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Enhanced efficacy and sensory properties of an anti-dandruff shampoo containing zinc pyrithione and climbazole

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Synopsis

Dandruff is a common complaint and is suffered by as much as half of the population at some time post puberty. The condition is characterized by the presence of flakes on the scalp and in the hair, and is often accompanied by itch. The most common treatment for dandruff is the use of shampoo formulations that contain fungistatic agents such as zinc pyrithione (ZPT) and octopirox. Whilst most antidandruff shampoos are effective in resolving the symptoms of dandruff these shampoos can often result in hair condition that is less than acceptable to consumers which can lead to a tendency for them to revert to use of a non-antidandruff shampoo. This can result in a rapid return of dandruff symptoms.

The aim of this investigation was to study the impact of using a combination of antidandruff actives and silicones on the resolution of dandruff and to deliver superior sensory properties to the hair. We have demonstrated that shampoo containing the dual active system of ZPT/Climbazole deposits both active agents onto a model skin surface (VitroSkin) and reduces Malassezia furfur regrowth in vitro. Clinical evaluation of the dual active shampoo demonstrated superior efficacy and retained superiority during a regression phase where all subjects reverted to using a non-antidandruff shampoo. We have also demonstrated that it is possible to deposit silicone materials from antidandruff shampoo uniformly over both virgin and damaged hair fibres that results in smoother hair fibres (as evidenced by reduced dry friction). This combination of antidandruff agents and conditioning silicones delivered from a shampoo provides subjects with superior antidandruff efficacy and desired end sensory benefits ensuring compliance and longer term dandruff removal.

Résumé

Les pellicules constituent un problème fréquent et concernent à peu près la moitié de la population à un moment post pubertaire. La condition est caractérisée par la présence de paillettes sur le cuir chevelu et les cheveux, et elle est souvent accompagnée de démangeaisons. Le traitement le plus courant pour les pellicules est l'utilisation de formulations de shampooing qui contiennent des agents fongistatiques comme le zinc pyrithione (ZPT) et octopirox. Alors que la plupart des shampooings antipelliculaires soient efficaces dans la résolution des symptômes des pellicules, ces shampooings peuvent souvent entraîner des états des cheveux non acceptables pour les consommateurs qui peuvent conduire à une tendance à revenir à l'utilisation d'un shampooing non-antipelliculaire. Cela peut entraîner un retour rapide des symptômes de pellicules. Le but de cette étude était d'étudier l'impact de l'utilisation d'une combinaison de principes actifs antipelliculaires et silicones sur la résolution de pellicules et de fournir de meilleures propriétés sensorielles pour les cheveux. Nous avons démontré qu'un shampooing contenant le système dual active de ZPT/Climbazole dépose les deux agents actifs sur une surface de la peau du modèle (VitroSkin) et réduit la repousse de *Malassezia furfur in vitro*. L'évaluation clinique du shampooing double actif a montré une efficacité supérieure et a conservé une supériorité lors d'une phase de régression où tous les sujets étaient revenus à un shampoing non-antipelliculaire. Nous avons également démontré qu'il est possible de déposer des matériaux en silicone d'un shampooing antipelliculaire uniformément sur les fibres capillaires vierges et/ou endommagées, ce qui se traduit par des fibres de cheveux lisses (comme on en témoigne par une réduction du frottement à sec). Cette combinaison d'agents antipelliculaires et silicones de conditionnement fournis à partir d'un shampooing apporte aux utilisateurs une efficacité antipelliculaire supérieure et les avantages sensoriels finaux souhaités, assurant l'utilisation durable et à long terme l'élimination des pellicules.

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Introduction

Dandruff is a common complaint and is suffered by as much as half of the population at some time post puberty [1]. The condition is generally characterized by the presence of flakes on the scalp and in the hair, and is often accompanied by itch. The severity of dandruff in the population can range from mild scale formation (similar to that of dry skin) to seborrhoeic dermatitis (SD) [2–4]. The central hypothesis of the aetiology of dandruff states that the lipophilic yeast, *Malassezia*, is the causal agent. The debate about the role of *Malassezia* in the formation of dandruff, and SD, has continued since the first report of the association of this species with dandruff in 1874 [5]. The evidence implicating *Malassezia* species in formation of dandruff has been generated over a number of years. Essentially, this comes from observations that the levels of

Malassezia (especially, *M. globosa* and *M. restricta*) species are elevated in dandruff, whereas the levels of other micro-organisms remain constant. For example, McGinley *et al.* found that *Malassezia* made up 46% of the microbial flora in normal subjects, 74% of the flora in patients with dandruff and 83% of the flora in cases of SD [6,7]. Further support for the association of *Malassezia* with the dandruff condition comes from the observation that the most effective anti-dandruff treatments are anti-fungal agents [e.g. zinc pyrithione (ZPT), piroctone olamine, selenium sulphide and ketoconazole] and that improvement of the dandruff condition with such ingredients is correlated with removal of the yeast [8–12].

Malassezia is a commensal organism that is found on healthy scalps as well as on dandruff scalps [13]. Initially, no pathogenic mechanism could be associated with the deterioration from a healthy to a dandruff scalp [14]. Subsequent work has recognized a role for lipid metabolism via lipase action from *Malassezia* species. More specifically, the role of oleic acid [15] as an initiator of dandruff has been proposed [13]. These observations imply that other factors must play a role in the development of dandruff on susceptible individuals. *Malassezia spp.* have also been found to trigger an innate immune response, possibly mediated by an upregulation of toll-like receptors (TLRs), especially TLR-2. It is possible that *Malassezia* may induce inflammation in the scalp to trigger dandruff symptoms by this mechanism [16,17]. It is true to say that dandruff has a multifactorial aetiology that includes *Malassezia* colonization, some underlying propensity to hyperproliferation, altered corneocyte maturation processes and a sub-clinical microinflammatory state. Furthermore, it has been shown that clear changes to the stratum corneum lipid composition are present in dandruff, in both their amount and relative composition and that there are significant changes to a range of biomarkers of inflammation, including IL-1α [18,19].

The dandruff state is also reflected in changes in the biophysical properties of the skin (which may be directly related to the changes in stratum corneum lipid content and ratio). For example, the transepidermal water loss of dandruff scalp is higher than that of healthy scalp [20]. This is indicative of a perturbed stratum corneum barrier. An investigation into the alteration of scalp stratum corneum intercellular lipid levels in response to a ZPT anti-dandruff shampoo has demonstrated restoration in scalp intercellular lipids [21]. ZPT treatment significantly increased levels of triglycerides, cholesterol and ceramides. These findings complemented those of a recent study in which ZPT was demonstrated to improve the scalp ultrastructure, normalizing the parakeratotic nature of dandruff skin [22]. It has been stated previously that *Malassezia* species are associated with dandruff formation. However, the observation that *Malassezia* are commensal organisms and that most people have *Malassezia* on scalp but not all people have dandruff, implies that other changes in the underlying scalp biology may be contributing to the development of dandruff [23].

The most common treatment for dandruff is the use of shampoo formulations that most often contain fungistatic agents. Whilst most anti-dandruff shampoos are effective in resolving the symptoms of dandruff, these shampoos can often result in hair condition that is less than acceptable to consumers [24] which, in turn, can lead to a tendency for them to revert to a non-anti-dandruff shampoo. This can have the effect of a rapid return of dandruff symptoms. To increase compliance, anti-dandruff shampoos must be formulated to deliver the anti-dandruff agent effectively to the scalp whilst providing excellent hair fibre properties.

The aim of the current investigation was to study the impact of using a combination of anti-dandruff actives on the resolution of dandruff and to deliver superior sensory properties to the hair. The anti-dandruff actives selected for this study comprised a combination of zinc pyrithione (1% w/w) and the anti-fungal agent, climbazole (0.5% w/w). Climbazole is an imidazole anti-fungal agent that has been demonstrated to be delivered effectively from anti-dandruff shampoos and to inhibit *Malassezia* growth [25,26]. The test formulations were further enhanced using a unique combination of silicones to maximize sensory properties. The anti-dandruff efficacy of this novel dual active system was compared with a commercial 1% zinc pyrithione-containing shampoo.

Materials and methods

Test shampoo formulations

Three shampoo formulations were tested.

- ZPT/climbazole shampoo: 1% (w/w) ZPT, 0.5% (w/w) climbazole, two silicone emulsions [Silicone 1: Dimethiconol (and) TEA-dodecylbenzenesulfonate; Silicone 2: Dimethicone (and) C12-15 Pareth-3 (and) C12-15 Pareth-23 (and) Poloxamer 407]
- ZPT shampoo: commercially available shampoo containing 1% (w/w) ZPT, dimethicone
- Standard beauty shampoo: commercially available shampoo without anti-dandruff actives

Generation of damaged hair switches

Damaged hair switches were prepared by bleaching and dyeing dark brown European hair switches. Bleaching solution was prepared according to the manufacturers instructions (L'Oréal Platine Precision) and applied to each side of the switches using a colouring brush. The switches were wrapped in aluminium foil and left for 30 min. The switches were then rinsed under tap water and left to dry for 1 h at 50°C. The switches were then washed in SLES solution and dried overnight at ambient conditions. The bleached switches were then dyed using Wella Koleston Perfect 710.

In vitro studies

Determination of silicone deposition to hair fibres

Silicone deposition from shampoo formulations was measured using X-ray fluorescence spectroscopy (XRF) with an Axios PW 1596 spectrometer with Super Q software. Dark brown, straight European hair switches, 3 g, (n = 5) were soaked in diethyl ether for 30 min and then swirled in 20% (w/w) hot sodium lauryl ether sulphate 1EO (SLES 1EO) to remove the ether. Diethyl ether was used as a safe and effective method to remove residual silicone on hair switches. The switches were then washed in SLES 1EO two further times and allowed to dry in a fume hood for approx. 20 min. Hair switches were placed in Petri dishes and had 540 μL water and 60 μL of test shampoo applied along the length of the switch. The switch was then massaged for 1 min and the lather left *in situ* for 1 min to simulate the washing process. The treated switches were then rinsed for 30 s with tap water (3 L min^{-1}). The switches were allowed to dry in ambient conditions overnight. Five replicates were prepared for each sample to be tested. Hair switches were divided into thirds to represent tip, middle and root prior to XRF analysis. Treated hair switch segments were placed in the XRF cups in a parallel manner and secured in the cup. Output from the XRF spectrometer (count rate) was converted to concentration of silicon with reference to a standard curve. Data were analysed using one

way ANOVA, a result was considered to be statistically significant if the *P*-value for the *F*-test was <0.05.

Determination of zinc deposition to VitroSkin™

The sheet VitroSkin™ was divided into 5 × 5 cm pieces and placed over one side of the smaller diameter XRF ring, with the rough topography facing downwards. The larger ring was then placed onto the smaller ring and pressed firmly to ensure a good seal, ensuring that the rough topography of the artificial skin was inside the cups. Distilled water (1.5 mL) and shampoo (0.5 mL) were pipetted into the XRF plastic cup and mixed onto the surface of the artificial skin using the stirring rod for 30 s (the surface of the stirring rod remaining in contact with the surface of the artificial skin). The shampoo solution was then removed using a plastic pipette and a rinse phase simulated using 2 mL of distilled water and a 30 s application time. Finally, all rinse water was removed using a pipette, and the XRF cups were allowed to dry overnight in ambient conditions. Output from the XRF spectrometer (count rate) was converted to concentration of zinc with reference to a standard curve. Data were analysed using one way ANOVA, a result was considered to be statistically significant if the *P*-value for the *F*-test was <0.05.

Determination of fungistatic activity

Fungistatic activity of the shampoo formulations was assessed by inoculation of *Malassezia furfur* onto VitroSkin that had been treated with shampoo in XRF cups in a manner similar to that described for assessment of zinc deposition. This is an attempt to gauge fungistatic activity on a surface following a wash and rinse procedure simulating a typical shampoo treatment. *Malassezia furfur* is commonly used as a model fungal species rather than using *M.restricta* or *M.globosa* as it is relatively easy to grow in culture on a VitroSkin surface. After allowing the treated XRF cells to dry overnight, the samples were placed into jars containing Modified Dixon agar. 200 μL of a suspension of *M. furfur* CB 1878 ($2–6 \times 10^6$ cells mL^{-1}) was inoculated onto the surface of the substrate and incubated for 24 h at 32°C. After this period of incubation, the cells were harvested with buffer solution and 100 μL of a 10-fold dilution spread onto replicate Modified Dixon Agar plates and incubated for 3–4 days at 32°C. Data were analysed using one way ANOVA; a result was considered to be statistically significant if the *P*-value for the *F*-test was <0.05.

Assessment of hair smoothness

Hair smoothness was measured using a Texture Analyser (TA.XT. Plus Stable Microsystems. Godalming, Surrey, U.K.). Hair switches (3 g; 16 cm × 4 cm) were secured in an aluminium frame and cleaned under tap water (37°C) at a flow rate of 4 L min^{-1}, for 5 s. 14% (w/w) sodium lauryl ether sulphate (2EO) (1.25 g) was applied to each of the switches and agitated for 30 s and then rinsed for 30 s under tap water. This wash cycle was repeated and each switch combed through to align the fibres. Following this pretreatment, the switches were treated with the treatment shampoo formulations using the same application protocol and tested after overnight drying (22°C, 50% RH). Smoothness of the hair switches was measured on the Texture Analyser by moving a probe for a total distance of 80 mm (2 × 40 mm) at 10 mm s^{-1} and 500 g load. The area of each friction hysteresis loop was calculated and reported as g.mm. Five switches were measured for each wash treatment. Data were analysed using Student's *t*-test. Results were considered to be significant at the 95% confidence level.

***In vivo* studies**

Anti-dandruff efficacy study design

The study was carried out in Bangkok, Thailand at the Unilever internal facility and was a double-blind, randomized, half-head design. The study was cleared by the Joint Research Ethics Committees, Bangkok, and all subjects gave their informed consent to participate. Men with dandruff were recruited and put onto a 4 week run-in phase where they used a beauty shampoo at home. Those men who still had dandruff at the end of the run-in phase continued onto the test phase of the study ($n = 69$). Subjects had their hair washed three times per week in a salon using a half-head procedure where half the head was washed with ZPT/climbazole shampoo and the other half was washed with ZPT-only shampoo. The side of head treated with each product was randomly allocated. Treatment continued for 4 weeks after which time subjects returned to use of a beauty shampoo at home for a further 2 weeks (regression phase). Sixty subjects completed the test phase of the study and 58 completed the regression phase. Dandruff was measured using the Unilever Total Weighted Head Score (TWHS) system [18] at baseline and at weekly intervals over the study. TWHS was assessed 48 hours after the final application of shampoo. The mean TWHS adhered flake (AF) score for each subject/treatment was used as a summary measure of treatment performance during the test phase and during the regression phase. The mean TWHS AF was only calculated for subjects with complete data. Analysis of covariance was used to analyse the mean test phase data and the mean regression phase data. Baseline measurement was treated as a covariate, treatment as a fixed effect and subject as a random effect. Results were considered to be significant if the *F*-test *P*-value was <0.05.

Results and discussion

Anti-dandruff active deposition

Table I shows the results of the deposition studies from zinc pyrithione/climbazole shampoo and zinc pyrithione shampoo. Both actives were readily detected on the VitroSkin™ after the simulated wash protocol. The zinc pyrithione/climbazole shampoo deposited significantly more zinc than the ZPT shampoo ($P < 0.05$).

In vitro inhibition of *Malassezia*

Inhibition of the growth of *Malassezia furfur* by the two zinc pyrithione-containing shampoos is presented in Fig. 1. The data are presented as log *Malassezia* number reduction. It can be seen that the zinc pyrithione/climbazole formulation was significantly more effec-

Table I Zinc and climbazole deposition to VitroSkin™

	Zinc (μg cm^{-2}) Mean ± SD	Climbazole (μg cm^{-2}) Mean ± SD
ZPT/Climbazole	14.83 ± 1.81	3.17 ± 0.41
ZPT	11.10 ± 1.59	ND

ND, not detected; ZPT, zinc pyrithione.

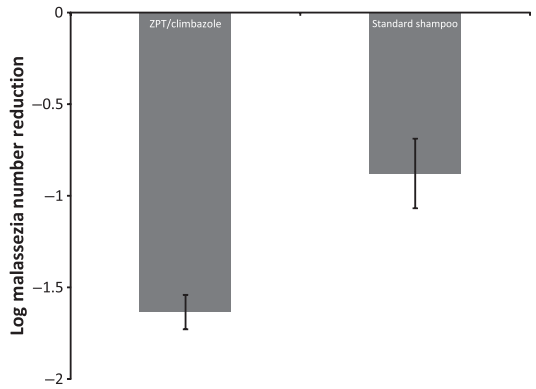

Figure 1 Malassezia inhibition on VitroSkin (mean ± SD, $P < 0.05$) zinc pyrithione (ZPT)/climbazole shampoo showed a significant inhibition of growth of *Malassezia furfur* in this test and in repeat studies, $P < 0.05$, when compared to the ZPT shampoo.

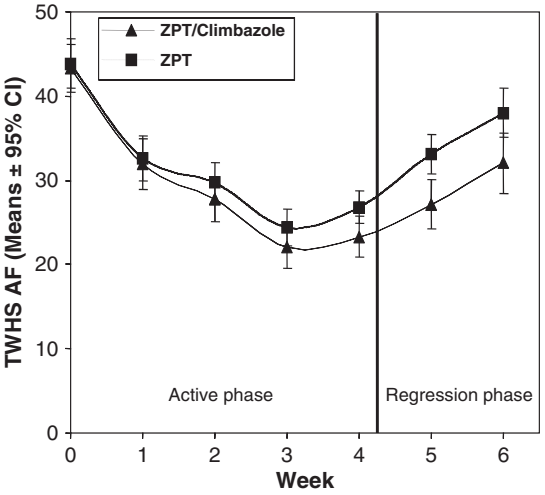

Figure 2 Antidandruff efficacy through active and regression phase. Dual active formulation (ZPT/climbazole) showed superior antidandruff efficacy compared to ZPT-only shampoo over the 4 week test period and over the 2 week regression phase.

tive at inhibiting growth of *Malassezia furfur* than the ZPT shampoo ($P < 0.05$).

Anti-dandruff efficacy

The results of the anti-dandruff study are presented in Fig. 2. Zinc pyrithione/climbazole shampoo was significantly more effective than the ZPT shampoo at reducing the clinically observed dandruff flakes over the 4 week test phase (95% CI −3.3 to −0.8, $P < 0.002$). During the regression phase, the zinc pyrithione/climbazole shampoo retained its anti-dandruff superiority over the ZPT shampoo (95% CI −7.4 to −4.1, $P < 0.001$).

Silicone deposition

Silicone deposition onto virgin and damaged hair is presented in Table II. The ZPT/climbazole shampoo, containing two silicone ingredients [Silicone 1: Dimethiconol (and) TEA-dodecylbenzenesulfonate; silicone 2: Dimethicone (and) C12-15 Pareth-3 (and) C12-15 Pareth-23 (and) Poloxamer 407] was found to deposit significant levels of silicone onto the whole of the hair fibre (root, middle and tip) when compared to a Standard shampoo (free from anti-dandruff actives but containing silicone) on both virgin hair and damaged hair. The level of deposition of silicone was significantly higher from the ZPT/climbazole shampoo than from the Standard shampoo ($P < 0.05$). No silicone deposition was detected from the Standard shampoo onto damaged hair, whereas silicone deposition was observed along all parts of the hair fibre from the ZPT/climbazole shampoo. The level of silicone deposition onto damaged hair was much lower than that measured on virgin hair.

Hair smoothness

Hair smoothness was assessed using a measure of the dry friction force following treatment with shampoo. The results are presented in Fig. 3. The ZPT/climbazole shampoo was found to generate significantly lower dry frictional force than the Standard shampoo ($P < 0.05$) on both virgin and damaged hair.

Conclusions

Dandruff is a common cosmetic complaint that afflicts many people at some point in their life. There are many shampoos available, in both supermarkets and pharmacies, to combat dandruff using cosmetic ingredients (for the majority of the world) or active ingredients

Table II Silicone deposition to virgin and damaged hair as determined by X-ray fluorescence

	Silicone deposition, $\mu g\ g^{-1}$ (mean ± SD)					
	Virgin hair			Damaged hair		
	Root	Middle	Tip	Root	Middle	Tip
ZPT/Climbazole	2888 ± 463	1755 ± 310	1766 ± 65	437 ± 109	428 ± 52	337 ± 23
Standard shampoo (No AD active)	2025 ± 688	1481 ± 317	852 ± 464	ND	ND	ND

ND, not detected.

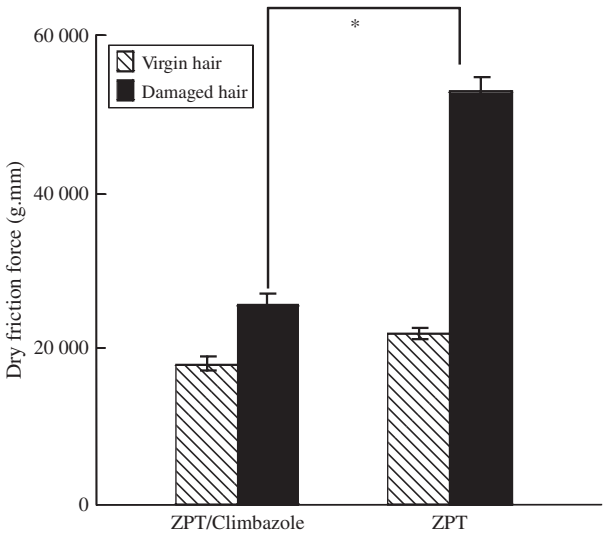

Figure 3 Dry friction force on switches treated with antidandruff shampoos (mean ± SEM, $*P < 0.05$) Dry friction force for the ZPT/climbazole shampoo was lower than that found with the shampoo containing ZPT alone. This result was significantly significant on damaged hair but not on virgin hair.

supported by the FDA Monograph in the United States (where anti-dandruff shampoos are considered to be drugs). The most common ingredient for treatment of the dandruff condition is ZPT. This fungistatic agent controls dandruff by limiting the regrowth of *Malassezia* species that have been strongly implicated in the aetiology of the condition. Despite its proven efficacy, the sensory properties of ZPT-based anti-dandruff shampoos leave much to be desired. The primary aim of many cosmetic shampoos is to clean the hair and allow the subject to generate their preferred hair style with the relevant sensory cues (such as conditioned benefit, control of fibre damage, fragrance.) [24]. In the case of ZPT-based anti-dandruff shampoos, there is a subject-perceived deficiency in the cosmetic/sensory performance. This perceived deficiency leads to subjects switching from an anti-dandruff shampoo to a standard shampoo in the belief that they will achieve superior cosmetic end benefits. Consequently, the dandruff condition will recur within a short period of time. The present investigations have been carried out to identify a formulation that provides both enhanced anti-dandruff efficacy and no loss in sensory and cosmetic properties on the hair. Such a formulation will prove invaluable in the prolonged control of dandruff as subject compliance with product use instructions will be enhanced.

A pre-requisite of any anti-dandruff formulation is to ensure that the active ingredient is: (i) deposited onto the scalp surface from a shampoo; (ii) reduces growth of the *Malassezia* species.

In the present research, we have demonstrated that the dual active system of ZPT/climbazole deposits both active agents onto a model skin surface (VitroSkin). Furthermore, the deposited ingredients also reduce *Malassezia furfur* regrowth. Taken together the deposition data and *Malassezia* inhibition data allow us to screen a formulation before moving onto an *in vivo* clinical study on human subjects. Clinical evaluation of the dual active system in the present study demonstrated that this novel anti-dandruff combination (ZPT/climbazole) is significantly better that ZPT-alone in reducing dandruff on the human scalp when delivered from shampoo formulations. The dual active system not only reduces dandruff flakes in the test phase but retains the superiority during a regression phase when subjects revert to using a non-anti-dandruff shampoo.

The sensory and fibre care properties of anti-dandruff shampoos are essential to ensure that subjects comply with usage instructions and continue to use the product to remove dandruff. By careful selection of conditioning ingredients (e.g. silicones), it is possible to achieve the aim of anti-dandruff efficacy and hair sensory benefits. We have been able to demonstrate that by selecting the correct combination of silicones, it is possible to coat uniformly over both virgin and damaged hair fibres. This is not achieved by the commonly used silicones in "beauty" shampoos (i.e. non-anti-dandruff shampoos) as exemplified by the Standard shampoo in the present investigation. This in turn results in smoother hair fibres (as evidenced by reduced dry friction), which drives consumer compliance. This combination of anti-dandruff agents and conditioning silicones provides subjects with anti-dandruff efficacy and desired end hair sensory benefits ensuring compliance and longer term remission from dandruff.

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