

Revisiting, in vivo, the hair regreasing process by the Sebuprint method

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Abstract

Background: The regreasing process of hair by sebum is daily observed by consumers. Methods to investigate this phenomenon were scarcely updated since the 1990s, despite the constant progresses in hair cleaning procedures or shampoo formulations.

Our objective was first, to develop an in vivo noninvasive method for quantifying the spread of sebum along the hair shaft. Secondly, we use this new method to define the overall kinetics of the hair-regreasing process among two cohorts of Chinese men with opposite self-perceptions of their scalp/hair greasiness (ie, greasy or not greasy).

Material and methods: One hundred and twenty-three Chinese men (aged 18–35 years) participated to the study. The technique used basically adapts the Sebumeter™ technology where supple polymer films are applied onto and along the hair shaft. The sampled hair sebum is further quantified by image analysis/increased transparency.

Results: The technique developed showed an adequate reproducibility under fixed conditions (pressure, investigators, scalp sites, etc.). In the two cohorts of subjects (eg, greasy, nongreasy), hair regreasing process was found sharing a same linear progression with time. The two cohorts of men presented significantly different values in the total amount of spread sebum by an approximately two-fold coefficient, with however comparable average values in the sebum amount present at the root region 48 hours post shampoo. At such timing, the spread of sebum reaches much longer distances in the greasy scalp cohort.

Conclusion: This technique appears promising for assessing the efficacy of cosmetic ingredients (or products) that aim at delaying a natural process that is daily and negatively perceived by consumers.

KEYWORDS

hair, hair greasiness, kinetics, sebum-coating

1 | INTRODUCTION

Some 100 000 human scalp hair follicles constantly deliver pre-formed sebum onto the human scalp ($\approx 650 \text{ cm}^2$ surface area in adults), leading to total amount of excreted sebum in the gram range, daily.^{1,2} Previous works²⁻⁴ have shown that, as compared to forehead, its neighboring region, the sebum excretion rate (SER) on scalp reaches much lower values. However, both sebum casual levels (SCL, equilibrium state of sebum in $\mu\text{g}/\text{cm}^2$) were found similar albeit of much different kinetics: the SCL of the forehead is reached in some 3 hours post cleaning, whereas in about 2 to 3 days on the scalp.^{2,3} The strong hormonal/androgenic dependence of sebum production (and its further excretion) naturally makes men stronger sebum producers than women.⁴⁻⁶ This hormonal dependence explains the scarcity of sebum on the skin and hair of prepubescent children.

Once produced by the sebaceous glands, sebum accumulates within the follicular duct and is further excreted sebum onto the whole scalp and progressively spreads along the $\approx 100\,000$ hair shafts present on the scalp. As previously published,² the root of these hairs (the emerging part) becomes sebum-coated 6 hours post shampoo (Figure 1 below). With time, sebum coats more distant hair regions, while some neighboring hairs appear being stuck/glued together.

This progressive spreading process, although scarcely studied,⁷ is driven by grooming (combing, touching, etc.) and individual hair characteristics (thickness, density, etc.). Post shampoo, this spread

of sebum becomes self-perceived by consumers, as a critical end-point when shampooing is felt necessary. In brief, hairs that were initially perceived as slightly greasy (ie, 1 day post shampoo) become later intimately judged as "dirty" since sebum is prone at trapping external compounds (dusts, volatiles from smokes or pollutants).^{8,9} In addition, sebum is highly sensitive to oxidization/per-oxidization processes through its poly-unsaturated compounds (squalene, fatty acids, etc.).¹⁰⁻¹² In overall, on longer times (3-4 days post shampoo), the head of hair becomes of a dull appearance, a condition that is quickly and easily abolished by a convenient shampooing procedure¹³⁻¹⁵: hairs then recover a clean and aesthetical appearance.

In some four decades, the act of shampooing has witnessed a clear trend in being performed with increased frequency, due to the successful improvements brought to shampoo formulae. From once a week (in Europe) forty years ago, the frequency of shampooing nowadays averages 4/week, possibly reaching 14 per week (morning and evening) in some Korean women. This frequency has an obvious incidence on the hair regreasing process since almost absent/unseen in subjects who wash their hair daily. However, it remains of a high prevalence among women whose shampoo frequency is usually much less for practical reasons (longer hairs need too much time for being dried off). In addition, some new developments in hair cleaning procedures (dry-shampoos, wipes, etc.) probably require additional criteria to being measured. All these reasons led us to revisit the kinetic aspects of such a process through a novel *in vivo* semiquantitative method that revisits and refines that used by the Lipometre® or Sebumeter® instruments^{2,16} by its adaptation to a previously described sampling method,⁷ slightly modified. The present paper aims at describing such approach and presenting its preliminary results.

2 | MATERIAL AND METHODS

2.1 | Subjects/Protocol

A total of 123 Chinese men (from Shanghai region) participated in the study. These were 18-35 years old and were recruited from local agencies specialized in part time occupations. Among these 123 subjects, 64 subjects were selected upon their self-perception of the greasiness status of their scalp (30 declaring "greasy" scalp and 34 declaring "nongreasy" scalp), 59 were selected as greasy scalp by measuring their sebum levels 1 day post shampoo, using the Sebumeter™ technique onto a small (approximately 1 cm^2) scalp region (vertex) that was preshaved in all subjects before the study. The scalp sebum levels of these greasy scalp subjects, according to a first sampling, exceeded $150 \mu\text{g}/\text{cm}^2$ (range $150\text{--}250 \mu\text{g}/\text{cm}^2$).

Aside from age criterion, exclusion criteria were the following: (1) free from a recurrent skin disease, (2) free from regular medication, and (3) able to visit our facilities (Pudong/Shanghai) with convenience, at different times according to the various scheduled protocols. They were all informed about the objective of the study and signed an informed consent.

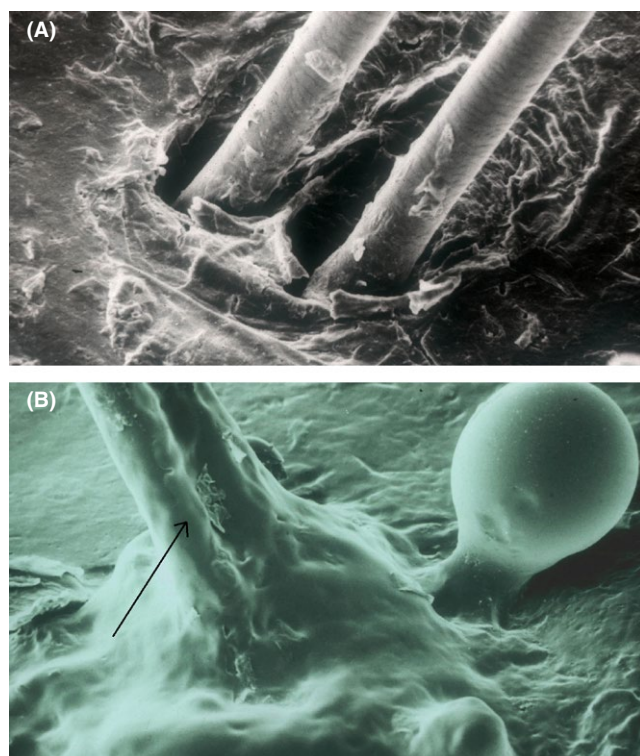


FIGURE 1 Imaging (S.E.M) a portion of the scalp surface, immediately after shampoo (a) and 24 h later (b) (L'Oréal Research & Innovation). The black arrow (1b) illustrates the coating of hair surfaces at their basal/root parts

Different protocols aimed at exploring different aspects of the developed technique, namely the site of hair sebum sampling, its variations with time (24 hours vs 48 hours), its dependence on manual pressure onto the hair surface, ie, possibly operator-dependent and the reproducibility of the measurements.

In all cases, subjects were shampooed in our facilities by trained technicians, using constant volumes of a bland Laureth-based shampoo (10 g, massaged for 3 minutes), followed by a copious rinse and hair drying. Subjects were asked to visit our facilities 24 and 48 hours later at about same times (± 1 hour according to transportation issues) allowing hair sebum to being sampled (see below). Before sampling, subjects were also asked to stay in a humidity and temperature-controlled environment for 20 minutes. Subjects were asked not to use any hair care product once the bland shampoo was applied, up to 48 hours, keeping on their grooming habits (combing, brushing).

2.2 | The process to being assessed

Figure 1A,B illustrate, through Scanning Electron Microscopy (S.E.M) of scalp surface replicas using a Silflo® polymer, the appearance of a clean scalp, immediately after shampooing (a) and 24 hours later (b), on the same subject. These replicas offer a perfect imaging of the skin or scalp surface relief, as previously published.¹⁶ The elevated sphere on Figure 1B is a water droplet, whereas sebum is clearly seen excreted as a magma-like material, spreading onto the emerging bases (the “roots”) of hairs that become to being stuck together. Of note, the sebum-coating prevents the cuticle scales to being observed.

2.3 | The Sebuprint® procedure

This procedure uses the mat tapes (0.8 cm width) provided by the Sebumeter™ technique (Courage & Kazakha, Köln, Germany), supplied as cartridges. Of a similar physical principle than that of previously published works^{2,7} that used frosted-glass plates, these polymer tapes become more transparent when covered by sebum. Hence, an increased transparency can be measured by photometry, the values of which being proportional to sebum amount (expressed as $\mu\text{g}/\text{cm}^2$). These tapes were selected for practical reasons; (1) commercially available and of a high reproducibility, (2) apt at being cut at different lengths, ie, prone at being applied onto hairs on rather long distances when needed (5, 10, 15, or 20 cm), (3) supple and deformable, they can easily adopt the natural curvature of hairs that follows the shape of the skull, (Figure 2A) from their root to more distant locations. When positioned onto a black background, the increased transparency of the tapes leads to increased black levels. In short, the blacker the tape, the higher the sebum amount.

2.4 | Hair sebum sampling

Similar to the Lipometre® or Sebumeter® techniques, sampling sebum along the hair shaft is therefore carried out under close

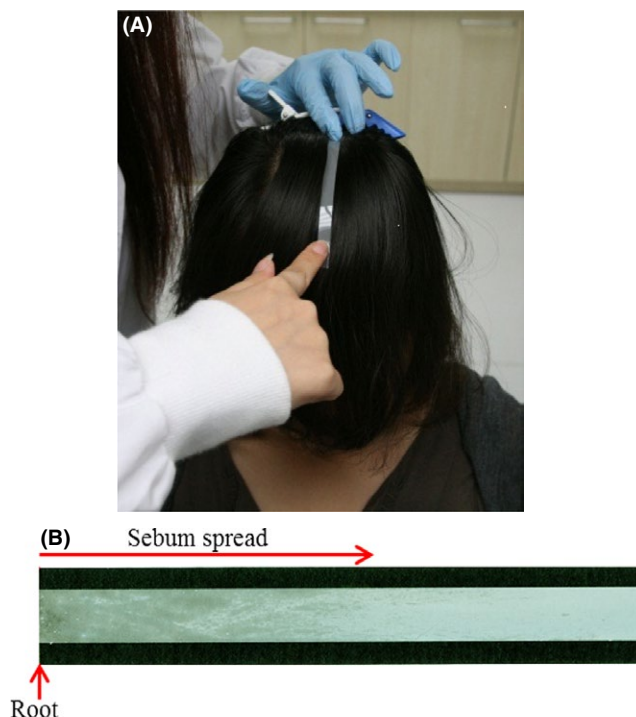


FIGURE 2 Illustrations of the hair sebum sampling procedure, from root to longer distances (A) and appearance of an applied mat tape post sampling (B)

contact, by applying a virgin mat tape onto the hairs, pressing all along the tape, with successive passages by a previously ethanol cleaned finger to avoid any deposit (lipids, dust, etc.) during a 5-second time of contact with the first 10 cm of the hair shafts. The use of latex gloves soon appeared as a source of variation as these did not slide evenly along the tape. Possible changes in hair thickness with length that could influence the hairs/tape contact were not assessed, assumed to being low along the first 10 cm.

Such sampling procedure cannot obviously collect all the sebum present along the hair shaft. It mostly aims at best reflecting the spread of sebum over hair length, as a semiquantitative index. As such, it allows evaluating the kinetics of the importance of sebum-coating along the fibers on a same site and exploring the possible influence of products (or ingredients) upon the progressive spread of sebum, post shampoo.

The sampling method is illustrated by Figure 2A,B.

2.5 | Measurements

Following sampling, the tape is positioned onto a flat and rigid cardboard of a black background (nil luminance value, $L^*=0$). A scanner (Scanjet G4010, HP, USA) allows the image of the tape to being recorded and stored. (Settings: Original Type: Photo, File Format: JPEG, Resolution: 600 dpi, Sharpness: Sharpen) Image is further selected from root up to the distance where sebum cannot be any more detected. The progressive decrease in blackness along the tape is quantified as black levels (=gray levels of a virgin

tape minus gray levels of a sampled tape), using the Photoshop™ CS4 Extended software. Repeated readings of the same tape led to comparable values of gray levels, ie, of low (2% to 3%) coefficients of variation ($SD/mean \times 100$), irrespective with the warming time of the scanner. Attempts to establish a strict relationship between the gray level of a given location of the tape and its respective amount of sebum (in $\mu\text{g}/\text{mm}^2$), through a solvent extraction, failed. These attempts faced two major drawbacks: (1) the high sensitivity of the polymer tapes to being solvent-degraded (heptane) and (2) mostly, the minute amount (μg range) of sebum present in a few mm^2 of the surface of the tape leads to a determination of an inherent low accuracy. Accordingly, the recorded black levels, reflecting the increase transparency of the tape, were assumed to directly reflect the density of sebum spread onto the tape, therefore taking the areas under the curves (AUC's) as acceptable indexes of the coating of sebum along the tape.

2.6 | Statistics

In all cases, Student *t* test (SPSS™ software package) and correlation coefficients were used for paired comparisons (between sampling procedures, cohorts, times) taking $P < .05$ as a threshold of statistical significance. All calculations were based by assuming a linear response, ie, allowing to express changes as % (cohorts, times), between the black levels of the recorded tapes and the amount of coated sebum, similar to the software included in the Sebumeter™ instrument.

3 | RESULTS

3.1 | Black level signals

Figure 3 shows, as example, the black levels along the tape, sampled from a same subject at 24 hours (green dots) and 48 hours post shampoo (red dots).

Figure 3 allows three major points to being noticed: (1) at 24 hours, sebum is undetectable at about 4.5 cm (referred here as

Y0) from the root, whereas still detected at 48 hours above 7 cm; (2) the root part in both cases shows rather close values, suggesting that sebum already saturated the root part 24 hours post shampoo in agreement with Figure 1B) and (3) the changes in black levels with distance seem, in both cases, following a linear ($Y = aX + b$) decrease where a negative “a” value indicates the slope of such decrease.

Accordingly, such observation led us to integrate the different black values with distance (x in the quoted equation), through the Photoshop™ CS4 extended, by calculating the area under curves (a.u.c), taken as the index of the total amount of hair-coating sebum.

3.2 | Influence of pressing by finger

To primarily ascertain the influence of pressing the tape onto hair, increased passages (5 and 10) with a finger were carried out on 31 subjects at 24 hours and 40 subjects at 48 hours post shampoo on two very close (<1 cm) scalp sites.

Figure 4 shows that increased passages lead to increased amount of sampled sebum. This likely results from a higher sebum collecting effect, possibly coupled with a more homogenous distribution of sebum along the tapes.

Figure 4 indicates that, at both 24 and 48 hours, all sampled amounts of sebum statistically differ ($P < .001$ according to paired

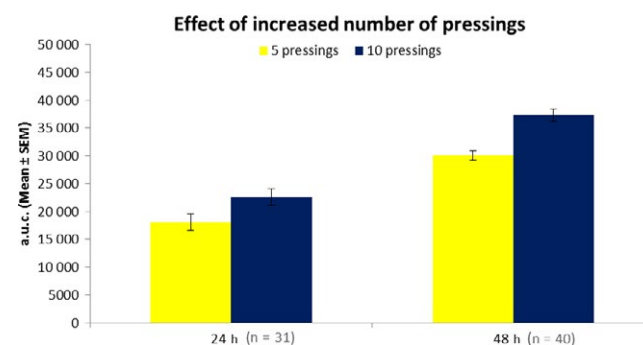


FIGURE 4 Effects of increased number of passages with finger along the sampling tape, at two times of samplings (24 and 48 h)

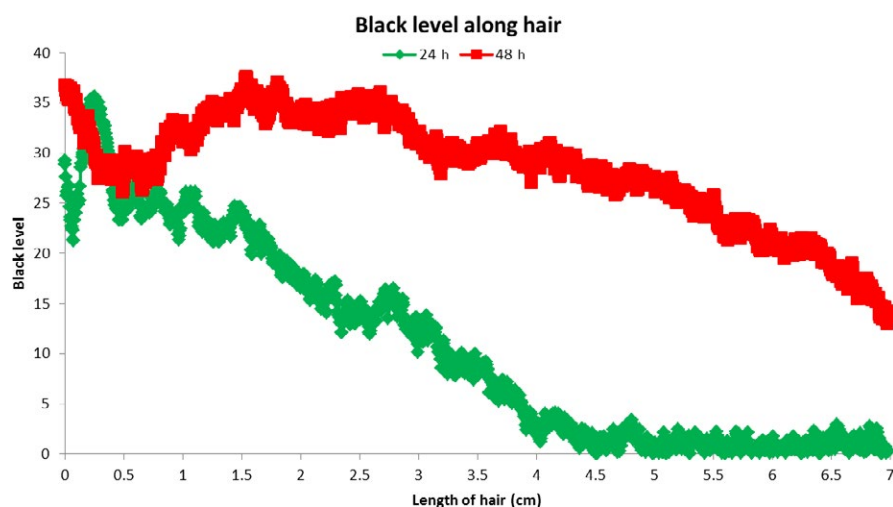


FIGURE 3 Variations in black levels along a tape that sampled hair sebum at 24 h (green) and 48 h (red) post shampoo on a same subject, as example

t test) and that 10 pressings allow significantly higher amounts of sebum by some 20%, assuming a linear response. Accordingly, the different protocols exposed in the following systematically adopted 10 passages. The development of a roller using a constant pressure is now under development for standardizing the contact and pressure between tape and hair surface, thus mitigating the possible variations between operators. However, the latter were assessed on a few subjects by two different operators and showed rather minor intervariations between the a.u.c.'s of sampled sebum.

3.3 | Influence of scalp locations

Sebum was sampled at three different scalp locations, as shown by Figure 5A, at different distances from the vertex/nose axis. Figure 5B illustrates the a.u.c obtained at 24 and 48 hours from 30 and 32 subjects, respectively. Colors used in Figure 5B correspond to those used in Figure 5A.

Figure 5B shows that, at 24 hours, the sampling location has an almost nil influence upon the amount of collected sebum, their a.u.c values being statistically identical. A slight but significant difference ($P = .011$) is observed at 48 hours between the center part (close to vertex, in blue) and the more lateral location (in green). Samplings carried out at 48 hours led to higher ($\approx +40\%$) and statistically different ($P < .001$) sebum values than those performed at 24 hours, resulting from the progression of sebum along the hair shaft. Hence, the middle location (close to vertex) was given privilege in the following studies exposed below.

3.4 | Reproducibility of the measurements

Eighteen subjects were asked to visit our facilities at 4 consecutive weeks, where sebum was sampled at 24 and 48 hours post shampoo by the same operator, using 10 passages with finger. Figure 6 shows highly reproducible a.u.c values. The three sets of data at 24 hours and 48 hours were, respectively, found not different, statistically. Once again, a.u.c values at 48 hours show same increased values as compared to those obtained at 24 hours, by about +45%.

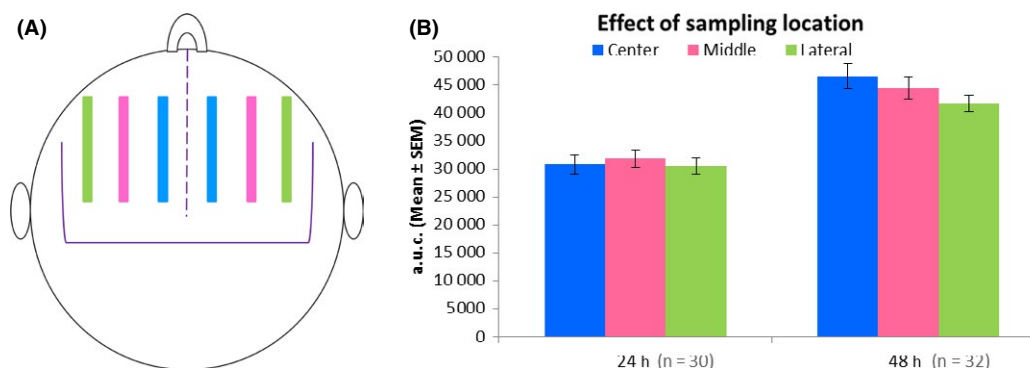


FIGURE 5 A,B, Three different scalp locations where sebum was sampled at 24 and 48 h. Figure 5 (regarding the new numerotation) summarizes the different a.u.c obtained on these three sites at the two sampling periods

3.5 | Agreement of measurements with self-declarations of subjects

Forty-seven subjects were selected for their different self-declarations with regard to their own feeling of hair greasiness: 23 self-declared presenting low hair greasiness, whereas 24 complained of greasy scalp or hairs. Sebum amounts were determined in the same conditions as mentioned above at 24 and 48 hours. Figure 7 summarizes the data obtained on these two cohorts, indicating that the values of a.u.c statistically differ ($P < .0005$), at both times. The “greasy scalp” group shows increased values by about 46% between 24 and 48 hours, whereas about twice more ($+119\%$) in the “non-greasy scalp” cohort. Of note, at 24 hours, the greasy scalp cohort shows a much higher (about twice) and significant sebum-coating than the one observed in the “nongreasy” scalp cohort.

4 | THEORETICAL AND PRACTICAL CONSIDERATIONS

The data afforded by the present technique suggest that the spread of sebum (ie, the black values) along the hair shafts of all subjects

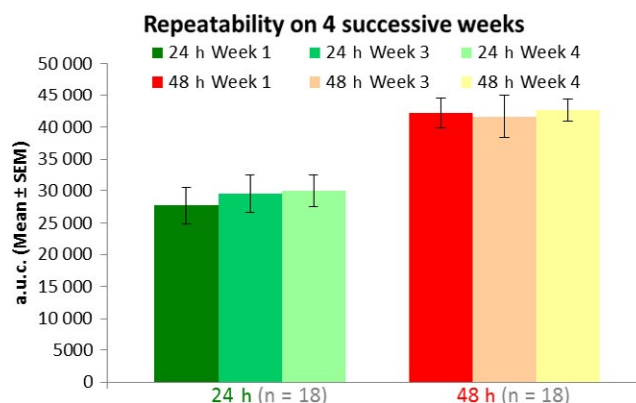


FIGURE 6 Repeatability, on four successive weeks on 18 subjects with greasy scalps of the collected amount of sebum at 24 and 48 h (18 subjects)

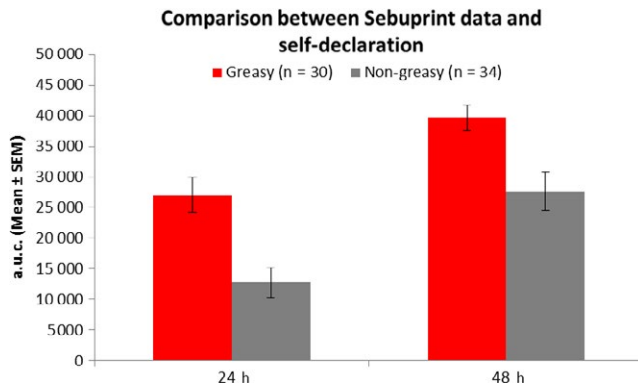


FIGURE 7 Hair sebum indexes (a.u.c.) sampled at 24 and 48 h post shampoo on two cohorts of subjects declaring opposite status of scalp greasiness

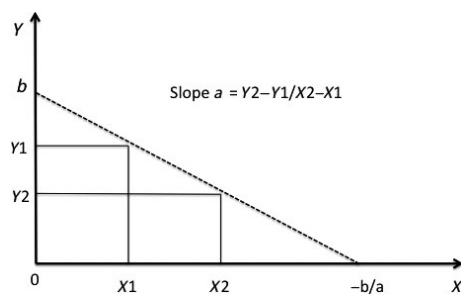


FIGURE 8 Theoretical approach of sebum spreading onto hair where Y = black level, X = distance (cm), “ a ” slope, b the value of black level at root, on a 24-h or 48-h collection

(perceived greasy or not) primarily obeys to a simple linear law (constant speed of sebum spreading) such as $Y = aX + b$ where “ a ” represents the slope (negative, in black level units/cm), X in cm, and b represents the black value of root as illustrated by Figure 8. In greasy scalp subjects, the hair root (approximately the first 5 mm of the band) is likely saturated by sebum as early as 24 hours post shampoo (Figure 1B, an amount constantly supplied by the continuous scalp sebum excretion). Statistical calculations of b , despite some intrinsic and unavoidable sources of errors (band inhomogeneity, uneven pressure, imprecise scanning etc.) show an acceptable constancy. On 96 individual data, gathering 24 hours and 48 hours values post shampoo, b was found rather constant, equal to 31.30 black level units ± 6.18 (SD) and/or ± 0.63 (SEM, standard error on the mean). The negative slope “ a ” (varying between 0 and $-\infty$) is calculated according to Figure 8. The global index of spread sebum (the a.u.c.) at a given time, once the slope “ a ” is determined, equals $-b^2/2a$ (“ a ” being negative) as the surface of the theoretical triangle.

Assuming that the averaged values of both groups at 24 hours and 48 hours (mean $\pm$ SEM) do reflect the typical kinetics of hair regreasing process, these were found significantly fitted ($r^2 = .87-.99$) with a $y = ax + b$ relationship, as shown in Figures 9 and 10. This linear fit may not be perfectly adequate at 24 hours in all subjects (Figure 10) with regard the very slightly curved shapes. Such representation allows the theoretical values of $-b/a$ to be determined,

ie, the distances (rounded data) from root (in cm) where sebum becomes absent along the hair shaft, indicated in Table 1. In short, greasy scalp subjects present a similar hair sebum spread at 24 hours than subjects with nongreasy scalp at 48 hours (8.4 vs 8.7 cm), suggesting an approximate two-fold regreasing rate.

The average black values at root (“ b ”) obtained in both groups seem similar (varying from 28.008 to 31.519 a.u.c.), at the exception of that obtained at 24 hours (19.371) in the cohort of nongreasy scalp. These data suggest that, in this cohort, the root part of the hair shaft at 24 hours post shampoo was not fully saturated by sebum.

The reference to the model proposed by Figure 8 allows the surfaces (eg, the area under curve) of the theoretical triangles to be calculated through the $-b^2/2a$ formula, as illustrated by Table 2. The latter clearly shows that, as index, the global amount of spread sebum in the greasy scalp cohort is about twice that of nongreasy ones at both timings. In addition, it confirms that, in each cohort, the spread of sebum is time-dependent at least up to 48 hours, ie, of a rather regular process.

Such theoretical approach, where all averaged data correlate with such linear regression of the $y = ax + b$ type (r^2 ranging .87-.99), indicates that sampling hair sebum at 24 hours post shampoo allows the progressive coating of hair by sebum to be assessed whereby the slope “ a ” translates the rate/speed of the sebum spread along the hair shaft.

5 | DISCUSSION

The present work aimed at revisiting the physical mechanism of a process visually perceived by consumers with regard to their own perception of greasy scalp, leading to hairs often seen as “dirt.” The semiquantitative methodology exposed here offers many positive aspects. Applicable in vivo, sensitive enough, reproducible, this technique seems a promising approach to both advanced and applied studies, once improved by a standardized method (operator-independent) of collection such as a pressuring roller or equivalent (under progress). The preliminary explorative steps carried out here allowed some major aspects in the natural hair regreasing process to be evaluated by analyzing the average values of two distinct cohorts of subjects (mean $\pm$ SEM). The somewhat large variations observed in individual values (S.D. of about 40% of the mean) are likely linked to different intersubjects sebum productions (as seen by the different sebum casual levels) and natural or uncontrolled factors (hair thickness, hair density, grooming, etc.), including the intrinsic variability of the methodology since the applied tape only collects an undetermined fraction (possibly varying with hair surface status) of total hair sebum, taken here as an index of spread sebum. Although the individual values follow comparable kinetics, they cannot shed some light on all the physical factors that fully govern the hair regreasing process. Hence, taking averaged values of black levels at various timings, the global kinetics may be established and defined by at least two parameters. At first, the slope by which sebum amount decreases along the hair shaft, and second,

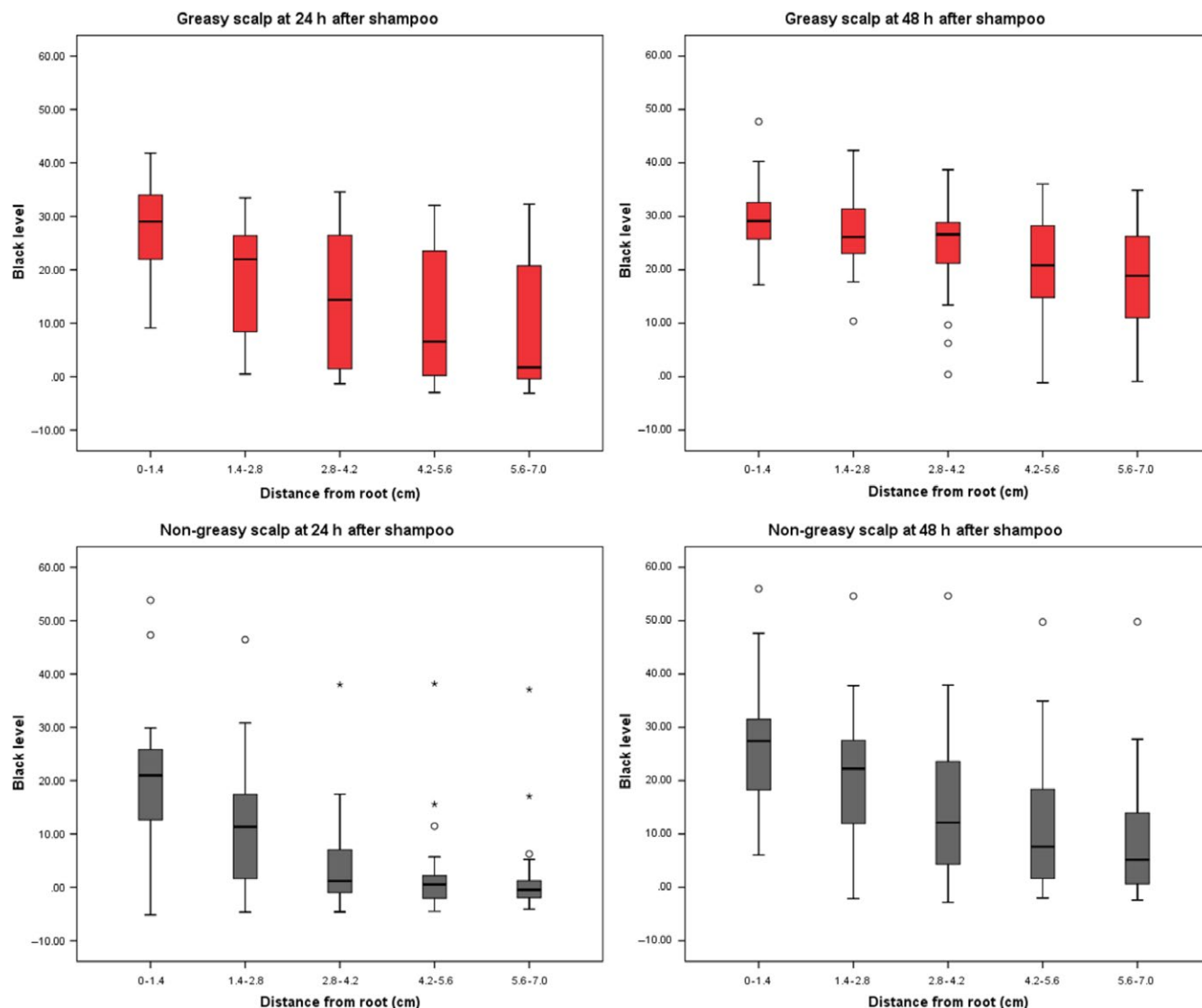


FIGURE 9 Average values ($\pm$ SD) of black levels at 24 and 48 h post shampoo in the greasy scalp cohort (N = 30, in red) and the nongreasy scalp cohort (N = 34, in black). °outlier values. *extreme values

the location from the hair root (in cm) where sebum cannot be detected ($-b/a$). In brief, combining both parameters define the average rate of sebum spreading along the hair fiber, according to the perceived scalp greasiness. The data here presented indicate that a 24-hour sampling is a reliable parameter since more prone at defining both parameters. A 48-hour sampling, although useful, does not allow the distance from the root where hair remains un-coated by sebum to being precisely determined—unless extrapolating a theoretical $Y = aX + b$ function. The present work has shown that the root part does not much differentiate “greasy scalp” subjects from “nongreasy scalp” group at 48 h. In the greasy scalp cohort, the root part seems in fact early (within 24 hours) saturated with sebum, a phenomenon partly observed in the nongreasy subjects. It should be emphasized that the observed transparency of the mat tape in this region may suffer from a technical bias where an increased density of collected sebum does not necessarily lead to an increased transparency by a possible saturation effect of the band.

Taking the calibration curve of the Sebumeter® as model, such saturation effect occurs at sebum amount above $300 \mu\text{g}/\text{cm}^2$ or $3 \mu\text{g}/\text{mm}^2$, assuming a homogeneous density of sebum. Whatsoever, this native emerging hair region appears continuously supplied with a complex lipid mixture that spreads along the hair shaft at an apparent regular rate. At least three primary factors that influence such sebum progression are, we believe, of importance: (1) the hair density that closely conditions the contacts between adjacent hairs, (2) the thickness of the hair shaft, and (3) grooming/comb/brushing procedures that may favor an additional spread out of sebum.

Chinese subjects, as many Asian subjects, present an average hair density of $200/\text{cm}^2$ of thicker (and rounder) hairs¹⁸ than other ethnics.^{18,19} It is reasonable to assume that, all other equal factors, thick hairs present a slower rate of regreasing process than thin hairs, by offering a larger coating surface. On such aspect, the determination of the slope “a” is of importance since integrating other factors, ie, the physico-chemical status of the hair surface (lipophilic/hydrophilic

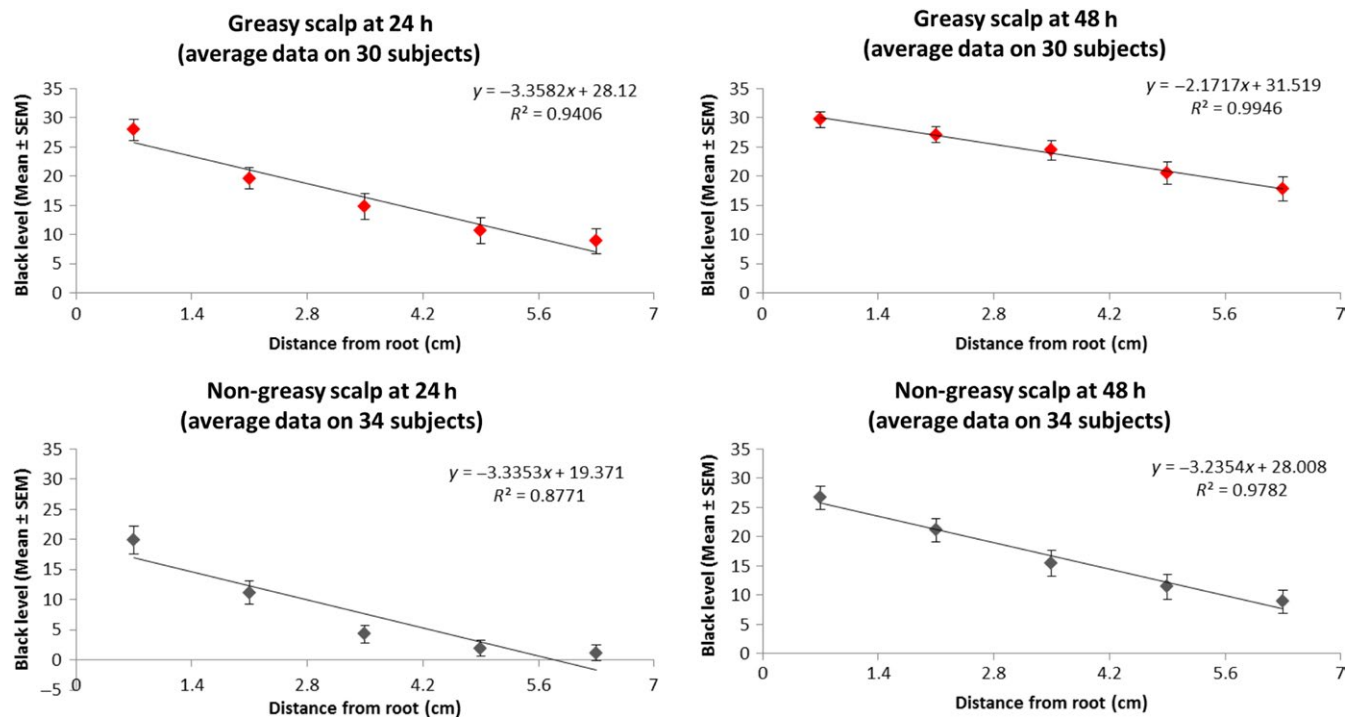


FIGURE 10 Linear correlations between averaged black level values ($\pm$ S.E.M) with time post shampoo and distance from hair root in the two cohorts, as extracted from Figure 9

TABLE 1 Distances ($-b/a$) where sebum becomes statistically absent in the two observed cohorts at 24 and 48 h. Calculated from the fitting equation $y = ax+b$

Group/Timing	$-b/a$ at 24 h (cm)	$-b/a$ at 48 h (cm)
Greasy scalp	8.4	14.5
Nongreasy scalp	5.8	8.7

TABLE 2 Calculations of the area under curve of black levels (rounded figures) through the formula $S = -b^2/2a$ found in both cohorts (G, Greasy scalp; NG, Nongreasy scalp)

Parameter/timing	24 h	48 h
a (NG)	-3.3	-3.2
a (G)	-3.4	-2.2
b (NG)	19.4	28.0
b (G)	28.1	31.5
Theoretical surface (in a.u.c)		
NG	57.02	122.5
G	116.1	225.5

balance) and the variable individual sebum productions. In short, whereas the slope “a” integrates various physical factors, the “b” value is likely more sensitive to biological (hormonal) or pharmacological factors (anti-androgens, 13-*cis* retinoic acid, etc.) that regulate the sebum synthesis by the sebaceous glands and its further excretion.^{5,6}

Overall, the methodology presented here seems offering a new approach for applied studies: any ingredient (or product) that

reduces the “a” slope would not only objectively appear as an efficient help in the management of greasy hairs but would also likely be self-appraised by consumers. On an applied viewpoint, further studies are needed to determine how some shampoo-based ingredients (other than surfactants) may favor—or not—the spread of sebum along the hair shafts. Besides, the present data clearly indicate that the self-appraisal of our studied subjects (eg, greasy vs nongreasy scalp) much reflect a reality, quantitatively speaking.

To summarize, the present work seems well revisiting a process the mechanism of which has been, since the 1970s, likely too neglected by cosmetic researchers despite its unaesthetical impact in real life and the continuous progresses in hair cleaning procedures.

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