follicular drug delivery are possible.

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