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Review Article

The role of oxidative damage in poor scalp health: ramifications to causality and associated hair growth

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Abstract

The oxidative stress element of unhealthy scalp leads to compromised pre-emergent hair formation and poorly formed hair as it grows. Only cosmetic solutions can minimize the impact of unhealthy hair and to achieve healthy looking and feeling hair, the scalp health must be normalized first. The objectives of this research were to both investigate whether oxidative stress was a relevant aetiological element in scalp dandruff and seborrhoeic dermatitis and whether scalp condition affects the quality of hair that grows from it. Further, this research was designed to determine whether an effective anti-dandruff shampoo would repair and protect the scalp and pre-emergent hair from oxidative stress. This study demonstrated that oxidative stress is an aetiological element relevant to the dandruff condition and that a potentiated ZPT shampoo effectively improves scalp condition, including a reduction in oxidative stress. The compromised hair condition associated with dandruff is concomitantly improved when the scalp condition is improved. It appears that there is a direct link between hair quality and scalp health.

Résumé

La composante de stress oxydatif du cuir chevelu en mauvaise santé compromet la qualité du cheveu en formation et celle du cheveu en croissance. Seules des solutions cosmétiques peuvent le minimiser et pour obtenir un cheveu à l'apparence et au toucher sains, le cuir chevelu doit être d'abord normalisé. L'objectif de cette recherche était à la fois de déterminer si le stress oxydatif est un élément étiologique pertinent dans le cuir chevelu à pellicules et la dermatite séborrhéique et si l'état du cuir chevelu affecte la qualité des cheveux qui y poussent. De plus, cette recherche a été conçue pour déterminer si un shampoing anti-pelliculaire efficace serait capable de réparer et de protéger le cuir chevelu et les cheveux avant leur croissance du stress oxydatif. Cette étude a démontré que le stress oxydatif est un élément étiologique pertinent à l'état pelliculaire et un shampooing au pyrithione de zinc améliore efficacement l'état du cuir chevelu, et permet une réduction du stress oxydatif. L'état de la chevelure abîmée par les pellicules est amélioré de façon concomitante à l'amélioration de l'état du cuir chevelu. Il s'avère qu'il y a un lien direct entre la qualité des cheveux et la santé du cuir chevelu.

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Introduction

The most common disorders of scalp health are dandruff and seborrhoeic dermatitis, often exceeding a combined 50% of the population [1]. The symptomology of D/SD includes flakes, itch, dryness, and sometimes redness. The modesty of these symptoms, however, belies the severity of the perturbation of the underlying scalp epidermis. Some of the structural alterations documented [2] to be relevant to D/SD are parakeratosis, irregular corneocyte envelope formation and unstructured and expanded intercellular lipid. Recent capabilities have now allowed description of perturbations at the molecular level [3]. In this work, dramatic perturbations were observed of biomarkers related to inflammation, hyperproliferation and barrier function.

Scalp hair originates approximately 4 mm deep in the skin with newly formed fibre residing for almost two weeks within the skin before reaching the scalp surface. This pre-emergent hair is in intimate contact with the surrounding scalp tissue while it matures and hardens during its journey from the bulb to the scalp surface. During this time, the severe perturbations accompanying poor scalp health such as D/SD may impact this pre-emergent hair. One objective of this work was to determine whether the health of the scalp affects the formative pre-emergent hair in a way that can be detected and quantified in samples of post-emergent hair.

Some degree of oxidative stress is an inherent attribute of physiological metabolism in an oxygen-based atmosphere. Whereas metabolic activity generates reactive oxygen species and free radicals, there is an innate defence system based on enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GP) to detoxify these reactive species. Under normal conditions, the pro- and antioxidant forces are in balance. However, many skin conditions are either accompanied by or caused by an imbalance of these forces resulting in an oxidative stress [4, 5]. The imbalance can be caused by excessive sources of oxidative damage – environmental, products, microbes, etc. – or by insufficient endogenous antioxidant activity which can have many causes, such as normal ageing. Another objective of this work was to determine whether oxidative stress is involved in D/SD and, if so, whether this stress can explain a linkage between scalp and hair health.

To assess the state of oxidative stress, we focused on the specific molecular species of lipid oxidation products rather than non-specific measures. Many components of the sebaceous lipids can become oxidized and thus have the potential to represent biomarkers of

oxidative stress. We have chosen to focus on a well-established [6, 7] marker of lipid oxidation – an oxidation product of linoleic acid called hydroxyoctadecadienoic acid, HODE.

Materials and methods

Clinical study design

This was a randomized, double-blind, multisite study conducted in Winnipeg, Canada and Minnesota, USA. The study was conducted in male and female subjects (18–75 years old) after obtaining the informed consent under the principles of Good Clinical Practice (see Table I for a summary of population demographics). The study consisted of 8 weeks pre-treatment phase followed by 16 weeks treatment phase. Special consideration was given to the duration of pre-treatment and treatment phases to ensure that collected hairs were grown under well-characterized scalp conditions so that an association could be made between scalp and hair conditions. The average hair growth rate is approximately 1 cm/month. For newly formed hair fibre to reach the scalp surface (approximately 4 mm journey), it is estimated to be approximately 2 weeks length of time. Thus, an 8-week pre-treatment phase was chosen to allow baseline hair to be collected that was grown from a well-characterized and equilibrated scalp condition. Likewise, 16 weeks of treatment allowed time for sufficient new hair to emerge under scalp conditions that reflect the impact of shampoo treatment. For analysis of hair samples, the most recently grown segment of hair was used (see *Assessment of biomarker of oxidative stress* for more details).

During the pre-treatment phase, all subjects were instructed to wash hair/scalp at least three times a week using the cosmetic shampoo provided by the study site for 8 weeks. Upon the completion of the pre-treatment phase, subjects refrained from shampooing for 72 h and returned to the study site and underwent the scalp assessment by the qualified dandruff grader using Adherent Scalp Flaking Score (ASFS) method (see Assessment of flaking below). Subjects with ASFS ≥24 were defined as Dandruff/Seborrhoeic Dermatitis subjects (D/SD) and those with ASFS ≤8 were defined as Non-dandruff subjects. Exclusion criteria included scalp diseases, scalp scarring, history of contact dermatitis, use of oral anti-fungal, immunosuppressant agents or anti-inflammatory drugs; use of anti-dandruff, anti-psoriatic or anti-seborrhoeic dermatitis shampoos within the previous 8 weeks and any other significant medical condition. Due to the measurements of markers of oxidative stress, subjects had to be non-smokers for 5 or more years and not have chemically treated their hair within 2 months of the start of the pre-treatment phase.

Table I Anti-dandruff clinical population parameters

	Dandruff/seborrhoeic dermatitis		
	Potentiated ZPT shampoo	Cosmetic placebo shampoo control	Non-dandruff control
Subjects randomized, *n*	120	120	60
Female, *n* (%)	58 (48.3%)	58 (48.3%)	30 (50.0%)
Male, *n* (%)	62 (51.7%)	62 (51.7%)	30 (50.0%)
Age in years, mean (SD)	48.3 (15.3)	50.2 (15.0)	51.1 (15.6)

n = sample size; SD, standard deviation.

Three hundred subjects meeting the entrance criteria ($n = 240$ in D/SD group and $n = 60$ in Non-dandruff group) underwent the procedures for collecting hair samples and tape strip samples for biomarker assessment. In addition, 120 subjects in the D/SD group participated in the procedure for measuring trans-epidermal water loss (TEWL). These samples and readings were used to generate baseline values prior to the treatment shampoo usage.

D/SD subjects were then randomized into one of the two treatment groups: potentiated ZPT shampoo group and placebo cosmetic shampoo group. These subjects were instructed to wash hair/scalp at least three times a week using the assigned shampoo product. Non-dandruff subjects did not participate in the treatment phase.

During the treatment phase, D/SD subjects returned to the study site for post-treatment assessments after refraining from shampooing for 72 h as follows: Tape strip sample collection at Weeks 3 and 16; Hair sample collection at Week 16; Dandruff flake assessment using ASFS at Weeks 3 and 16; and TEWL assessment (the same subset as baseline) at Week 16.

Measurement of standard dandruff efficacy parameters

Assessment of flaking severity

Dandruff flaking severity was measured using the Adherent Scalp Flaking Score utilizing a scale ranging from 0 to 80 units by qualified graders as reported elsewhere[8].

Assessment of biomarkers of scalp health

Samples of scalp surface biomarkers were collected using D-squame® tape strips. In D/SD subjects, these tape strip samples were collected from the highest flaking octant as determined by the qualified grader at baseline ASFS assessment. In Non-dandruff subjects, the tape strip samples were collected from the octant that received ASFS score of 0 at baseline. The tape strip samples at the post-treatment time points were collected from the same octant as baseline. The extracts from the tape strip samples were analysed for histamine by high-performance liquid chromatography tandem mass spectrometry. A resulting histamine value was divided by the protein content in the same extract to generate normalized histamine value, which was used for statistical analysis. The details of sampling and quantitation methods were previously described [3, 9]. The inflammatory biomarker IL-1ra:IL-1α ratio was also measured as previously described [3].

Assessment of HODE biomarker of oxidative stress

Tape strips (single) obtained from the scalp of human subjects were placed into individual polypropylene amber vials or glass amber vials, and then extracted with extraction solvent (methanol with 0.1% butylated hydroxytoluene, w/v) using vortexing for 10 min.

Hair samples were collected from an octant at the back of the head closest to the neck. One 1 inch by 1 inch area was identified within the octant and a cosmetologist cut the bundle of hair as close to the scalp as possible without damaging the skin. At Week 16, the cosmetologist repeated the same procedure from the same site. Hair samples were cleaned prior to HODE analysis to remove superficial dirt and sebaceous lipids. Cleaning procedures consisted

of taking 2-cm segment of hair from the root end and placing in 20 ml of methyl acetate for 60 min. The methyl acetate was decanted, and 5 ml of water was added. After 5 min, the water was decanted and samples were allowed to equilibrate for 48 h in a constant temperature and humidity room (~50% RH/70°C). The cleaned hair samples were simultaneously pulverized and extracted using the extraction solvent in a bead-based device (BeadRuptor).

The standards and the extracts of the scalp tape strips and hair extracts were analysed using gradient reversed-phase high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) operating under multiple reaction monitoring (MRM) conditions. Analytes, (±) 9 or 13-Hydroxy-10E, 12Z-octadecadienoic acid (HODE) and Linoleic acid (LA, 18:2n-6), and their ISTDs (HODE-d4 and LA-d4) are monitored by negative ion electrospray (ESI). A standard curve was constructed by plotting the signal, defined here as the peak area ratio (peak area analyte/peak area ISTD) for each standard versus the mass of each analyte for the corresponding standard. The mass of each analyte in the calibration standards and human scalp and hair extract samples were then back calculated using the generated regression equation. The resulting values were normalized by dividing the amount of HODE by the amount of linoleic acid (the parent lipid) found in the respective extract (results expressed as ng HODE/ng linoleic acid).

Assessment of trans-epidermal water loss

The 1 inch by 1 inch area that was clipped for hair sampling was exposed using a hair a clip. Subjects were acclimated in a controlled-temperature (70 ± 2°F) and controlled-humidity (30–45%) room for a minimum of 30 min. For given subject, TEWL measurements at each time point were made by the same trained operator using the same AquaFlux instrument (Model AF200, Biox Systems Ltd, London UK).

Test products

The test products used in this study were two commercial shampoos, one an anti-dandruff shampoo and the other a cosmetic shampoo functioning as a placebo control product. The anti-dandruff shampoo used was based on potentiated 1% zinc pyrithione technology ('potentiated ZPT shampoo') [10]. The main formula components of these two products are summarized in Table II. The formulas have a number of common elements, being based on similar surfactants, hair conditioning agents and deposition aids.

Statistical analysis

After completion of the clinical study, data were checked for accuracy and completeness. All subjects were evaluated for compliance with study protocol at each visit, and their evaluability was determined prior to database locking. Statistical analyses were performed on evaluable data. Dandruff and Non-dandruff groups were compared at baseline visit using analysis of covariance method, adjusted for age, gender and study site. For post-baseline data, potentiated ZPT shampoo group was compared to the cosmetic shampoo group using analysis of covariance, adjusted for its baseline value, baseline interaction with treatments, age, gender, study sites and age. Logarithm transformation was applied to biomarker and TEWL data prior to analysis to stabilize the variance. A statistical test was determined to be significant if a two-sided *P*-value was <0.05.

Table II Summary of key formula components of commercial products evaluated

Function	Materials	'Potentiated ZPT'	Cosmetic shampoo
Surfactants	Sodium lauryl sulphate	X	X
	Sodium laureth sulphate	X	X
	Cocamidopropyl betaine	X	X
Hair conditioner	Polydimethylsiloxane	X	X
Thickener	Ethylene glycol distearate	X	X
Active	Zinc pyrithione	1.0%	
Non-active functional materials	Guar hydroxypropyl trimonium chloride	X	X
	Zinc carbonate	X	

Results

Evaluation of oxidative stress in scalp and newly grown hair

Dandruff vs. non-dandruff

HODE was quantified in scalp and newly grown hair samples at baseline from the D/SD population and the control healthy scalp Non-dandruff group after 8 weeks of standardized cosmetic shampoo usage. The results are summarized in Table III and Fig. 1. The data show a significant increase in HODE levels in both the scalp and newly grown hair of a dandruff population as compared to the control healthy scalp, indicating the elevated presence of oxidative stress state in dandruff.

Dandruff treatment: potentiated ZPT vs. cosmetic shampoo

The effects of potentiated ZPT shampoo were examined in dandruff subjects. In the scalp, a significantly greater reduction in HODE level was observed in potentiated ZPT shampoo group compared to placebo cosmetic shampoo group after 3 weeks of usage. Continued use of potentiated ZPT shampoo resulted in further lowering of HODE level, achieving >70% reduction relative to baseline with a highly significant treatment difference relative to placebo cosmetic shampoo. In hair samples, potentiated ZPT shampoo group also showed a significantly greater reduction in HODE level compared to placebo cosmetic shampoo group. The results are summarized in Table IV and Fig. 2. The data show that the potentiated ZPT sham-

Table III Summary of HODE levels in scalp and hair dandruff vs. non-dandruff

	HODE level*, normalized, log (SE)	
	Scalp	Hair
D/SD	−2.33 (0.028)	−1.57 (0.014)
Non-dandruff	−2.87 (0.062)	−1.72 (0.031)
P-value	<0.0001	<0.0001

*Expressed as ng HODE/ng linoleic acid.

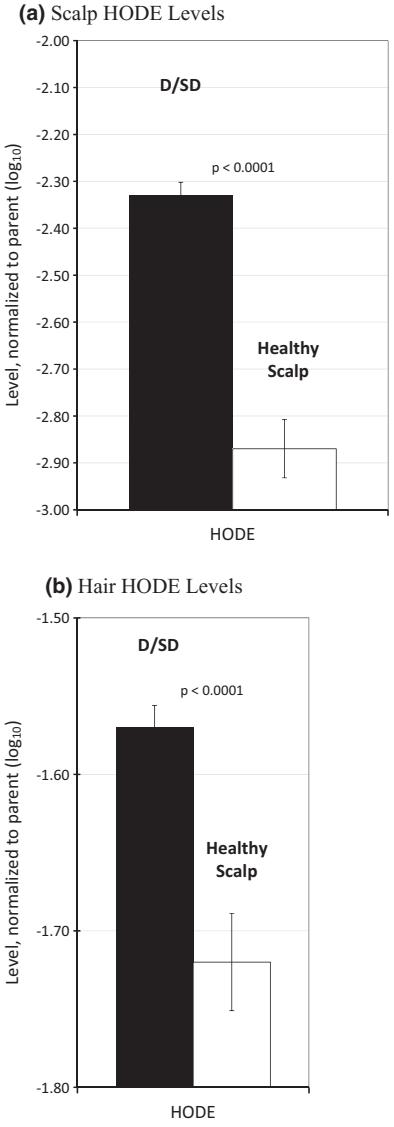

Figure 1 Summary of HODE levels in (a) scalp and (b) hair dandruff vs. non-dandruff.

poo is very effective at reducing the oxidative stress associated with the dandruff scalp condition both on scalp and in newly grown hair.

Standard dandruff efficacy parameters

To confirm that the anti-dandruff shampoo behaved as expected with regard to dandruff scalp health improvement, standard anti-dandruff efficacy parameters were assessed, and the responses were compared to the cosmetic shampoo control. These parameters comprised expert-assessed adherent scalp flaking score (ASFS), objective determinations of established biomarkers of skin condition (histamine, IL-lra:IL-1α ratio) and trans-epidermal water loss (TEWL) as indicator of scalp barrier function. The use of anti-dandruff shampoo was accompanied by a reduction in flaking, histamine level, IL-lra:IL-1α ratio and trans-epidermal water loss, indicative of return to healthier scalp. When these changes were compared to those observed in the placebo cosmetic shampoo group, the differ-

Table IV Reduction in HODE levels as a function of treatment

	Improvement in HODE level*, % (+SE, −SE)		
	Scalp		Hair
	Week 3	Week 16	Week 16
Potentiated ZPT	−53.5 (4.3, 3.9)	−71.2 (2.8, 2.6)	−36.5 (3.0, 2.9)
Placebo control	−22.1 (7.3, 6.7)	−27.5 (7.4, 6.7)	−18.9 (4.0, 3.8)
P-value	<0.0001	<0.0001	0.0003

*Reduction from baseline.

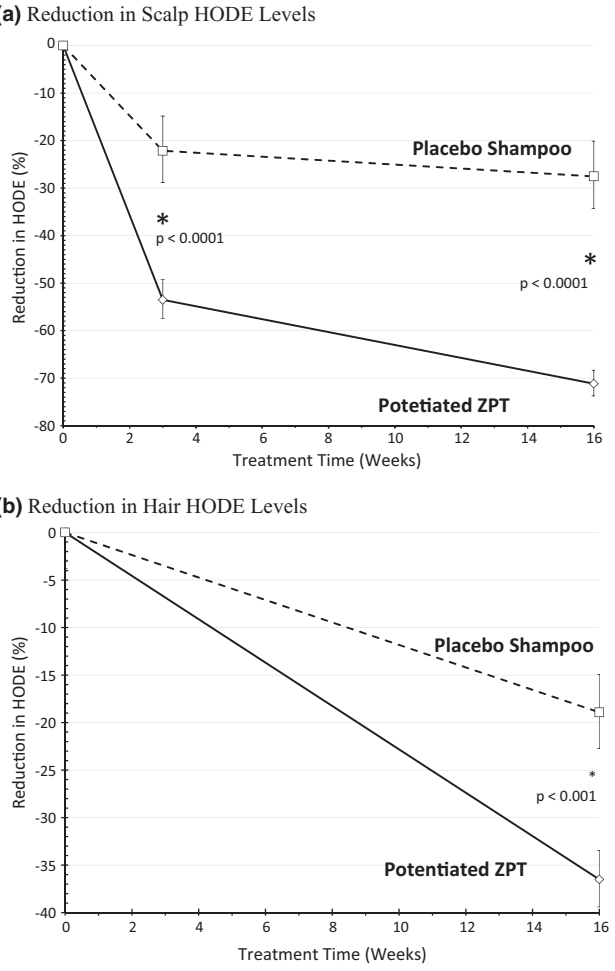

Figure 2 Reduction in HODE levels in (a) scalp and (b) hair as a function of treatment with either potentiated ZPT shampoo of placebo shampoo.

Table V Summary of common anti-dandruff efficacy measures relative to cosmetic shampoo control at week 16 of treatment

Anti-dandruff efficacy parameter	Strength of significance of advantage for potentiated ZPT vs. placebo shampoo (*P*-value)
Reduction flake appearance (ASFS)	$P = 0.0001$
Reduction in histamine	$P = 0.0031$
Reduction in IL1ra:Il-1α	$P = 0.0001$
Reduction in TEWL	$P = 0.0007$

ences were significant (Table V), confirming the performance of the anti-dandruff shampoo.

Discussion

The current state of knowledge [11] in D/SD considers the aetiology as originating in *Malassezia*-derived lipase activity which liberates fatty acids from sebaceous triglycerides. The fatty acids then trigger an inflammatory skin reaction leading to hyperproliferation and barrier impairment. The symptoms emanating from this sequence of events are itch/redness, flakes and dryness. Is this aetiological description complete or does oxidative stress play an unappreciated role? Are there additional consequences to an unhealthy dandruff scalp beyond those conventionally accepted? Is hair quality impacted?

The skin is the interface with the environment, which can be a substantial source of reactive oxygen species from pollution, ultraviolet light and oxidizing products. Endogenous sources of oxidative stress include *Malassezia* yeasts, energy-generating metabolism and emotional stress. Given the prevalence of sources of oxidative stress, it is not surprising that it has been demonstrated [4, 5] to be a factor in a wide range of skin diseases: atopic dermatitis, psoriasis, acne, irritant contact dermatitis and physical urticaria. Is it also an aetiological component of D/SD?

Over the last 40 years, there has been a wide range of epidemiological studies evaluating hair from healthy scalps vs. that from unhealthy scalps, such as psoriasis and seborrhoeic dermatitis. Four studies using scanning electron microscopy (SEM) and morphometric analysis to compare the hair from psoriasis sufferers with that from a normal control group demonstrated the psoriasis-derived hair had a reduced diameter [12, 13], surface pitting and roughness [12, 14, 15] and cuticle breakage [14]. Using the more sensitive technique of atomic force microscopy (AFM), hair from psoriasis sufferers was observed [16, 17] to have thicker cuticles, a rougher surface and more surface pitting. This same technique was used to characterize the hair from individuals with atopic dermatitis [17] or seborrhoeic dermatitis [16, 18] where increased cuticle thickness and roughness were observed as well. Finally, a range of techniques were used to demonstrate [19] hair from seborrhoeic dermatitis had a more brittle surface and reduced shine. Although there may be many reasons why an unhealthy scalp leads to hair with more surface irregularities, oxidative stress is one mechanism known to be able to cause this [20].

To determine whether oxidative stress was an aetiological element of the D/SD condition and whether, by its propensity for self-propagation, the damage caused by oxidative stress in the scalp could lead to collateral damage to pre-emergent hair, we developed the capability to assess the level of oxidative damage in both scalp and newly grown hair. The methodology involves collection of relevant scalp and hair samples under well-defined condition followed by analytical quantitation of an oxidized version of linoleic acid, HODE. A clinical protocol was developed to enable comparative assessment of HODE levels in both D/SD and healthy scalp populations as well as whether an effective scalp care shampoo could improve HODE levels in scalp and hair relative to a cosmetic placebo shampoo.

The D/SD population had a significantly higher level of scalp oxidative stress (as indicated by HODE level) than the healthy scalp population control (Table III, Fig. 1a). This demonstrates that oxidative stress is indeed an attribute associated with the D/SD condition [21], as observed in many other common skin conditions [4, 5]. Whether or not this oxidative stress is an early initiation event or a downstream ramification is unclear. However, there is

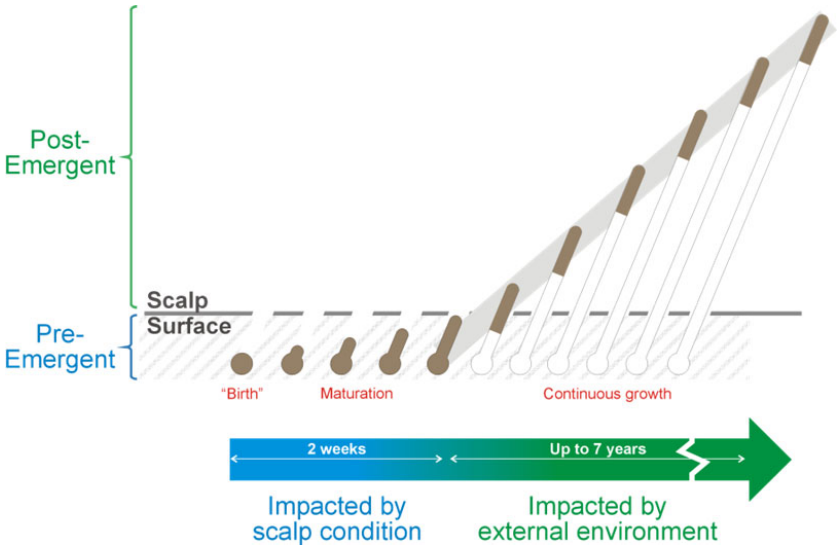

Figure 3 Demonstration of propagation of the impact of compromised pre-emergent hair formation.

increasing evidence that oxidative stress is also involved in the pathogenesis of acne [22, 23] which is significant as D/SD share many common aetiological elements with acne. The awareness that oxidative stress is a component of D/SD aetiology may expand therapeutic strategies as exogenous factors (such as pollution and stress) that are sources of reactive oxygen species can potentially be exacerbating factors.

The newly grown hair obtained from the dandruff and healthy populations likewise demonstrated (Table III, Fig. 1b) that the hair growing from an unhealthy (dandruff) scalp is also under oxidative stress. This is a significant observation as it suggests there is a direct link between scalp condition and the quality and health of the hair that grows from it. This may help explain the many previous epidemiological observations from psoriasis, atopic dermatitis and seborrhoeic dermatitis populations that showed compromised hair surface attributes.

Having established that oxidative stress is a new relevant aetiological component of D/SD, we sought to determine whether an effective scalp care shampoo could reduce the oxidative stress that is part of the unhealthy scalp D/SD condition and whether this improvement would impact the growing hair as well. The potentiated ZPT shampoo used in this study improved the standard measures of scalp health during a 16-week treatment regimen (Table V). Focusing on oxidative stress as indicated by HODE level, this shampoo also significantly reduced the oxidative stress that was elevated prior to treatment (Table IV, Fig. 2a). This indicates that the potentiated ZPT shampoo improves all measured attributes associated with the unhealthy dandruff scalp condition.

In the first study of its kind, the hair growing from a dandruff scalp was shown to be improved as a result of improving the scalp condition (Table IV, Fig. 2b). HODE level decreased significantly vs. cosmetic placebo control at the end of the study. This indicates that there is a causal linkage between scalp and resultant hair condition, with oxidative stress a potential underlying factor. This is supported by observations [24] that conditions such as hair loss are also tied to abnormalities in oxidative stress in the scalp. This is the first treatment study that demonstrates that an improvement in hair quality can result by use of a product that targets the improvement of the scalp condition.

The beneficial effects of the potentiated ZPT shampoo towards reducing oxidative stress likely originate from at least two product attributes. In addition to the zinc pyrithione, the product also contains zinc carbonate; zinc is known to deliver antioxidant activity directly [25]. In addition to this direct antioxidant activity, the anti-fungal activity of ZPT reduces the population of *Malassezia* which has been shown to exert some oxidative stress [26, 27].

In summary, the oxidative stress element of unhealthy scalp leads to compromised pre-emergent hair formation. This is important because this poorly formed hair will be propagated as the hair grows (Fig. 3). Once poorly formed hair emerges from the scalp, there is no way to fundamentally repair it, and only cosmetic solutions can minimize the impact of the unhealthy hair. Thus, to achieve healthy looking and feeling hair, the scalp health must be normalized first.

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