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Investigation of Differences in Follicular Penetration of Particle- and Nonparticle-Containing Emulsions by Laser Scanning Microscopy

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Abstract—Hair follicles represent a long-term storage of topically applied drugs and cosmetics in the skin. Analyzing the penetration of particle- and nonparticle-containing formulations by laser scanning microscopy, it was found, surprisingly, that particles at a size similar to the thickness of the keratin cells of the hair penetrate more efficiently into the hair follicles. These results were obtained from in vitro and in vivo investigations. It seems that the moving hairs in the follicles act as a geared pump because of the zigzag structure of the surface of the hairs. This pumping effect probably pushes particles with the corresponding size deep into the hair follicles.

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INTRODUCTION

The penetration and storage of topically applied substances in the human skin is essential for the efficacy of skin treatment in dermatology and cosmetology.

In the past, it was assumed that topically applied substances penetrate via the intercellular pathway inside the lipid layers in and through the skin barrier (stratum corneum) [1–3]. Recently, it was found that hair follicles can also represent an efficient pathway for the penetration and storage of topically applied drugs and cosmetic products [4–6]. Topically applied substances are mainly located in the uppermost cell layers of corneocytes in the stratum corneum [7]. Because of the renewing of the skin, approximately one layer of corneocytes is depleted daily. Therefore, topically applied substances can be efficiently stored in the horny layer for only one or two days.

In contrast to the stratum corneum, hair follicles represent a long-term reservoir for topically applied substances, because the depletion of the hair follicles takes place only through sebum production and hair growth [8]. Both are slow processes. Therefore, the hair follicles represent an important target for drug delivery.

Laser scanning microscopy (LSM) is an efficient tool to investigate the distribution of topically applied fluorescent dye labeled substances in hair follicles [9]. Furthermore, it can be used for the analysis of the surface structure of hairs and the stratum corneum in the upper part of the follicles.

An analysis of the penetration and storage of topically applied substances by LSM showed that microparticles penetrate more efficiently into the hair follicles than nonparticle-containing emulsions [10, 11]. This was shown in vitro on pig ear skin and in vivo on human skin, using the noninvasive method of differential stripping [10] recently described by Teichmann et al. [12]. Using this procedure, the follicle content was able to be removed selectively from the skin, and the storage kinetic of topically applied emulsions into the hair follicles could be determined.

The present paper demonstrates that different laser spectroscopic and microscopic methods represent efficient tools to investigate the follicular penetration pathway of particle and nonparticle substances.

MATERIALS AND METHODS

Formulations

Two formulations based on the same hydrogel were utilized for the investigation. Both contained the same concentration of the fluorescent dye, sodium fluorescein, whereas one contained the sodium fluorescein in particulate and the other in nonparticulate form (both were prepared by the Department of Biopharmaceutics

and Pharmaceutical Technology, University of Saarland, Saarbruecken). The size of the particles was 320 nm.

Volunteers and Porcine Skin

The investigations were performed in vitro on porcine skin (which is a suitable model for human skin because of its similar morphology and skin structure [13]) and in vivo on the calves of six healthy volunteers. Approval for this study was obtained from the Ethics Committee of the Charité and from the Veterinary Board of Control, Berlin, Treptow-Köpenick. The study was conducted in accordance with the ethical rules stated in the Declaration of Helsinki Principles. The volunteers participating in the study gave their written consent.

Study Designs

Study design A. Comparison of the follicular penetration depth and storage behavior of particulate and nonparticulate sodium fluorescein determined by LSM.

In vitro: application of 2 mg/cm^2 of the particulate and nonparticulate formulations by means of massage [10]. After a one-hour penetration time, 3-mm punch biopsies were removed. These were frozen, and histological sections were obtained and investigated by laser scanning microscopy (LSM 2000, Carl Zeiss, Jena, Germany). Ar^+ laser radiation at 488 nm was used to excite the dye. The fluorescent signal was detected at a wavelength ≥560 nm.

In vivo: application of 2 mg/cm^2 of the particulate and nonparticulate formulations by means of massage [10]. After different penetration times (1, 4, 8, and 10 days), application of differential stripping [12], which means the removal of the upper part of the SC by tape stripping, as described by Weigmann et al. [7] and the removal of the follicular content by cyanoacrylate skin surface biopsies. These were punched to a constant size of 15 mm in diameter and afterwards extracted in ethanol (Uvasol, Merck, and Darmstadt, Germany) using ultrasound (Sonorex Super RK102H, Bandelin Electronic, Berlin, Germany) and centrifugation (at 4000 rpm for 10 min at 20°C, Centrifuge MR1812, Jouan GmbH, Unterhaching, Germany), followed by the determination of the concentration of the sodium fluorescein using fluorescence measurements (Luminescent LS 50B, Perkin Elmer, Überlingen, Germany). The fluorescence signal was detected in the spectral range from 480 to 600 nm. The maximum fluorescence intensity was detected at a wavelength of 510 nm.

Study design B. Structure analysis of the hair was determined by LSM and scanning electron microscopy.

The surface structure of the hairs on pig ear skin was analyzed in vitro and on human calf skin in vivo using the dermatological laser scanning confocal microscope *Stratum*, Optiscan Ltd., Melbourne. The radiation of an Ar^+ laser at 488 nm was used to excite the topically applied fluorescent dye fluorescein. The radiation was transferred by an optical fiber to the probe, which contained the scanning system. The investigated field of vision was 200 × 200 μm.

The optical window of the handpiece was set directly onto the skin surface. The fluorescence emission was collected by the objective lens and transferred by the optical fiber to a photodetector.

Scanning electron microscopy (LEO Gemini 1530, Zeiss, Oberkochen) was used to analyze the surface structure of the human hairs in vitro at a high magnification.

RESULTS AND DISCUSSION

An efficient penetration and long-term storage in the skin is the prerequisite for a high efficacy of topically applied drugs and cosmetics. In the stratum corneum, the topically applied substances are stored only in the upper layers of the corneocytes. These layers are a short-term reservoir, because, every day, one layer of corneocytes is worn off. In contrast, follicles represent a long-term reservoir, as the depletion takes place only by hair growth and sebum production, which are slow processes.

The results obtained by LSM showed that the penetration of particulate substances is much more efficient if massage is applied (see Fig. 1). The distribution depth of both formulations was determined in 50 hair follicles. For the particulate formulation, the penetration was significantly deeper (approximately 1500 μm) compared to the nonparticulate formulation (approximately 500 μm).

These results were confirmed by in vivo measurements on human skin, where the storage time of the particles was significantly longer than that of the nonparticle-containing formulation (10 days compared to 4 days).

Surprisingly, particles with a diameter in the range of 300 nm penetrate much more efficiently into the hair follicles than the nonparticulate formulation (see Fig. 1). The reason seems to be the surface structure of the hairs and the stratum corneum in the upper part of the hair follicles, which are similar in human and pig ear skin. From Figs. 2 and 3, it can be seen that the cuticle produced by keratinocyte desquamation forms a structure surface of the hair, which can be approximated by a zigzag structure. This structure is determined by the thickness of the keratin cells, which is between 500 and 800 nm. A corresponding structure is formed by the surface of the stratum corneum in the upper part of the hair follicles. If the hairs are moved, the hair follicles probably act as a geared pump. In vivo, such a movement of the skin is always present. However, in vitro, this movement can be stimulated by a massage. Therefore, if massage is applied, the penetra-

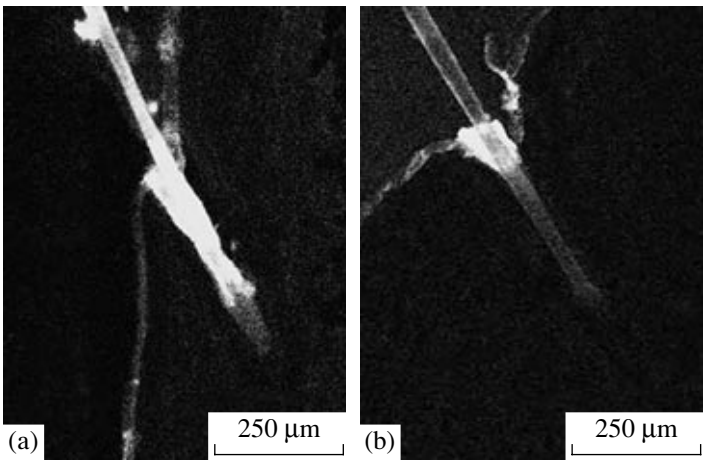

Fig. 1. Histological sections demonstrating the penetration depth of the particle- and nonparticle-containing emulsions in the hair follicles of pig ear skin (LSM measurements). Left: particle-containing emulsion; right: nonparticle-containing emulsion.

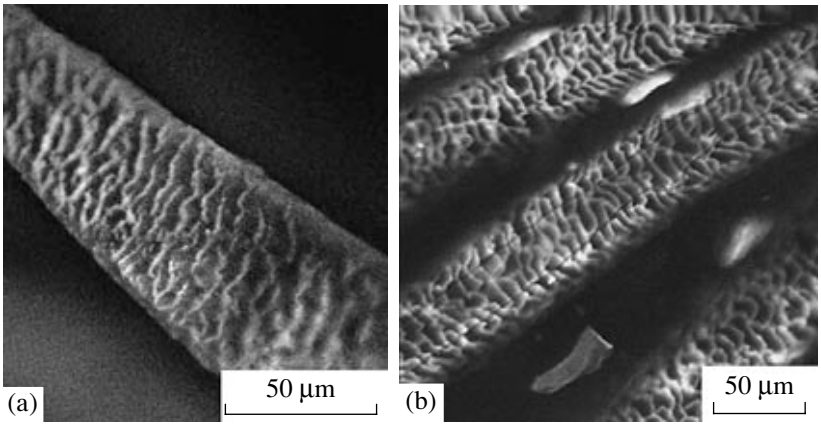

Fig. 2. LSM image of the surface structure of the hair (pig ear skin). Left: pig ear skin; right: human skin.

tion of the particle-containing formulation in a porcine ear skin is increased.

Taking into consideration the results obtained by Toll et al. [14], the smallest particles investigated, which had a diameter of about 700 nm, penetrated more efficiently into the hair follicles than larger sized particles. The results reported in the present paper demonstrate that nanoparticles with a diameter of 320 nm penetrate better into the hair follicles than nonparticle formulations. Consequently, the optimum size of particles for follicular penetration should be between 320 and 700 nm. This dimension corresponds to the thickness of the keratin cells of the hairs, which supports the

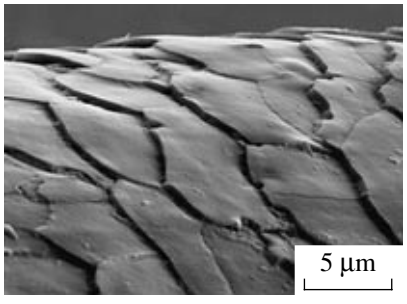

Fig. 3. Electron microscopic image of the surface structure of the hair (human skin).

hypothesis of the geared pump mechanism for drug delivery into the hair follicles.

CONCLUSIONS

The long-term storage of topically applied substances is important for several applications of drugs and cosmetics. Using laser scanning microscopy, it was demonstrated that hair follicles represent a much more efficient long-term reservoir for topically applied substances than the stratum corneum. Surprisingly, it was found that the particles, whose size was similar to the thickness of the keratin cells forming the hair surface structure, penetrated much more efficiently and deeper into the hair follicles than the nonparticle-containing formulation. It seems that the hair follicles with moving hair acting as a geared pump push the particles with the corresponding size deep into the hair follicles. The observed results are important for the optimization of topically applied drugs and cosmetics, which should remain active in the skin for a significant length of time.

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