Received: 13 October 2020 | Revised: 21 December 2020 | Accepted: 24 December 2020

DOI: 10.1111/srt.13008

ORIGINAL ARTICLE

WILEY

Analysis of the morphometric parameters of pig ear hair follicles

Alexandra Zambrano | Anna Lena Klein | Alexa Patzelt

Department of Dermatology Venereology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Charité – Universitätsmedizin Berlin, Corporate member of Freie Universität Berlin, Charité Universitätsmedizin Berlin, Berlin, Germany

Correspondence
Anna Lena Klein, Department of Dermatology Venereology and Allergology, Center of Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany.
Email: anna-lena.klein@charite.de

Abstract

Background: Porcine ear skin is used in studies of percutaneous penetration as a substitute for human skin. The objective of the present study was to determine the structure of the hair follicles on the dorsal area of porcine ear skin and make a morphometric comparison with the hair follicles of human skin.

Materials and Methods: Sections of frozen biopsies were cut vertically to the skin surface in longitudinal sections using a cryotome and were investigated using microscopy. For each hair follicle, various parameters were determined.

Results: The follicular density in porcine ear skin varies according to the area studied, and the length of most of the follicles was approximately 1458 ± 286 μm. The size of the follicular orifice was also determined in a total of 305 follicles. It showed a diameter of roughly 113 ± 43 μm.

Conclusion: The results showed a very good similarity between human and pig hair follicles. Therefore, porcine ear skin can be considered as a very suitable model of human skin in dermal and especially follicular penetration studies.

KEYWORDS

hair follicle morphology, human skin, in vitro model, porcine ear skin

1 | INTRODUCTION

In recent years, the relevance of hair follicles in the process of skin penetration has been demonstrated in many studies. The stratum corneum is the outermost layer of the skin and represents the main skin barrier. The stratum corneum is discontinuous in the region of the hair follicles, which facilitates faster absorption of certain substances and drugs in these areas.[1] Additionally, the presence of numerous blood capillaries, in close contact with the hair follicle, enables a rapid uptake of various actives into the bloodstream.[2,3] This previous statement could be corroborated in a study conducted by Otberg et al demonstrating that, after a caffeine application for 2 minutes in a shampoo formulation, caffeine penetrated through the hair follicles and the stratum corneum. However, absorption through the hair follicles and relevant levels of caffeine in the blood was already found 5 minutes after topical application. Comparable levels could not be obtained before 20 minutes when caffeine penetrated exclusively through the stratum corneum of the interfollicular epidermis.[4] Whereas the intercellular penetration pathway along the lipid layers around the corneocytes can still be considered as the most relevant one for most topically applied substances, the follicular penetration pathway offers entrance to further target structures of interest. The epidermal stem cells, for example, which are located in the bulge region of the hair follicle, are of special interest for regenerative therapy approaches, the dendritic cells, which are surrounding the infundibular part of the hair follicle, can be targeted if transfollicular immune therapy is desired.[5]

Likewise, in recent years, a growing interest has been developed in nanometer-sized drug delivery systems in the field of medicine. Due to their small size, nanocarriers can improve the

site-specific drug delivery and therapeutic effect of drugs that suffer from poor solubility, poor stability, and unwanted toxicity. This helps to generate better tissue distribution and pharmacokinetics and thus a reduction of the adverse effects of certain drugs in the body.[6]

However, it has been observed that not all of the topically applied particles or substances can cross the epidermis. Consequently, the hair follicles play a fundamental role again in their penetration, storage, and transport. The results of many studies suggested that particles located in the stratum corneum were nearly quantitatively removed after 1 day.[7] In contrast, particles in the hair follicles remained for more than 10 days. This could be observed, for example, in a study carried out with Itraconazole applied topically onto the forearm of male and female volunteers. 2 weeks after the last application of the substance, relevant concentrations of it were observed in the hair follicles, stating that these represent a long-term reservoir for topically applied substances.[8] It has also been suggested that nanoparticles after penetrating the skin might be taken up by local lymph nodes and then transported into the blood.[7] Another aspect that is important to highlight when we talk about skin penetration through hair follicles is the size of the particle. In a study by Patzelt et al, it was determined that the particles of medium size (643 and 646 nm) penetrated deeper into porcine hair follicles than smaller (122 nm) or larger particles (860 nm).[9] It was also concluded that by varying the particle size, different sites within the porcine hair follicle can be targeted selectively.[9] Studies conducted with human hair follicles revealed similar results, showing that particles with a size of approximately 750 nm penetrated deeper into the terminal follicles of human Scalp.[10] Due to the confirmed impact of the hair follicles in the process of transdermal penetration, different authors have carried out multiple in vivo or ex vivo studies on the morphology of the hair follicles and the follicular penetration of various substances into human skin.[11,12]

However, access to human skin as a skin model is usually limited, and its use has certain disadvantages for follicular penetration studies.[13] For example, upon excision, human skin shrinks due to the contraction of the elastic fibers, producing a contraction and permanent occlusion of the hair follicular orifice and decreasing the follicle reservoir, significantly limiting the penetration process by up to 90%.[14]

Therefore, and in search for an appropriate animal skin model to extrapolate skin penetration studies in humans, different comparative studies of hair follicle morphology and skin structure in different species have been carried out in the recent past. The morphology and skin penetration of various substances through the hair follicles in mammals generally vary according to the species and area of the body.[15] However, porcine skin and the hair follicle morphology in the area of the ear have high similarity with human skin in contrast to other pig skin regions. For this reason, it has been concluded that porcine ear skin should be a suitable ex vivo model for percutaneous penetration studies, especially when the follicular penetration pathway is investigated.[16] Studies comparing the penetration behavior of various substances in human skin, as well as pig skin, revealed that the penetration process in both models is similar.[17,18]

Unfortunately, the morphological description of hair follicles is only available for human skin[10] but not for porcine ear skin, yet. Therefore, the objective of the present study was to determine the morphometric characteristics of the hair follicles in pig ears in order to enable a more specific comparison of human and porcine hair follicles, and to allow a better extrapolation of data obtained from pig skin experiments to the in the human situation.

2 | MATERIALS AND METHODS

The morphometry of porcine ear hair follicles was investigated on 345 longitudinal sections of hair follicles of n = 10 individuals by determining seven different parameters. The ear skin was selected, as porcine ear skin is a frequently utilized ex vivo skin model to investigate the intercellular and especially the follicular penetration of topically applied compounds. Thus, the knowledge of the exact morphometry is very relevant but only partly available yet.

2.1 | Porcine ear skin

The investigations were performed using ear skin (dorsal region) from 10 pigs (German domestic pig, 6 months old). The ears were kindly provided by a regional butcher and were investigated on the day of slaughtering. After arrival, the ears were cleaned using cold water. The experiments were authorized by the Federal Ministry of Consumer Protection and Agriculture, Landkreis Dahme-Spreewald. In preparation for the experiments, the ears were washed under cold tap water and dried using soft paper towels.

2.2 | Preparation of longitudinal hair follicle sections

As hair follicle density is verifiably varying in different parts of porcine ears, each ear was divided into three areas (A, B, and C), whereby area A was determined to be the caudal end of the ear, area B the middle part, and area C the cranial end of the ear. For each area, 2 fields of 6 cm^2 were marked in the central region and 8 square biopsies sized 5 mm × 5 mm were removed from each field, as demonstrated in Figure 1.

The follicular density of each biopsy was determined macroscopically. Afterward, the biopsies were shock-frozen in liquid nitrogen and stored at −20°C. The frozen biopsies were cut vertically to the skin surface in longitudinal sections of 7 μm thickness using a cryotome (Microm cryo-Star HM 560, MICROM International GmbH). The sections were transferred to glass slides (Menzel Gläser Superfrost®, Thermo Fischer Scientific Inc Waltham). For

each hair follicle, various sections were prepared. For each area (A, B, and C) of each porcine ear skin sample, a minimum of 10 hair follicles was investigated.

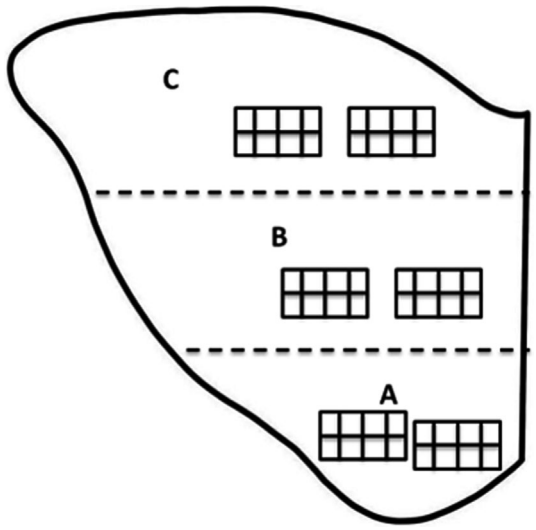

FIGURE 1 Schematic representation of the division of the porcine ears

2.3 | Determination of the morphometric parameters of the hair follicles

Using a microscope (BX60, Olympus), Camera (XC10, Olympus), and the software "cellSens Dimension" (Olympus), multiple parameters were determined for each hair follicle: the length of the hair follicle (a), the length of the infundibulum (b), the length and position of the bulge region (c and d), the opening of the hair follicle (e), the thickness of the hair follicle epithelium in the area of the infundibulum (f) and the thickness of the interfollicular epidermis (g) as depicted in Figure 2.

As the longitudinal sections of the hair follicles often did not display the complete hair follicle, the numbers of hair follicles assessed for the specific parameters are presented in Table 1.

2.4 | Statistical results

The statistical analyses were performed using the software program Microsoft® Excel 2007 and SPSS 24 IBM Statistic software (IBM Corporation). Mean values and standard deviations were calculated. The descriptive statistical analysis Wilcoxon test was used to

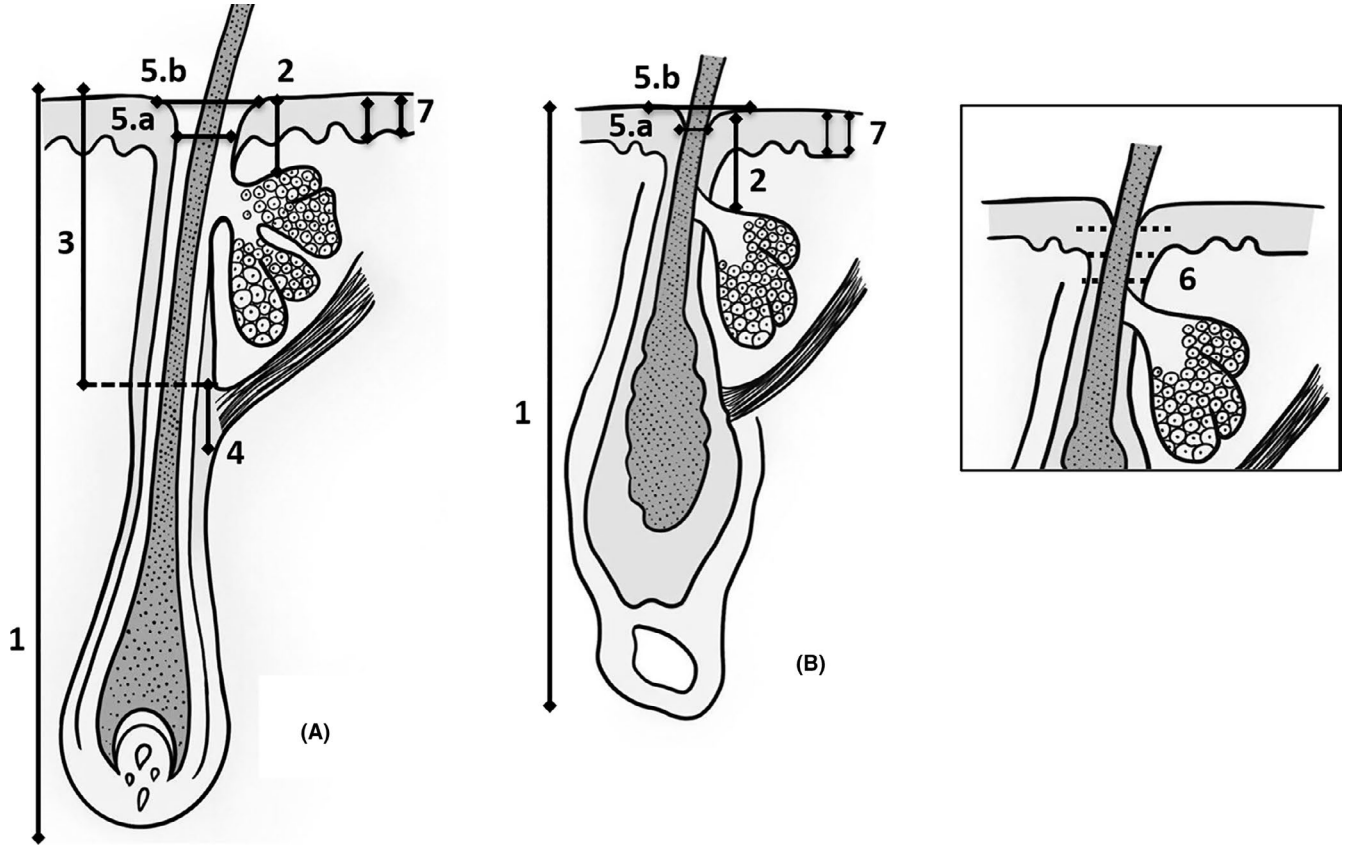

FIGURE 2 Morphometric measurements on porcine hair follicles from the dorsal auricular region. Total length of the hair follicle (1), length of the infundibulum (2), position and the length of the bulge region (3,4), hair follicle opening at the skin surface (5.a without epithelium, 5.b with epithelium), thickness of the hair follicle epithelium in the area of the infundibulum: upper, middle and lower part at the entrance of the sebaceous gland (6) and thickness of the interfollicular epidermis with intervals of 150 and 200 μm from the opening of the follicle (7). Figure A representation of the anagen phase and figure B telogen phase hair follicle

determine the significance of the differences between the different parameters in three areas of the ear (A, B and C).

3 | RESULTS

3.1 | Hair follicle density

The density of the hair follicles in the dorsal skin area of the porcine ears was determined. The results revealed that the number of hair follicles varied according to the area of the ear. The area indicated as zone C (cranial region) had the highest density of hair follicles per cm^2, while the area with the lowest density of follicles was the most caudal area (area A). The results are presented in Table 2.

3.2 | Length of the hair follicle

A total of 345 follicles were investigated. The total length of the hair follicles was only determined in n = 259 follicles and they were classified according to their anagen and telogen growth phases. Histologically, it is possible to recognize the follicles in the anagen phase, because these follicles are long, very straight and angled. In the telogen phase, the hairs are shorter, and the hair shaft is closer to the surface of the skin.[19] Figure 3 shows an example of a microscopic image that was used for the evaluations. 64% of the hair follicles (n = 166) were in the anagen phase, and 36% were in the telogen phase (n = 93). The follicles were also classified into two groups according to their length (<2000 μm and >2000 μm). 64% of the hair follicles in the anagen phase had a length <2000 μm (MV 1458 ± 296 μm), whereas 92% of the hair follicles in the telogen phase measured less than 2000 μm (MV 1240 ± 359 μm). No significant difference in follicle length was found for the different ear skin areas ($P > .05$).

TABLE 1 Numbers of hair follicles evaluated by parameter per area in the dorsal region of porcine ear skin

Parameter	Area A	Area B	Area C
Hair follicle density	50	62	90
Length of the hair follicle	88	87	84
The opening of the hair follicle	106	103	96
Length of the Infundibulum	63	54	55
Epithelium in the area of the infundibulum	60	45	47
Length of the bulge region	33	15	28
Position of the bulge region	26	15	26
Epidermis interfollicular	100	91	93

Note: The values by category were combined for the total sum of follicles evaluated.

TABLE 2 Mean density of hair follicles per cm^2 in the dorsal area of the porcine ears

Ear area	Number of hair follicles per cm^2
A	8 ± 2
B	10 ± 1
C	15 ± 3

Note: Data are presented as mean ± SD.

3.3 | Length of the infundibulum

The length of the infundibulum was determined in a total of 172 follicles. For the anagen hair follicles with a total length of <2000 μm, the infundibulum length was 373 ± 72 μm (n = 64), for telogen hair follicles (<2000 μm), the infundibulum length was 458 ± 131 μm (n = 64).

In hair follicles >2000 μm, the length of the infundibulum was 516 ± 107 μm (n = 38) in anagen hair follicles and 619 ± 152 μm (n = 6) in telogen hair follicles. There were no statistical differences ($P > .05$) for the infundibulum length between the different skin areas A, B, and C.

The descriptive statistical analysis with the Wilcoxon test revealed that there was a significant difference ($P < .05$) between the two hair follicle groups (<2000 μm and >2000 μm) concerning the infundibulum length. This was observed in follicles in the anagen phase as well as in the telogen phase. All results concerning the total follicular length and the length of the infundibulum are summarized in Table 3.

3.4 | Length and position of the bulge region

The bulge region is generally viewed as a prominent epithelial protuberance of the outer sheath of the follicle root. It is located between the opening of the sebaceous gland and the attachment site of the arrector pili muscle. In most cases, it is challenging to identify this lateral protrusion.[19,20] The parameters were only determined in the follicles in the anagen phase because the size of the follicle during this growth phase made it easier to identify it.

The position of the bulge region could be identified in 67 follicles. For these follicles, it was found at a depth of 881 ± 208 μm from the surface of the skin. The length of the bulge region was 272 ± 36 μm (n = 76). There was no significant statistical difference between the three areas of the ear ($P > .05$). To assess the position and length of the bulbar region, all the follicles found were added, without classifying them by their total length, because the values were similar in all the follicles. The results are summarized in Table 4.

3.5 | Opening of the hair follicle

The size of the follicular orifice in the infundibulum area was determined in a total of 305 follicles. Measurements were made with and without the infundibular epithelium. The mean diameter of the opening of the hair follicle, including the infundibular epithelium, was 326 ± 58 μm, and

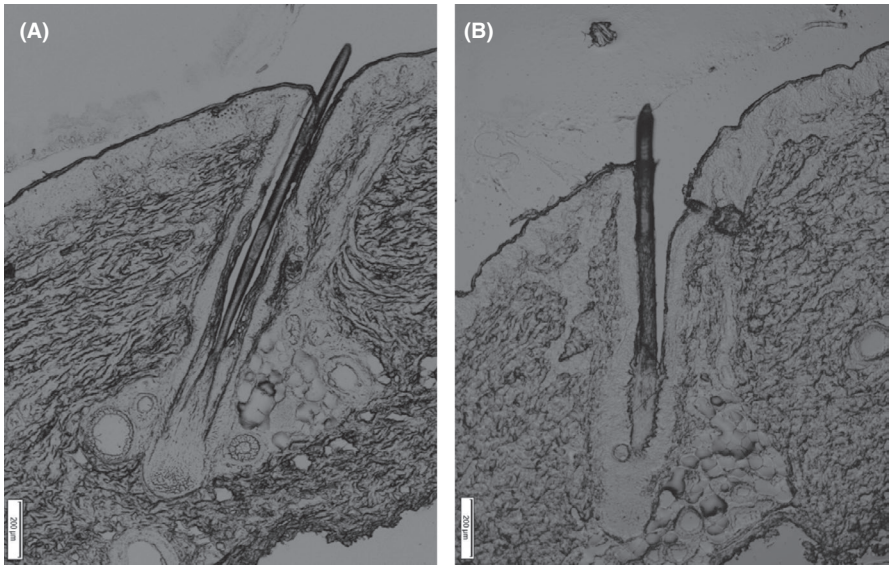

FIGURE 3 Photographic representation of the hair follicles in pig ears in their different growth phases (A. anagen phase and B. telogen phase)

TABLE 3 Summary of the data obtained for total follicular length (LHF) and length of the infundibulum (LI)

	Anagen (n = 166)			Telogen (n = 93)		
	LHF		LI	LHF		LI
HF	MV±	n=	MW±	MV±	n=	MV±
Area A						
<2000 μm	1440 ± 300	37	402 ± 79	1237 ± 388	27	412 ± 138
>2000 μm	2380 ± 242	21	486 ± 84	2137 ± 58	3	567 ± 102
Total	1780 ± 532	58	438 ± 91	1237 ± 475	30	418 ± 140
Area B						
<2000 μm	1440 ± 308	35	357 ± 64	1296 ± 301	34	460 ± 111
>2000 μm	2506 ± 272	16	559 ± 125	2384 ± 150	2	823 ± 0
Total	1775 ± 577	51	386 ± 104	1331 ± 390	36	482 ± 124
Area C						
<2000 μm	1498 ± 274	32	355 ± 58	1313 ± 373	25	512 ± 140
>2000 μm	2416 ± 290	25	536 ± 115	2163 ± 68	2	698 ± 51
Total	1901 ± 536	57	446 ± 129	1376 ± 423	27	518 ± 140
Total A + B + C < 2000 μm	1458 ± 286	63% 104	373 ± 72	1240 ± 359	92% 86	458 ± 131
Total A + B + C > 2000 μm	2427 ± 275	37% 62	516 ± 107	2215 ± 144	8% 7	619 ± 152
Total A + B + C <2000 μm + >2000 μm	1820 ± 551	100% 166	426 ± 111	1314 ± 432	100% 93	472 ± 140

Note: Values are given in μm. The LHF and LI were determined on histological sections of the dorsal area of pig ears. The follicles were divided into different categories according to their length (HL) and growth phase (anagen and telogen). Data are presented as mean ± SD.

without the epithelium 113 ± 43 μm. No statistical differences were observed for the different porcine ear areas ($P > .05$) (Figure 4).

3.6 | Epithelium in the area of the infundibulum

Also, the thickness of the infundibular epithelium was measured. The measurements were made at three different regions of the infundibulum. In the upper part of the infundibulum, the epithelium was 59 ± 11 μm thick. In the middle part, it was 51 ± 10 μm thick and in the lower part at the entrance of the sebaceous gland 46 ± 11 μm thick. The measurements were made on the right and left side of each hair follicle. No significant differences were found in the thickness between both sides as well as between the 3 different areas (A, B, and C) of the ear ($P > .05$) (Figure 5).

3.7 | Thickness of the interfollicular epidermis

The thickness of the interfollicular epidermis was also determined in the three areas of the ear (A, B, and C) with a lateral distance on both sides of the follicular orifice of 150 and 200 μm. The following results were obtained: Right side of the follicular orifice 101 ± 2 μm and 100 ± 1 μm; left side of the follicular orifice 101 ± 2 μm and 100 ± 0 μm. No significant difference between the three areas of the ear ($P > .05$) could be determined (Figure 6).

TABLE 4 Summary of the data obtained for the position and length of the bulge region of porcine hairs per area on histological sections of dorsal area of pig ears

	Position of the bulge region	Length of the bulge region
Area A	924 ± 196	270 ± 37
Area B	803 ± 176	281 ± 36
Area C	883 ± 223	270 ± 34
Total A + B + C	881 ± 208	272 ± 36

Note: Values are given in μm, data are presented as mean ± SD.

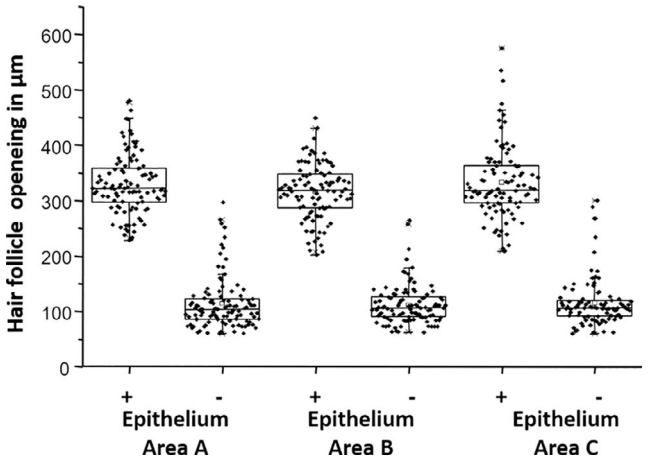

FIGURE 4 Size of the hair follicular orifices. The diameter of the hair follicular orifices was measured with epithelium (+) and without epithelium (–) at the skin surface. Data are presented as mean ± SD. ($P > .05$)

3.8 | Comparing results: human skin vs porcine ear skin

Table 5 compares the results obtained from the measurements of the different structures of the hair follicle in humans and pig ears. The data from humans originate from the study of Vogt et al.[10]

In pig ear, a greater variation in the length of the hair follicles could be found. Due to this, the follicles were classified into two groups. The classification was based on whether the hair was longer or shorter than 2000 μm. Only follicles in the anagen phase were evaluated. The other results obtained in this study by parameters were grouped into a single category in order to enable a clearer representation and comparison of the values.

4 | DISCUSSION

The morphometry of the hair follicles in three different central dorsal areas of 10 pig ears was studied, as this skin area is frequently utilized as an ex vivo skin model for penetration investigations and a complete overview of the morphometric data was not available yet but would be useful to evaluate obtained data correctly.

The results of this study showed that the average density of hair follicles per cm^2 is different depending on the skin area under investigation. The cranial zone presented on average a higher number of hairs per cm^2, with a maximum number of 18. The area with the lowest average number of hairs per cm^2 was the caudal area with 10 hairs. The results obtained were compared to other publications determining the follicular density in pig ears. The density of hair follicles per cm^2 varied in these studies; however, their results are similar. Some described the presence of 11-25 hair follicles per cm^2 [16]; others 11-42 per cm^2.[21] Unfortunately, the authors did not specify

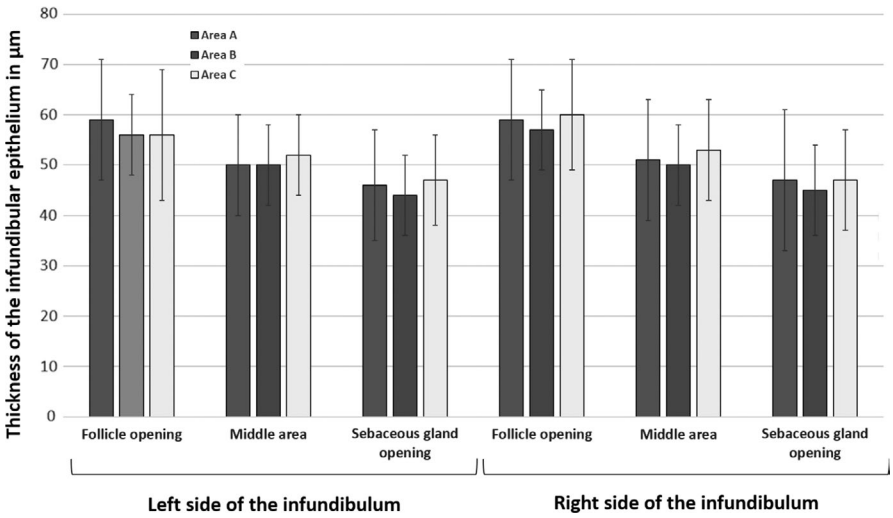

FIGURE 5 Thickness of the infundibular epithelium of porcine hair follicles. The thickness was measured at three different points and on both sides of the infundibulum from the skin surface down to the entry level of the sebaceous duct. The diagram illustrates that the thickness of the infundibular epithelium decreases continuously with increasing depth of the hair follicle

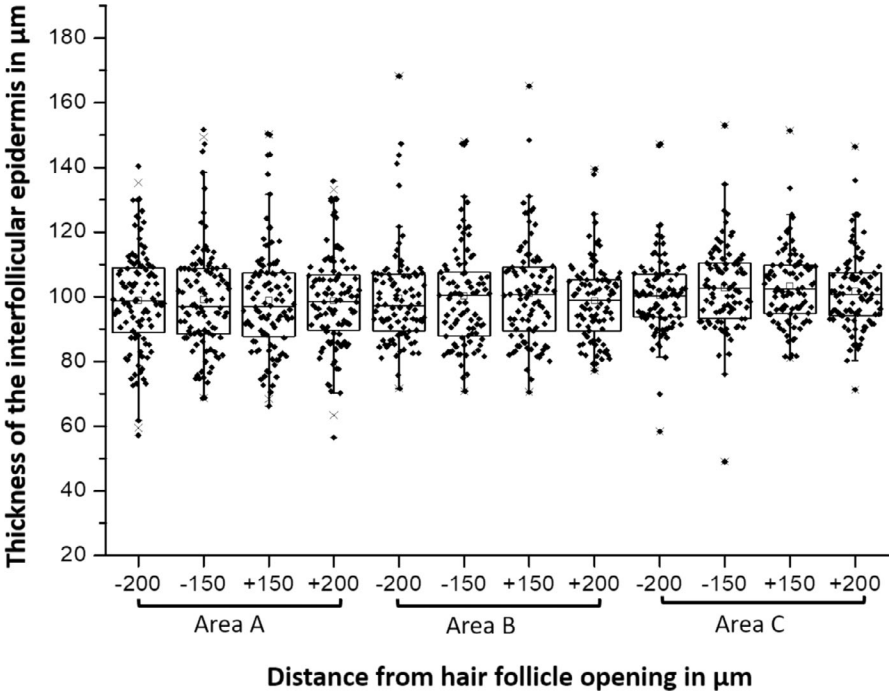

FIGURE 6 Thickness of the interfollicular epidermis on both sides of the hair follicle (+right side, −left side). The thickness was measured at 150 µm and 200 µm from the hair follicular orifice of porcine ear skin. Data are presented as mean ± SD

TABLE 5 Comparison of human and porcine hair follicles

	Human skin	Pig ear
Length of the hair follicle (anagen stage)	3864 ± 605	1458 ± 296 2427 ± 275
Length of the infundibulum (anagen stage)	580 ± 84	426 ± 111
Position of the bulge region (anagen stage)	1191 ± 23	881 ± 208
Length of the bulge region (anagen stage)	240 ± 52	272 ± 36
Diameter of the hair follicle opening at skin surface level	172 ± 70	113 ± 2
Interfollicular epidermis	136 ± 37	101 ± 2

Note: Values are given in µm, data are presented as mean ± SD (includes data from humans originate from the study of Vogt et al[10]).

the area of the ear in which the hair follicles were quantified. In principle, it should be noted that macroscopically the density of hairs at the edge of the ear is much higher. However, typically this area is not utilized for penetration studies, as the skin is not sufficiently flat within this area.[22]

In humans, the number of hair follicles likewise varies depending on the area of the body. On the calf region, on average a number of 14 hair follicles per cm^2 can be found, whereas a higher density can be determined in the region of the chest (25 hair follicles/cm^2), on the back (29 hair follicles/cm^2), on the arms (18-32 hair follicles/cm^2), on the thigh (17 hair follicles/cm^2), or on the abdomen (70 hair follicles/cm^2). The highest density has been described on the forehead with an average of 292 follicles/cm^2.[11,23] As most in vivo skin experiments are performed on the arm, it can be stated that with regard to follicular density, the porcine ear skin is well suitable as ex vivo skin model. Other models of animal skin are less suitable compared to human skin due to their much higher density of follicles per cm^2, which is, for example, 367 in dogs, 627 in cats, 1598 in rats, and approx. 1728 in rabbits.[15]

A further similarity between porcine skin and human skin is that the hair follicles undergo the same growth cycle and the hairs show the same growth stages.[16] At any given time, the vast majority of the hair follicle in healthy human scalp are considered to be in the anagen stage (80%-90%) and between 10% and 20% in the telogen one.[24] In the present study, 64% of the total number of follicles was found to be in the anagen stage and 36% were in the telogen phase. No data were found to compare our results with other studies in pig skin. These results are interesting for future skin penetration studies using pig skin as a model because in previous studies, it could be demonstrated that the growth stage of a hair follicle has an influence on the penetration process. It was shown that only hair follicles, which are in the anagen stage and thus show hair growth activity, are open for follicular penetration.[12]

As already described in the introduction part, different areas of the hair follicle are of specific interest for new therapeutic approaches. Selective targeting of these areas within the hair follicle can be reached by selecting a specifically sized drug delivery system such as nanocarriers.[25] Therefore, it is crucial to know the exact localization of the corresponding structure within the hair follicle. In principle, the structure of human and porcine hair follicles is comparable as both provide an infundibulum, an isthmus, a bulge region, and a bulb region. A sebaceous gland and an erector pili muscle are also associated with the hair follicles of both species.[25] It has not been investigated if these structures within the hair follicles are localized in the same position and have a comparable size in human and porcine hair follicles.

In the present study, we decided to classify the hair follicles into two groups (group 1: >2000 µm and group 2: <2000 µm), as

classification into terminal and vellus hairs as available for human hair follicles is not usual in pigs.

The average length for the hairs of group 1 (>2000 μm) was 2427 ± 275 μm, the average length for hairs of group 2 (<2000 μm) was 1458 ± 297 μm. In humans, the total length of terminal hairs in the anagen stage is even greater, with a mean dermal depth of approx. 3000 μm.[10] If we compare the results, we can see that in pig ear we can find hair follicles of great size just like in human skin. However, there is a variation in the length of the hair follicles of the pig skin, with an average length of 1820 ± 551 μm. In humans, the length of vellus hair follicles is smaller 646 ± 140 μm.[10] Vellus hairs, which are defined as short, non-pigmented and generally non-medullated hairs, were not found in the pig ear.[26]

The length of the infundibulum of the hair follicles in the anagen phase varied depending on the total length of the hair follicle. Hair follicles <2000 μm had an infundibulum length of approximately 373 ± 72 μm, hair follicles >2000 μm provided a longer infundibulum, with an average length of 516 ± 107 μm. These results are in concordance with previous studies in pigs, where the length of the infundibulum was approximately 500 μm.[16] In humans, the length of the infundibulum is comparable with an average value of 500 μm in terminal hairs, which are in the anagen phase. The length of the infundibulum in human vellus hair is 225 ± 34 μm.[10]

Due to the importance of the bulge region as a reservoir of stem cells, which participate in the process of proliferation and regeneration of both the hair follicles and the epidermis,[27] the position and length of the bulge region were also studied. In pig hair follicles, the bulge region was located at 881 ± 208 μm from the skin surface and in humans at 1191 ± 23 μm, which is well comparable. The length of the bulge region was also well comparable in porcine hair follicles and human hair follicles (272 ± 36 μm and 240 ± 52 μm).[10]

The follicular orifice on the skin surface also demonstrated similarity between porcine ear skin and human skin. Concerning the diameter of the entrance hole of the follicle in the infundibular area, in pigs, the opening diameter of the follicle was 113 ± 2 μm and in humans 172 ± 70 μm.

Additionally, the thickness of the follicular epithelium in the area of the infundibulum was determined and was shown to decrease continuously with increasing depth of the hair follicle. This phenomenon can also be observed in human follicles.[16] Data for human and porcine hair follicles were demonstrated to be relatively comparable (upper part of hair follicle: human 65 μm, pig: 59 μm; distal part of infundibulum: human 53 μm, pig: 46 μm.[10] The thickness of the interfollicular epidermis was approximately 100 μm in the present study. In other studies, also performed with pig skin, values of 72 μm were given.[16] However, both results are similar to human data available although the thickness of interfollicular epidermis also varies according to the region of the body: buttock 97 μm, shoulder 81 μm, scalp and retroauricular region 136 μm, forearm dorsal 75 μm.[10,28]

However, all studies confirmed that the thickness of the infundibular epithelium is lower than the thickness of the interfollicular epidermis with decreasing thickness in the lower part of the infundibulum. Therefore, it can be assumed that the barrier properties continuously decrease inside the hair follicles with increasing depth and that percutaneous absorption can be enhanced when substances reach deeper parts of the hair follicles.

5 | CONCLUSION

In summary, our results reveal that the morphometric characteristics of the central part of the porcine hair follicles have good similarity with the human hair follicles. Thus, it can be stated that the porcine ear skin is a good ex vivo skin model for skin penetration studies. Furthermore, it can be concluded that, apart from the follicular density, there are no significant differences in the investigated morphometric data of the hair follicles between the 3 areas of the central ear.

ACKNOWLEDGMENTS

We would like to thank Mr Gerald Nusche for providing the porcine ears for the experiments. Open access funding enabled and organized by ProjektDEAL.

ORCID

Anna Lena Klein https://orcid.org/0000-0002-8169-2541
Alexa Patzelt https://orcid.org/0000-0002-8350-6757

REFERENCES

1. Vogt A, Blume-Peytavi U. Die Biologie des menschlichen Haarfollikels. *Der Hautarzt*. 2003;54(8):692-698.
2. Lademann J, Richter H, Meinke M, Sterry W, Patzelt A. Which skin model is the most appropriate for the investigation of topically applied substances into the hair follicles? *Skin Pharmacol Physiol*. 2010;23(1):47-52.
3. Wosicka H, Cal K. Targeting to the hair follicles: current status and potential. *J Dermatol Sci*. 2010;57(2):83-89.
4. Otberg N, Teichmann A, Rasuljev U, Sinkgraven R, Sterry W, Lademann J. Follicular penetration of topically applied caffeine via a shampoo formulation. *Skin Pharmacol Physiology*. 2007;20(4):195-198.
5. Mak WC, Patzelt A, Richter H, et al. Triggering of drug release of particles in hair follicles. *J Controlled Release*. 2012;160(3):509-514.
6. Grazu V, Moros M, Sánchez-Espinel C. Nanocarriers as nanomedicines: design concepts and recent advances. *Front Nanosci*. Elsevier; 2012;4:337-440.
7. Nohynek GJ, Lademann J, Ribaud C, Roberts MS. Grey goo on the skin? Nanotechnology, cosmetic and sunscreen safety. *Crit Rev Toxicol*. 2007;37(3):251-277.
8. Lademann J, Patzelt A, Schanzer S, et al. Non-invasive analysis of penetration and storage of isoconazole nitrate in the stratum corneum and the hair follicles. *Eur J Pharm Biopharm*. 2012;80(3):615-620.
9. Patzelt A, Richter H, Knorr F, et al. Selective follicular targeting by modification of the particle sizes. *J Controlled Release*. 2011;150(1):45-48.
10. Vogt A, Hadam S, Heiderhoff M, et al. Morphometry of human terminal and vellus hair follicles. *Exp Dermatol*. 2007;16(11):946-950.

11. Hueber F, Schaefer H, Wepierre J. Role of transepidermal and transfollicular routes in percutaneous absorption of steroids: in vitro studies on human skin. *J Skin Pharmacol Physiol*. 1994;7(5):237-244.
12. Lademann J, Otberg N, Jacobi U, Hoffman RM, Blume-Peytavi U. Follicular penetration and targeting. *J Investig Dermatol Symp Proc*. 2005;10(3):301-303.
13. Jacobi U, Toll R, Sterry W, Lademann J. Do follicles play a role as penetration pathways in in vitro studies on porcine skin?-: An optical study. *Laser Phys*. 2005;15(11):1594-1598.
14. Patzelt A, Richter H, Buettemeyer R, et al. Differential stripping demonstrates a significant reduction of the hair follicle reservoir in vitro compared to in vivo. *Eur J Pharm Biopharm*. 2008;70(1):234-238.
15. Mangelsdorf S, Vergou T, Sterry W, Lademann J, Patzelt A. Comparative study of hair follicle morphology in eight mammalian species and humans. *Skin Research Technology*. 2014;20(2):147-154.
16. Jacobi U, Kaiser M, Toll R, et al. Porcine ear skin: an in vitro model for human skin. *Skin Research Technology*. 2007;13(1):19-24.
17. Frum Y, Eccleston GM, Meidan VM. In-vitro permeation of drugs into porcine hair follicles: is it quantitatively equivalent to permeation into human hair follicles? *J Pharm Pharmacol*. 2008;60(2):145-151.
18. Schmook FP, Meingassner JG, Billich A. Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption. *Int J Pharm*. 2001;215(1-2):51-56.
19. Alonso L, Fuchs E. The hair cycle. *J Cell Sci*. 2006;119(119):391-393.
20. Wang X, Shi Y, Zhou Q, Liu X, Xu S, Lei T. Detailed histological structure of human hair follicle bulge region at different ages: a visible niche for nesting adult stem cells. *J Huazhong Univ Sci Technol*. 2012;32(5):648-656.
21. Turner NJ, Pezzone D, Badylak SF. Regional variations in the histology of porcine skin. *Tissue Eng Part C Methods*. 2015;21(4):373-384.
22. Meyer W, Kacza J, Zschemisch N-H, Godynicki S, Seeger J. Observations on the actual structural conditions in the stratum superficiale dermidis of porcine ear skin, with special reference to its use as model for human skin. *J Ann Anat-Anatomischer Anzeiger*. 2007;189(2):143-156.
23. Otberg N, Richter H, Schaefer H, Blume-Peytavi U, Sterry W, Lademann J. Variations of hair follicle size and distribution in different body sites. *J Investig Dermatol*. 2004;122(1):14-19.
24. Oh JW, Kloepper J, Langan EA, et al. A guide to studying human hair follicle cycling in vivo. *J Investig Dermatol*. 2016;136(1):34-44.
25. Patzelt A, Knorr F, Blume-Peytavi U, Sterry W, Lademann J. Hair follicles, their disorders and their opportunities. *Drug Discovery Today Dis Mechanisms*. 2008;5(2):e173-e181.
26. Erdogan B. Anatomy and Physiology of Hair. 2017.
27. Ohyama M. Hair follicle bulge: a fascinating reservoir of epithelial stem cells. *J Dermatol Sci*. 2007;46(2):81-89.
28. Sandby-Moller J, Poulsen T, Wulf HC. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. *Acta Derm Venereol*. 2003;83(6):410-413.

How to cite this article: Zambrano A, Klein AL, Patzelt A. Analysis of the morphometric parameters of pig ear hair follicles. *Skin Res Technol*. 2021;27:730–738. https://doi.org/10.1111/srt.13008