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Follicular Delivery of Caffeine from a Shampoo for Hair Retention

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Abstract: A key factor in the prevention of hair loss is the provision of optimal conditions on the scalp. In this regard, reduction of oxidative stress on the scalp is one critical requirement to support the hair follicles to function optimally. Recently, a novel shampoo formulation technology containing anti-oxidants such as piroctone olamine has been demonstrated to improve hair retention based on micellar degradation and coacervation effects. Caffeine has also been shown to exhibit anti-oxidant activity including the ability to inhibit lipid peroxidation. As with piroctone olamine, it is expected that follicular delivery of caffeine will enhance its anti-oxidant activity in a region that will be beneficial for hair retention. In this study, two shampoo formulations as well as a control formulation were applied to the calf area of $n = 9$ male participants. The technique of differential tape stripping was applied to obtain the caffeine penetrated to the stratum corneum and to the hair follicles. Isotope-dilution liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was performed to demonstrate caffeine follicular delivery from the shampoo formulas. The results showed that the percentage of caffeine recovered in the hair follicles was 8–9% of the caffeine absorbed into the skin and matched an existing caffeine-based shampoo. In conclusion, a novel shampoo formulation technology has been developed that effectively delivers beneficial anti-oxidants to improve hair retention. This new shampoo is expected to be especially useful in the goal of retaining hair during aging.

Keywords: hair loss; caffeine; shampoo; hair follicles

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1. Introduction

Hair loss is a problem that essentially all humans face. It may be genetically determined, associated with a disease or simply a function of normal human aging [1,2]. As early as our 30s, the amount of hair on our heads begins to decline [3]. Focusing on age-related hair thinning, the efficiency of replacement of normally shed hairs decreases. The cumulative effect of incomplete shed hair replacement is initially hard to perceive, but increasing scalp (especially part line) visibility as well as the feel of fullness eventually become apparent.

Once hair loss becomes apparent, significant loss has already occurred. While there are potentially successful drug treatments to reverse hair loss [4–6], prevention of initial

loss is likely a more effective means of maintaining hair amount through aging. One key factor in loss prevention is providing optimal scalp conditions for hair to grow, be optimally retained and replaced after normal shedding [7]. Oxidative stress can negatively influence normal hair growth and impede its retention [8,9].

Cosmetic treatments are needed to enable optimum scalp conditions and support the hair follicles to function optimally. Reduction of oxidative stress on the scalp is one critical requirement as it has been shown [10] that oxidative stress occurring on the scalp is transmitted to the pre-emergent hair fiber leading to impaired structure and function of that fiber [11].

The oxidative stress experienced by the scalp can have both exogenous and endogenous origins. One manifestation of this stress is the generation of per-oxidized lipids (including those of linoleic acid and squalene [12]). Commensal *Malassezia* yeasts are known to be capable of causing lipid oxidation [13] and are also known to reside in the hair follicle [14,15]. Thus, it is likely that the *Malassezia* metabolic activity that leads to lipid peroxidation may be especially detrimental to hair since it occurs within the follicle.

A novel shampoo formulation technology has been developed [16] to aid the delivery of benefit agents to the scalp surface as well as the follicle. This technology is based on a decreased micellar stability, which leads to a weaker association between actives and micelles as well as coacervation due to an increased charge density of cationic polymers.

The advanced delivery technology was shown to effectively deliver piroctone olamine to the scalp surface as well as the hair follicle. This material was shown to reduce scalp oxidative stress and was also demonstrated to improve hair retention [17].

Caffeine has also been shown to exhibit anti-oxidant activity [18,19], including the ability to inhibit lipid per-oxidation [20]. As with piroctone olamine, it is expected that follicular delivery of caffeine will enhance its anti-oxidant activity in a region that will be beneficial for hair retention.

Caffeine follicular delivery has been evaluated previously [21–23] by a number of methods; we have used herein differential tape stripping [24,25] and cyanoacrylate biopsy [26,27] techniques with LC-MS/MS quantitation to demonstrate caffeine follicular delivery from this new shampoo technology, thereby delivering dual anti-oxidants (caffeine and piroctone olamine) to the hair follicle.

2. Materials and Methods

2.1. Shampoo Formulations

The shampoo formulations P, C and K are commercially available products with the compositions summarized in Table 1. In the context of the present study, formulation P had the function of a control, while formulation C contained caffeine and formulation K contained caffeine and piroctone olamine. The caffeine contained in the formulations was of standard commercial grade.

Composition K is based on a unique formulation technology enabling increased deposition efficiency as presented in [16].

No information about the composition and the caffeine content of the formulations was supplied to the investigators since this study was placebo-controlled and investigator-blinded.

2.2. Study Characteristics and Volunteers

For the in vivo studies, ethics approval was granted by the Ethics Committee of the Charité-Universitätsmedizin Berlin (EA4/042/22). All procedures complied with the Declaration of Helsinki; the study is registered in the German Clinical Trials Register (DRKS00029226). The study was executed as a randomized placebo-controlled trial on 10 volunteers at Charité-Universitätsmedizin Berlin, Department of Dermatology, Venereology und Allergology, Berlin. The study was blinded for all subjects and the investigator. The criteria for inclusion were: Male participants between 18 and 60 years with a Fitzpatrick skin type between I and III. All participants were free of pathological skin conditions, had given their written informed consent and had been informed about the aims of the

study and the nature of the examinations. Persons with tattoos in the area of the calves and persons who cannot make decisions on their own responsibility were excluded from this study.

Table 1. Summary of shampoo compositions used in the present study.

	Formula P	Formula C	Formula K
Caffeine (%)	None	1%	1%
Surfactants			
Sodium Alkyl Sulfates	X	X	X
Cocamidopropyl Betaine	X		X
Disodium Laureth Sulfosuccinate		X	
Sodium Lauroyl Glutamate		X	
Nonionic Surfactants	X	X	
Hair Benefit Agents			
Panthenol, Panthenyl Ethyl Ether	X	X	X
Hydrolyzed Wheat Protein		X	
Skin Benefit Agents			
Niacinamide		X	X
Tocopherol		X	
Zinc PCA		X	
Glycerin	X		
Piroctone Olamine			X
Ginger Root Extract			X
Cationic Polymer Deposition Aids	X	X	X
Sensates			
Menthol		X	

2.3. Experimental Setup and Application of the Formulations

In total, 4 areas of 6 cm × 6 cm (2 areas per calf) were marked with a waterproof pencil. The hairs within the marked areas were shortened previously by using surgical scissors. The shampoo formulations P, C and K were applied in a randomized manner at a concentration of 2 mg/cm^2 by using an insulin syringe. The fourth area remained untreated. For the application of the formulations, the index finger of the investigator was covered with a latex finger cot. The finger cot was subsequently moistened by dipping the finger into water. Afterwards, the formulation was massaged for 2 min at a frequency of 250 beats per minute using a metronome app (Pro Metronome, EUMLab Xanin Technology GmbH, Berlin, Germany). The skin area was then slowly and homogenously rinsed with 100 mL of lukewarm water without touching the skin. Subsequently, the area was blow dried with cold air.

2.4. Differential Stripping of the Treatment Areas

A Teflon film was applied to the skin to cover the area around a defined cutout for the tape stripping procedure. Subsequently, the skin was stripped once using adhesive tape (tesafilm®, Tesa, Norderstedt, Germany) to remove excess formulation. Then, 50 tape strips followed by one cyanoacrylate tape strip were performed (Figure 1) and the tapes were affixed to slide frames. The number of tape strips was determined in a preliminary experiment, in which it was observed that the caffeine recovery did not show any significant change in this range as a function of the number of tape strips. The tape stripping procedure [24] was performed by pressing the adhesive tapes onto the skin using a rubber roller weighing about 700 g. This roller was moved 10 times back and forth with a paper towel between the taped area and the roller. Subsequently, the tape was removed from the skin in one swift movement. For the cyanoacrylate tape strip, a small amount of cyanoacrylate

glue (UHU® Sekundenkleber, UHU GmbH, Bühl, Germany) was homogeneously applied onto the area within the Teflon foil. After that, an adhesive tape was pressed onto the glue and fixed by moving the roller 10 times back and forth. After 5 min, the tape was removed in one swift movement.

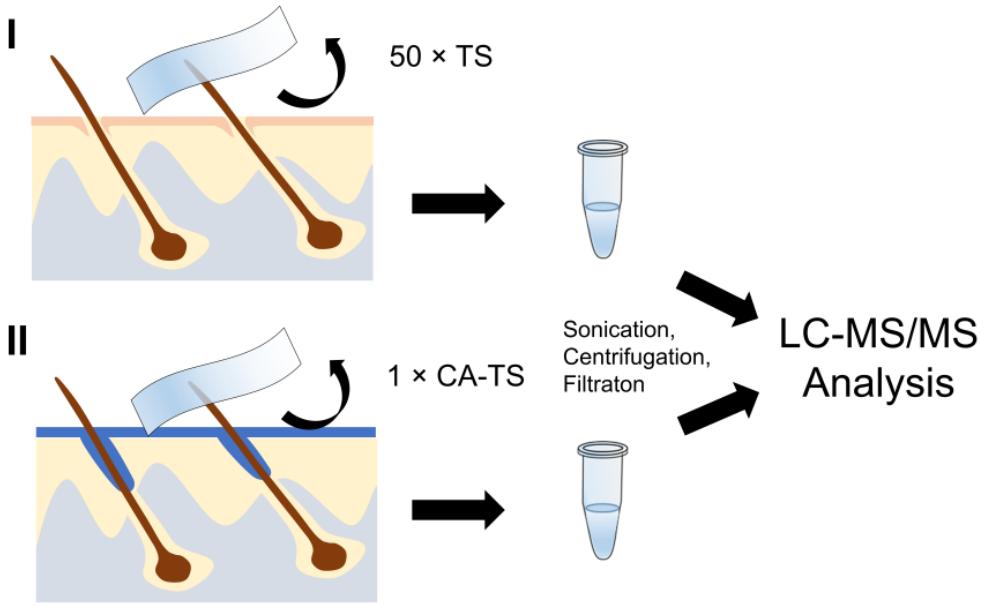

Figure 1. Scheme for the representation of the methodical procedure. Firstly, 50 tape strips (TS) were taken after skin treatment and the tapes were dissolved in ethanol (**I**). Then, one cyanoacrylate tape strip (CA-TS) was taken and the tape was transferred into ethanol (**II**). Both solutions were sonicated, centrifuged and filtered. Subsequently, caffeine content was determined by isotope-dilution liquid chromatography coupled with tandem-mass spectrometry (LC-MS/MS).

2.5. Extraction of Caffeine from the Tapes

2.5.1. Cyanoacrylate Tape Strip

The tape was removed from the slide frame and the edges were cut off with a scalpel resulting in a tape of 3.4 × 1.9 cm^2 in size. Each tape was then cut into 10 pieces to enable the extraction in a low amount of ethanol. The individual parts were placed in a 2 mL Eppendorf tube® and filled with 2 mL ethanol (Uvasol® Ethanol for spectrometry, Merck KGaA, Darmstadt, Germany) followed by an ultrasonic bath for 10 min and centrifugation for further 10 min at 1920× *g* and 20 °C (Universal 320 R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The supernatant was removed using a Pasteur pipette and transferred to another 2 mL Eppendorf tube®. The tubes were sealed with para film and stored at −20 °C until further analysis.

2.5.2. Stratum Corneum Tape Strips

To extract caffeine from the 50 tape strips of one area, falcon tubes of 50 mL were filled with 15 mL ethanol for spectrometry.

The edges of the tape were then removed using a scalpel and the 3.4 × 1.9 cm^2 tapes were cut into four pieces each. The first piece of tape was transferred into the 50 mL tube using tweezers and the tube was closed. The tube was then gently shaken to wet the piece with ethanol. The same procedure was repeated with pieces 2–4 of the tape. This prevented the individual pieces from sticking together. In this way, all 50 tapes were dissolved. At the end, the solution was shaken using a vortex device.

After all 4 samples of 50 tapes had been sunk in the 50 mL tubes, the tubes were placed into the ultrasonic bath for 10 min and then centrifuged at 1920× *g* and 20 °C for 10 min. The supernatant of the centrifuged solutions was filtered with a cell sieve (MACS® SmartStrainers, 70 µm, Miltenyi Biotec B.V. & Co. KG, Teterow, Germany) and

transferred to new 50 mL tubes. In this way, any residue of the dissolved adhesive film could be collected in the cell sieve. Using a pipette, approximately 1.8 mL of the solution was transferred into 2 mL Eppendorf tubes®, sealed with para film, and stored at −20 °C for further analysis.

2.6. Analysis of Caffeine Content by Isotope-Dilution Liquid Chromatography Coupled with Tandem-Mass Spectrometry (LC-MS/MS)

LC-MS/MS was performed to determine the caffeine content in the hair follicles and stratum corneum of the subjects after each treatment. Frozen extracts of tape-stripped skin were thawed at room temperature, ultrasonicated on ice for 10 min and centrifuged at 3000× *g* for 3 min (4 °C). Subsequently, 45 µL of each sample was mixed with 5 µL of the internal standard d_9-caffeine (100 nM in methanol) (CDN Isotopes, Pointe-Claire, Canada). Samples were vortexed and aliquots were subjected to LC-MS/MS caffeine quantification applying the multiple reaction monitoring (MRM) approach. Chromatographic separation was achieved on a 1290 Infinity II HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a Poroshell 120 EC-C18 column (3.0 × 150 mm, 2.7 µm; Agilent Technologies) guarded by a pre-column (3.0 × 5 mm, 2.7 µm) of identical material. Water (eluent A) and acetonitrile (eluent B), both acidified with 0.1% formic acid, were pumped with 0.4 mL/min. Elution of caffeine and its internal standard was achieved with a 10-min linear gradient from 5% to 90% eluent B. Total run-time was 16 min including re-equilibration of the LC system. MS/MS analyses were carried out using an Ultivo (G6465B) triple-quadrupole mass spectrometer (Agilent Technologies) operating in the positive electrospray ionization mode (ESI+). The following ion source parameters were set: sheath gas temperature, 350 °C; sheath gas flow, 12 L/min of nitrogen; nebulizer pressure, 50 psi; drying gas temperature, 100 °C; drying gas flow, 7 L/min of nitrogen; capillary voltage, 2.5 kV; and nozzle voltage, 0 kV. The following mass transitions were recorded (fragmentor voltage [FV] and collision energies [CE] in parentheses): caffeine (example presented in Figure 2A): m/z 195.1→42.1 (FV: 112 V, CE: 40 eV), m/z 195.1→109.9 (FV: 112 V, CE: 24 eV), m/z 195.1→138.0 (FV: 112 V, CE: 20 eV, quantifier); d_9-caffeine (example presented in Figure 2B): m/z 204.1→45.1 (FV: 128 V, CE: 40 eV), m/z 204.1→116.0 (FV: 128 V, CE: 24 eV), m/z 204.1→144.0 (FV: 128 V, CE: 20 eV, quantifier). Peak areas were determined with Mass-Hunter Software (Agilent Technologies) and caffeine was directly quantified via its internal standard d_9-caffeine that was concentrated to 10 nM in the samples.

2.7. Statistical Analysis

Mean value comparisons were performed using SPSS for Windows (IBM Corp., Armonk, NY, USA), considering $p \leq 0.05$ as significant. The data set was checked for outliers to be excluded from the analysis. Outliers were present if the values reached the third quartile plus factor 1.5 of the interquartile range or the first quartile minus factor 1.5 of the interquartile range. Next, the normal distribution of the data set was checked utilizing the Shapiro–Wilk test. In the case of multiple groups, the Friedman test was conducted. Multiple comparisons with a Bonferroni–Holm adjustment were executed in the framework of a post hoc analysis. In the case of two groups, either the Wilcoxon test (non-normal distribution) or a *t*-test for associated samples was utilized.

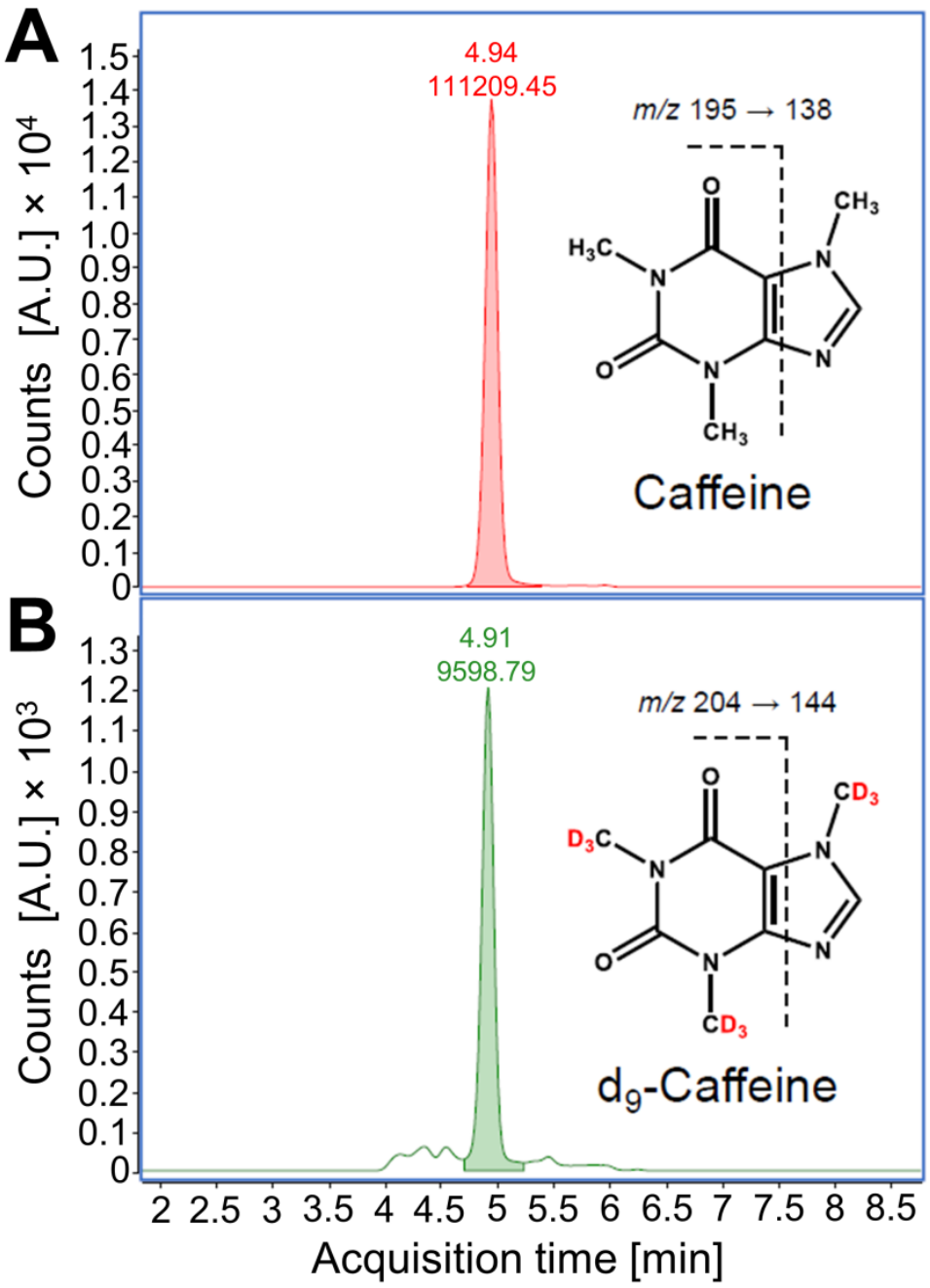

Figure 2. Representative LC-MS/MS peak examples of the analyte caffeine (**A**) and the internal standard d_9-caffeine (**B**) showing abundance at approximately 5 min acquisition time. The quantifier mass transition is given along with the structural formulas of caffeine and the internal standard d_9-caffeine as inset.

3. Results

In the framework of the retrospective data analysis, the caffeine concentration found in the stratum corneum of volunteer 6 showed a remarkably high value after application of formulation C (>50 nmoL/cm^2). An outlier analysis revealed that the amount of caffeine found in the stratum corneum of volunteer 6 was outside the rest of the value distribution (the third quartile plus factor 1.5 of the interquartile range was reached). Therefore, we excluded volunteer 6 from all further statistical tests and evaluated the caffeine amounts for n = 9 volunteers.

In Figure 3 and Table 2, it is shown that no significant amounts of caffeine were found either for the untreated area or after treatment with formulation P. However, the caffeine concentration was significantly increased in the stratum corneum and in the hair follicles after application of formulations C and K compared to the untreated area and formulation P. Formulations C and K delivered caffeine equivalently effectively. We assume that the cyanoacrylate tape stripping only removes the content of the hair follicles. Preliminary investigations on two excised human skin samples have shown that the concentration in the removed tapes > 50 was comparable to the ones from untreated skin.

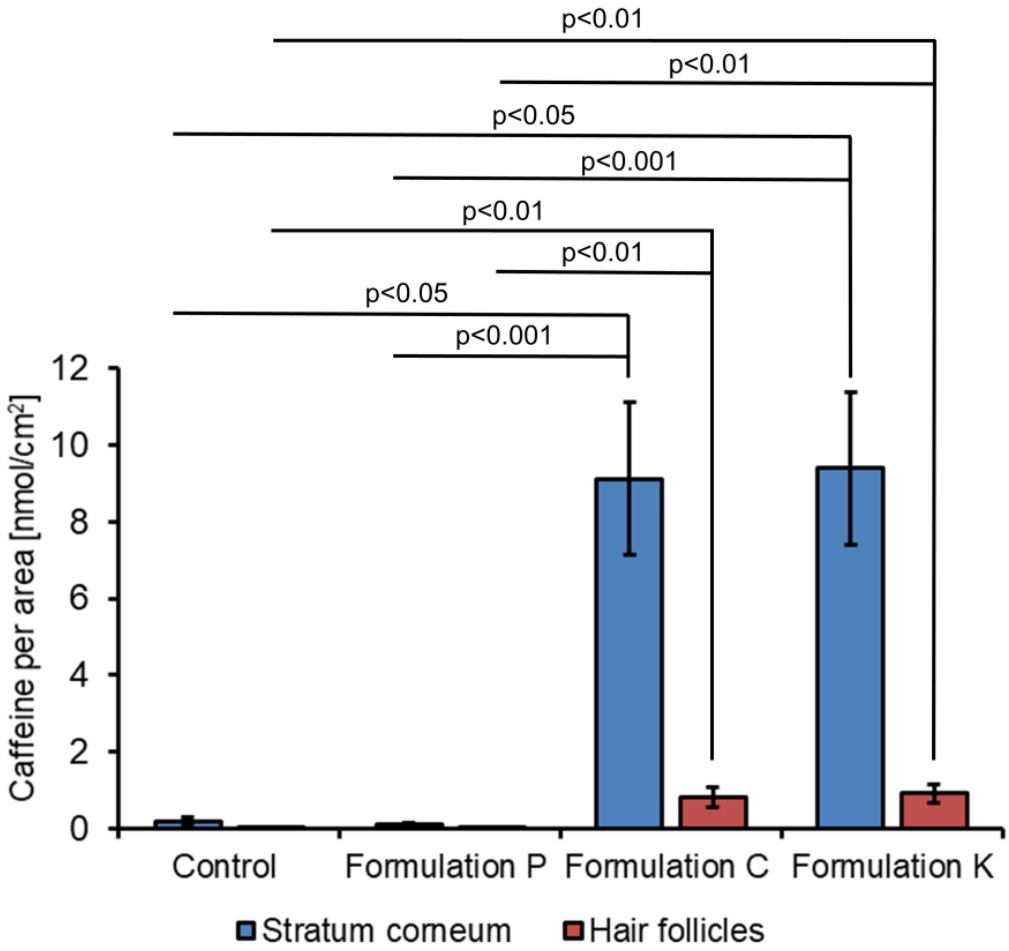

Figure 3. The area-related caffeine concentration in nmoL/cm^2 is depicted for the stratum corneum (blue) and the hair follicles (red) for the control (untreated area) as well as formulations P, C and K. Significantly increased concentrations of caffeine in the stratum corneum and in the hair follicles were found after application of formulation C and K. No significant differences were found between formulation C and K. Data are shown as mean values ± SEM of n = 9 volunteers with corresponding p-values between the different groups.

Table 2. Summary of the caffeine delivery data showing mean values ± SEM.

	Control	**Formula P**	**Formula C**	**Formula K**
Caffeine Level (nmoL/cm^2)				
Stratum corneum	0.18 ± 0.11	0.09 ± 0.03	9.13 ± 1.98	9.40 ± 1.99
Hair Follicles	0.01 ± 0.01	0.02 ± 0.01	0.80 ± 0.26	0.91 ± 0.23

Furthermore, the averaged ratios between the caffeine amount in the hair follicles and in the stratum corneum (Figure 4A) or the hair follicles and the total amount (Figure 4B)

were the same for formulations C and K. From this it can be assumed that there was no significant difference between both formulations regarding the anatomical distribution of the caffeine.

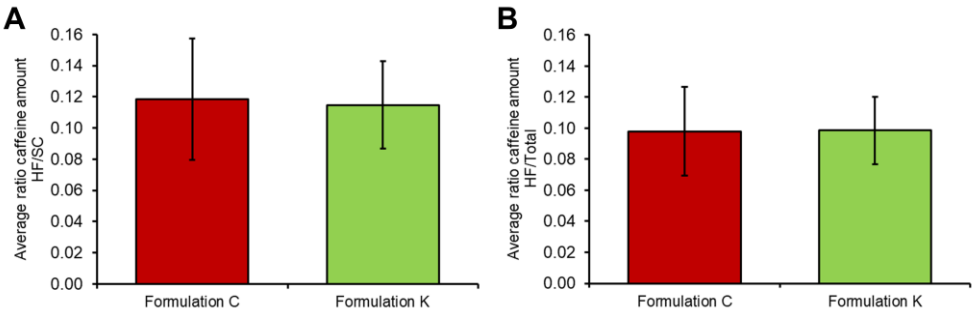

Figure 4. (**A**): The averaged ratios in caffeine amount between hair follicles (HF) and stratum corneum (SC) are shown for formulation C and K. (**B**) shows the averaged ratios in caffeine amount between hair follicles (HF) and total caffeine amount for both formulations. No significant differences between the ratios were found. Data are shown as mean values ± SEM of $n = 9$ volunteers.

The total proportion of caffeine absorbed from the formulation into the skin was 9.6 ± 1.9% for formulation C and 10.0 ± 2.1% for formulation K. The amount of caffeine from the formulation that penetrated into the hair follicles was 0.78 ± 0.25% (Formulation C) and 0.88 ± 0.23% (Formulation K), respectively. Thus, 8–9% of the total recovered amount of caffeine was present in the hair follicles (Figure 5). Since the calculation of the average ratio of the caffeine amount in the hair follicles and the total caffeine (Figure 4B) was based on averaging the single ratios for each volunteer in order to obtain a SEM, the values are slightly differing (approximately 10% in Figure 4B).

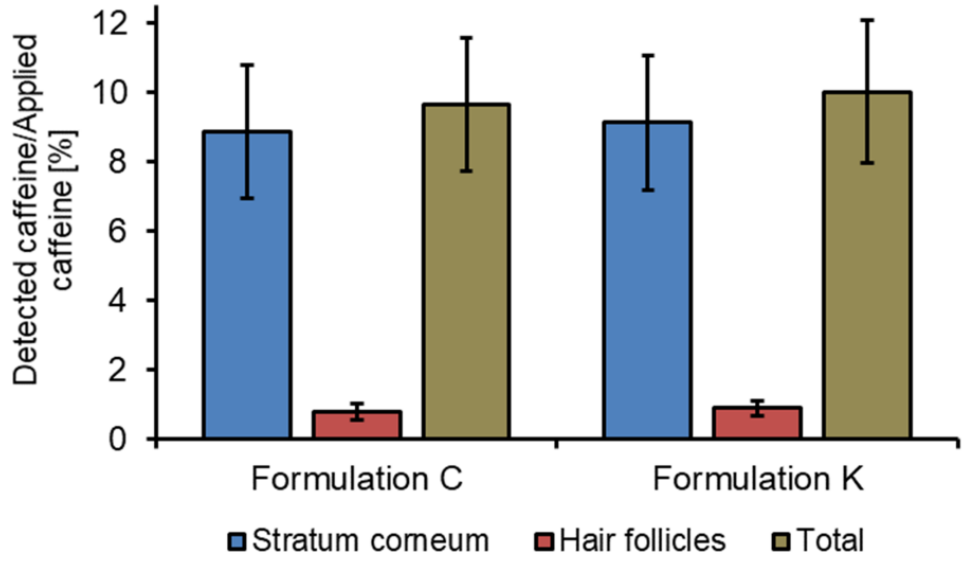

Figure 5. The proportion of caffeine recovered from the shampoo formulations is depicted for the stratum corneum (blue), the hair follicles (red) and in total (green) for the formulations C and K. No significant differences were found between formulations C and K; 8.1% (C) and 8.8% (K) of the total recovered amount of caffeine was present in the hair follicles. Data are shown as mean values ± SEM of $n = 9$ volunteers.

4. Discussion

Hair loss often leads to anxiety and distress [28]. Since age-related hair loss can begin in the early 30s [3], effective products are needed to reduce premature hair loss. Because this is a long-term chronic problem requiring continuous preventative treatment, convenient

solutions are required. The most convenient product form is a shampoo to avoid the requirement for new usage habits and practices. The technological challenge with the shampoo form is that it is primarily designed to remove materials (i.e., clean), not deliver benefit agents. Development of effective hair retention shampoos requires such products to leave behind specific benefit agents both on the scalp and in the hair follicle to achieve the effects that lead to the benefits.

Premature hair loss can be initiated as a result of excessive scalp oxidative stress [8,9]. Thus, a proven approach for improving hair retention from shampoos is the delivery of benefit agents that reduce scalp, and consequently hair, oxidative stress [17,29]. A new shampoo formulation technology has been developed to increase the efficiency of benefit agent delivery, which has demonstrated improved delivery of one such benefit agent, piroctone olamine, to the scalp and follicle [16]. The underlying mechanism here was decreased micellar stability as well as coacervation due to increased charge density of cationic polymers, which led to a weaker association between the agent and micelles, resulting in more efficient agent retention on the scalp surface and delivery to the hair follicles.

As caffeine is also an effective anti-oxidant [18,20], we sought to determine whether the new shampoo formulation technology could effectively deliver it to the hair follicle as well. Caffeine follicular delivery has been demonstrated from a conventional shampoo previously [21–23].

In addition to piroctone olamine and caffeine, the formulations contained several other cosmetic ingredients known for their beneficial effects on skin and hair (Table 1). Niacinamide is known for its anti-inflammatory and barrier-stabilizing effects [30] and hydrolyzed wheat protein has conditioning effects on the skin and hair based on ionic interactions [31]. The further contained panthenol also bears hair- and skin-conditioning functions [32]. Tocopherol is well-known for its antioxidative effects in skin [33], while glycerin is used as a humectant [34].

By using differential tape stripping [24] and cyanoacrylate biopsy [26] techniques, we have shown that the new shampoo formulation technology (formula K) also effectively delivers caffeine to both the stratum corneum and the hair follicle. Delivery was as effective as a conventional commercial shampoo with 1% caffeine (formula C) while the control shampoo that did not contain caffeine (formula P) resulted in no caffeine recovery.

In the present study, 8–9% of the total recovered amount of caffeine could be found in the hair follicles. Teichmann et al. [24] investigated the penetration behavior of fluorescein sodium by differential stripping and fluorescence spectroscopy on the backs of eight volunteers. In this study, the intrafollicular proportion of the total amount recovered was 5%. However, the comparability of both substances and methods is limited. Although the percentage of follicular orifices on the skin surface is comparable for the back and the calf region according to Otberg et al. [30], the infundibular volume of the hair follicles in the calf region is significantly higher, which could explain the higher intrafollicular proportion of caffeine in the present study. Furthermore, hair follicle density on the scalp is much higher compared to the calf, and hair follicle size is comparable [35]. Thus, the proportion could be enhanced for the hair follicle penetrated amount at the scalp.

This work has demonstrated the effective delivery of caffeine from a novel shampoo composition which also effectively delivers the second anti-oxidant piroctone olamine. We would expect that such a product will be especially beneficial to help solve the near-universal human problem of hair loss with age.

5. Conclusions

Novel shampoo formulation technology has been developed that effectively delivers beneficial anti-oxidants to improve hair retention. This formula has been previously shown to effectively deliver piroctone olamine to the scalp and follicles. Data from the current study show coincident effective delivery of caffeine to the skin surface and follicles. The percentage of caffeine recovered in the hair follicles was 8–9% of the caffeine absorbed into

the skin and matched an existing caffeine-based shampoo. This new shampoo is expected to be especially useful in the goal of retaining hair during aging.

Author Contributions: Conceptualization, J.R.S., K.P. and M.C.M.; methodology, H.R., S.B.L., F.S., B.K. and M.C.M.; formal analysis, L.B.; investigation, L.B., A.L.K., S.S., H.R. and F.S.; resources, J.R.S. and K.P.; data curation, L.B. and F.S.; writing—original draft preparation, L.B., J.R.S., K.P. and F.S.; writing—review and editing, L.B., A.L.K., J.R.S., K.P., H.R., S.S., S.B.L., F.S., B.K. and M.C.M.; visualization, L.B., A.L.K. and F.S.; supervision, M.C.M. and B.K.; project administration, L.B., A.L.K., S.B.L. and M.C.M.; funding acquisition, A.L.K. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin (EA4/042/22; Date of final approval: 21 April 2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data are available from the corresponding author upon reasonable request.

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Conflicts of Interest: J.R.S. and K.P. are employees of The Procter & Gamble Company. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Mulinari-Brenner, F.; Bergfeld, W.F. Hair loss: An overview. *Dermatol. Nurs.* **2001**, *13*, 269. [PubMed]
2. Alessandrini, A.; Bruni, F.; Piraccini, B.M.; Starace, M. Common causes of hair loss–clinical manifestations, trichoscopy and therapy. *J. Eur. Acad. Dermatol. Venereol.* **2021**, *35*, 629–640. [CrossRef] [PubMed]
3. Robbins, C.; Mirmirani, P.; Messenger, A.G.; Birch, M.P.; Youngquist, R.S.; Tamura, M.; Filloon, T.; Luo, F.; Dawson, T.L., Jr. What women want–quantifying the perception of hair amount: An analysis of hair diameter and density changes with age in Caucasian women. *Br. J. Dermatol.* **2012**, *167*, 324–332. [CrossRef] [PubMed]
4. Otberg, N.; Restrepo, I.; Shapiro, J. Possibilities and limitations for reversal of age-related hair loss. In *Aging Hair*; Springer: Berlin/Heidelberg, Germany, 2010; pp. 193–205.
5. Kaufman, K.D.; Olsen, E.A.; Whiting, D.; Savin, R.; DeVillez, R.; Bergfeld, W.; Price, V.H.; Van Neste, D.; Roberts, J.L.; Hordinsky, M.; et al. Finasteride in the treatment of men with androgenetic alopecia. *J. Am. Acad. Dermatol.* **1998**, *39*, 578–589. [CrossRef]
6. E Buhl, A.; Waldon, D.J.; A Baker, C.; A Johnson, G. Minoxidil sulfate is the active metabolite that stimulates hair follicles. *J. Investig. Dermatol.* **1990**, *95*, 553–557. [CrossRef]
7. Tosti, A.; Schwartz, J.R. Role of scalp health in achieving optimal hair growth and retention. *Int. J. Cosmet. Sci.* **2021**, *43*, S1–S8. [CrossRef]
8. Trüeb, R.M. Oxidative stress and its impact on skin, scalp and hair. *Int. J. Cosmet. Sci.* **2021**, *43*, S9–S13. [CrossRef]
9. Trüeb, R.M.; Henry, J.P.; Davis, M.G.; Schwartz, J.R. Scalp condition impacts hair growth and retention via oxidative stress. *Int. J. Trichol.* **2018**, *10*, 262. [CrossRef]
10. Schwartz, J.R.; Henry, J.P.; Kerr, K.M.; Mizoguchi, H.; Li, L. The role of oxidative damage in poor scalp health: Ramifications to causality and associated hair growth. *Int. J. Cosmet. Sci.* **2015**, *37* (Suppl. S2), 9–15. [CrossRef]
11. Schwartz, J.R.; Henry, J.P.; Kerr, K.M.; Flagler, M.J.; Page, S.H.; Redman-Furey, N. Incubatory environment of the scalp impacts pre-emergent hair to affect post-emergent hair cuticle integrity. *J. Cosmet. Dermatol.* **2018**, *17*, 105–111. [CrossRef]
12. Niki, E. Lipid oxidation in the skin. *Free Radic. Res.* **2015**, *49*, 827–834. [CrossRef] [PubMed]
13. Ambaw, Y.A.; Pagac, M.P.; Irudayaswamy, A.S.; Raida, M.; Bendt, A.K.; Torta, F.T.; Wenk, M.R.; Dawson, T.L., Jr. Host/malassezia interaction: A quantitative, non-invasive method profiling oxylipin production associates human skin eicosanoids with malassezia. *Metabolites* **2021**, *11*, 700. [CrossRef] [PubMed]
14. Meyer, L.E.; Otberg, N.; Tietz, H.-J.; Sterry, W.; Lademann, J. In vivo imaging of Malassezia yeasts on human skin using confocal laser scanning microscopy. *Laser Phys. Lett.* **2005**, *2*, 148–152. [CrossRef]
15. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The human skin microbiome. *Nat. Rev. Microbiol.* **2018**, *16*, 143–155. [CrossRef] [PubMed]

16. Johnson, E.S.; Chang, D.W.; Schwartz, J.R.; Blume-Peytavi, U.; Henry, J.P.; Caterino, T.L.; Talley, A.L. Enhanced piroctone olamine retention from shampoo for superior anti-dandruff efficacy. *Int. J. Cosmet. Sci.* **2023**, *45*, 236–245. [CrossRef]
17. Davis, M.G.; Piliang, M.P.; Bergfeld, W.F.; Caterino, T.L.; Fisher, B.K.; Sacha, J.P.; Carr, G.J.; Moulton, L.T.; Whittenbarger, D.J.; Punyani, S.; et al. Scalp application of the antioxidant piroctone olamine reduces hair shedding in an 8-week randomized, double-blind, placebo-controlled clinical study. *Int. J. Cosmet. Sci.* **2021**, *43*, S26–S33. [CrossRef]
18. Shi, X.; Dalal, N.S.; Jain, A.C. Antioxidant behavior of caffeine: Efficient scavenging of hydroxyl radicals. *Food Chem. Toxicol.* **1991**, *29*, 1–6. [CrossRef]
19. Herman, A.; Herman, A.P. Caffeine's mechanisms of action and its cosmetic use. *Skin Pharmacol. Physiol.* **2012**, *26*, 8–14. [CrossRef]
20. Devasagayam, T.; Kamat, J.; Mohan, H.; Kesavan, P. Caffeine as an antioxidant: Inhibition of lipid peroxidation induced by reactive oxygen species. *Biochim. Biophys Acta* **1996**, *1282*, 63–70. [CrossRef]
21. Otberg, N.; Teichmann, A.; Rasuljev, U.; Sinkgraven, R.; Sterry, W.; Lademann, J. Follicular penetration of topically applied caffeine via a shampoo formulation. *Skin Pharm. Physiol.* **2007**, *20*, 195–198. [CrossRef] [PubMed]
22. Teichmann, A.; Richter, H.; Knorr, F.; Antoniou, C.; Sterry, W.; Lademann, J. Investigation of the penetration and storage of a shampoo formulation containing caffeine into the hair follicles by in vivo laser scanning microscopy. *Laser Phys. Lett.* **2007**, *4*, 464–468. [CrossRef]
23. Lademann, J.; Richter, H.; Schanzer, S.; Klenk, A.; Sterry, W.; Patzelt, A. Analysis of the penetration of a caffeine containing shampoo into the hair follicles by in vivo laser scanning microscopy. *Laser Phys.* **2010**, *20*, 551–556. [CrossRef]
24. Teichmann, A.; Jacobi, U.; Ossadnik, M.; Richter, H.; Koch, S.; Sterry, W.; Lademann, J. Differential stripping: Determination of the amount of topically applied substances penetrated into the hair follicles. *J. Investig. Dermatol.* **2005**, *125*, 264–269. [CrossRef]
25. Knorr, F.; Lademann, J.; Patzelt, A.; Sterry, W.; Blume-Peytavi, U.; Vogt, A. Follicular transport route–research progress and future perspectives. *Eur. J. Pharm. Biopharm.* **2009**, *71*, 173–180. [CrossRef] [PubMed]
26. Blume-Peytavi, U.; Vogt, A. Human hair follicle: Reservoir function and selective targeting. *Br. J. Dermatol.* **2011**, *165*, 13–17. [CrossRef] [PubMed]
27. Mills, J.O.H.; Kligman, A.M. The follicular biopsy. *Dermatology* **1983**, *167*, 57–63. [CrossRef] [PubMed]
28. Hadshiew, I.M.; Foitzik, K.; Arck, P.C.; Paus, R. Burden of hair loss: Stress and the underestimated psychosocial impact of telogen effluvium and androgenetic alopecia. *J. Investig. Dermatol.* **2004**, *123*, 455–457. [CrossRef]
29. Davis, M.G.; Piliang, M.P.; Bergfeld, W.F.; Caterino, T.L.; Fisher, B.K.; Sacha, J.P.; Carr, G.J.; Moulton, L.T.; Whittenbarger, D.J.; Schwartz, J.R. Scalp application of antioxidants improves scalp condition and reduces hair shedding in a 24-week randomized, double-blind, placebo-controlled clinical trial. *Int. J. Cosmet. Sci.* **2021**, *43*, S14–S25. [CrossRef]
30. Gehring, W. Nicotinic acid/niacinamide and the skin. *J. Cosm. Dermatol.* **2004**, *3*, 88–93. [CrossRef]
31. Burnett, C.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Safety Assessment of Hydrolyzed Wheat Protein and Hydrolyzed Wheat Gluten as Used in Cosmetics. *Int. J. Toxicol.* **2018**, *37* (Suppl. S1), 55S–66S. [CrossRef]
32. Scott, L.N.; Fiume, M.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.G.; Shank, R.C.; Slaga, T.J.; et al. Safety Assessment of Panthenol, Pantothenic Acid, and Derivatives as Used in Cosmetics. *Int. J. Toxicol.* **2022**, *41* (Suppl. S2), 77–128. [CrossRef] [PubMed]
33. Rinnerthaler, M.; Bischof, J.; Streubel, M.K.; Trost, A.; Richter, K. Oxidative stress in aging human skin. *Biomolecules* **2015**, *5*, 545–589. [CrossRef] [PubMed]
34. Becker, L.C.; Bergfeld, W.F.; Belsito, D.V.; Hill, R.A.; Klaassen, C.D.; Liebler, D.C.; Marks, J.J.G.; Shank, R.C.; Slaga, T.J.; Snyder, P.W.; et al. Safety Assessment of Glycerin as Used in Cosmetics. *Int. J. Toxicol.* **2019**, *38* (Suppl. S3), 6S–22S. [CrossRef] [PubMed]
35. Jimenez, F.; Ruifernández, J.M. Distribution of human hair in follicular units: A mathematical model for estimating the donor size in follicular unit transplantation. *Dermatol. Surg.* **1999**, *25*, 294–298. [CrossRef]