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Characterizing Scalp Stratum Corneum Turnover： A Novel Application of Dansyl Chloride Photography

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Abstract

INTRODUCTION: Dandruff, a common scalp condition affecting a significant portion of the global population, is linked to altered stratum corneum (SC) turnover. SC turnover time is reportedly faster in those with dandruff compared to healthy scalps. Current methods for assessing SC turnover are often invasive, highlighting the need for innovative approaches. This research explores and refines a novel, non-invasive method using dansyl chloride (DC) staining and photographic imaging (optimized for scalp application) to investigate stratum corneum turnover time differences between dandruffaffected and healthy scalps, potentially advancing anti-dandruff research.

METHOD: Fifteen participants (six healthy, nine with dandruff) were enrolled. A 5% DC solution was applied to the shaved areas of the scalp for 12 hours in a controlled environment (21±1°C, 45±5% RH). Prior to DC application, a twoweek washout period using a standardized non-anti-dandruff shampoo was implemented. Post-staining, fluorescence images were captured using a dermascope under UV light after each shampoo (non-anti-dandruff shampoo) application (three times per week). The time for fluorescence to decrease by 95% was measured, representing the SC turnover time.

RESULTS: The 5% DC application proved successful and reproducible on the scalp, demonstrating no significant difference in fluorescence intensity or area between healthy and dandruff-affected scalps immediately after staining. The stratum corneum turnover time, measured by 95% fluorescence fadeaway, was significantly faster in dandruff-affected scalps (10.6±2.8 days) than in healthy scalps (17.7±2.6 days). This faster turnover rate in dandruff-

affected scalps was also reflected in the more rapid decline of the fluorescent area after repeated shampooing.

DISCUSSION & CONCLUSION: This research validates the novel application of the DC method for quantifying stratum corneum turnover specifically on the scalp, offering a non-invasive alternative to traditional biopsy techniques. The successful differentiation of turnover times between dandruff-affected and healthy scalps, with a quantifiable analysis of fluorescence fading, highlights the method's sensitivity and potential for future research. This innovative approach provides a promising new tool for investigating scalp conditions like dandruff and opens exciting avenues for developing targeted anti-dandruff treatments.

Key Words: Dansyl Chloride, Stratum Corneum Turnover Rate, Dandruff, Malassezia, Barrier Function.

1. Introduction

Dandruff is a very common scalp condition, with upwards of 50% of the global adult population reported as affected, irrespective of gender and ethnic group. Dandruff normally appears as white flaky scales on the scalp and hair roots. Dandruff scale is a cluster of corneocytes that cohere together and detach from the stratum corneum [1-5]. *Malassezia* is a lipid-dependent genus of yeasts widely reported as the key factor in dandruff, while it is also present on healthy scalps [6-8]. Previous investigations have elucidated that *Malassezia* degrades sebum, freeing multiple fatty acids from triglycerides, consuming specific saturated fatty acids for its proliferation, and leaving behind the unsaturated fatty acids [9]. One of the *Malassezia* species, *Malassezia restricta* is reported to be strongly correlated with dandruff. Research on both Chinese and French populations have published that there is a higher incidence of *M. restricta* on dandruff-affected scalps compared to healthy scalps [3,4]. However, individual differences in the abundance of *M. restricta* among populations with and without dandruff cannot be ignored. The balance of the microbiome environment plays an important role in scalp health. A growing body of studies reveals that dandruff is not a single-factor condition. Multiple factors interact with each other in dandruff, including the balance of the microbiome, sebum secretion, the barrier function of the scalp and individual susceptibility [9-12].

Stratum corneum turnover time is a functional indicator of the epidermis. SC (stratum corneum) turnover rates, of course, vary among regions of the body based on their horny layers and the completion of functional structure. The stratum corneum that is mainly composed of proteins and intercellular lipids is the main barrier to percutaneous absorption [1] and prevents trans-epidermal water loss (TEWL). The lipid component forms an important part of the skin barrier [1]. Skin barrier function can break down as a result of the penetration of modified sebaceous secretion into the stratum corneum. Recent data have demonstrated that the changes in sebum composition over time are a direct result of Malassezia metabolism [9]. The dandruff severity is consistent with impaired barrier function indicated by higher trans-epidermal water loss [9]. In most of the body regions in revealed studies, the stratum corneum turnover time is around 2 weeks [13]. The stratum corneum turnover time of dandruff-affected scalp is reported to be faster than that of healthy scalp [14]. Maintaining the complete functional structure of the barrier is important for resisting irritants. Impaired skin barrier function results in inflammation

leading to epidermal proliferation [10]. A deeper understanding of stratum corneum turnover rate in the scalp is important for the study and treatment of dandruff.

Generally, the biopsy method labeled with tritiated thymidine has been reported to determine the stratum corneum turnover rate on the scalp. It has limitations in application to cosmetics due to its radioactivity and invasiveness. Dansyl Chloride (5-dimethylamino-1-naphthalene-sulphonyl chloride) functions as a fluorescent marker through its reaction with amino groups, enabling the measurement of stratum corneum renewal time. Previous investigations have proved that dansyl chloride is a noninvasive and non-radioactive method to observe the turnover rate of the horny layer [15-17]. Dansyl chloride (DC) can stain the horny layer but will not stain deeper than the granular layer [16]. By tracking the fluorescence fading period, we can determine the turnover rate of the stratum corneum. Most recorded applications of DC are on the forearm and facial skin. The application of DC on the scalp is rather scarce. It's a new attempt to apply a DC patch on the scalp in this study to determine the stratum corneum turnover time of the scalp.

Accordingly, this work aimed to (i) enhance the dansyl chloride staining method on the scalp and (ii) investigate the stratum corneum turnover time between dandruff-affected scalp and healthy scalp.

2. Material and Methods

Subject recruitment and dandruff scoring

This study adhered to the tenets of the Declaration of Helsinki. All subjects were formally informed and signed consent letters.

A mixed-gender group of subjects aged between 19 and 55 years took part in the study. Subjects who accepted to have two 2.5cm by 2.5cm areas shaved on their vertex were enrolled, excluding those with seborrheic dermatitis and allergies to aluminum, adhesives, and tapes. Scalps were classified as healthy (<0.5) or dandruff-affected (≥1.5) by a trained expert according to a dandruff atlas. The scalp was divided into 8 areas (Fig. 1). Each area was graded (0-5, 6-point scale) separately, and the average score of the 8 areas was calculated. A total of 15 subjects (8 men, 7 women) were recruited, of whom 6 were in the healthy group and 9 were in the dandruff-affected group.

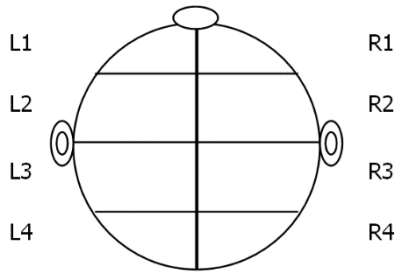


Figure 1. Adherent dandruff grading test area

Dansyl Chloride (DC) Patch Treatment

A 5% DC solution was prepared by mixing DC powder with Paraffin liquid. The mixture was dropped onto the filter of an 8mm occlusive patch chamber until saturated. The occlusive patch with 5% DC solution was applied to the vertex of the scalp as demonstrated in Figure 2, which had already been shaved (2.5cm by 2.5cm) before treatment. Subjects were instructed to stay in a controlled environment room (21±1°C, 45±5% RH) for 12 hours. After 12 hours, a DC fluorescence image was taken using a dermascope, which is a photographic tool that captures magnified images of the scalp under ultraviolet illumination at 395 nm wavelength. This image served as the baseline image.

Figure 2. Example of 5% DC patch applied on shaved area (2.5cm by 2.5cm) of vertex.

Shampoo Treatment

The test shampoo used in this study was a regular cleansing shampoo without anti-dandruff claims. Before the DC application, subjects were required to use the test shampoo for 2 weeks as a washout stage to standardize the scalp condition of enrolled subjects. All shampoo applications (3 times per week) were performed by a technician on-site after DC staining. 12g of shampoo was applied to the scalp and hair according to a standard shampooing procedure. This procedure aimed to standardize the entire study process to ensure quality.

Fluorescence Fadeaway Time (Days)

Fluorescence area was computed using a dermascope tool in ultraviolet light illumination mode by measuring the number of fluorescent pixels. The baseline level (T^0) was captured immediately after 12 hours of occlusive application of the 5% DC patch. The subsequent fluorescence images (T^1) were captured after each shampoo washing. Fluorescence fadeaway time (in days) was recorded when the fluorescence fadeaway rate fell below 5%. The test duration depended on the time it took for the fluorescence to fade away completely.

$$Fluorescence\ Fadeaway\ Rate\ (\%) = \frac{T^1{}_{\text{number of fluorescence pixels}}}{T^0{}_{\text{number of fluorescence pixels}}} \times 100\%$$

Statistics

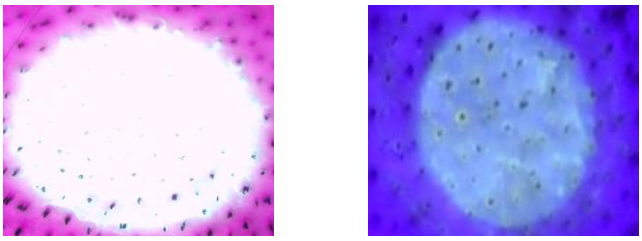
All quantitative data are expressed as mean ± standard deviation (SD). Student's t-test was used, with $P < 0.05$ considered as the threshold for statistical significance.

3. Results

DC Patch Application on Scalp

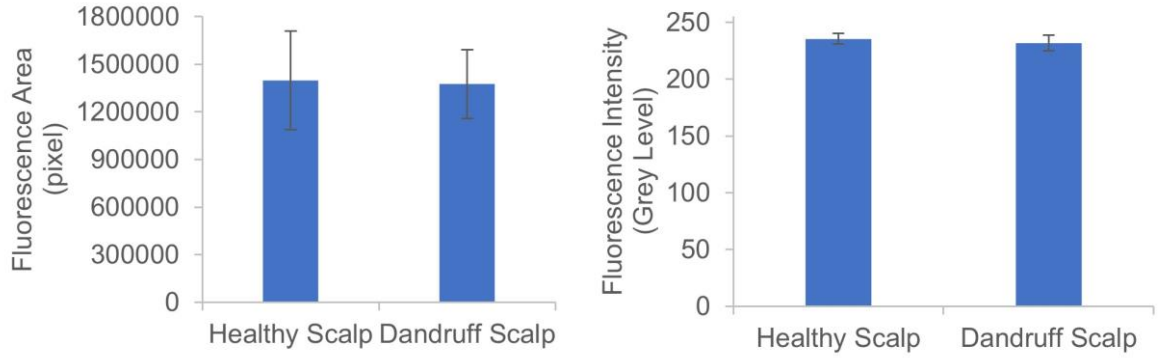
The DC fluorescence photographs were shown in Figure 3, where Figure 3a was captured by a dermascope in ultraviolet illumination mode and Figure 3b was captured by a digital camera with an ultraviolet light source. The effects presented by the two approaches were quite similar. The fluorescence on the scalp after staining was easily observable under ultraviolet light for both methods.

Quantitative assessment of the fluorescence area immediately after 5% DC patch application showed no significant difference between the two groups (healthy scalp vs. dandruff-affected scalp) (Fig. 4a). This indicated that DC application on the scalp was reproducible. There was no significant difference in fluorescence intensity between the two groups immediately after staining (Fig. 4b), indicating that the staining depth was similar between the two groups. These results demonstrated that the new approach of applying a 5% DC patch on the scalp in a controlled environment was feasible.



(a) (b)

Figure 3. The DC fluorescence effect photographs captured by (a) dermascope in ultraviolet illumination mode and (b) digital camera together with ultraviolet light source.



(a) (b)

Figure 4. The comparison of fluorescence area (a) and fluorescence intensity (b) immediately after 12h application of 5% DC patch between healthy scalp and dandruff affected scalp.

Stratum Corneum Turnover Rate of Scalp

The mean fluorescence fadeaway time (days) of healthy scalp (17.7±2.6 days) was significantly longer than that of dandruff-affected scalp (10.6±2.8 days; $P<0.05$) (Fig.5a). This detected that the stratum corneum turnover rate of dandruff-affected scalp was significantly faster than that of healthy scalp. The dynamics of average fluorescence area between the two groups were illustrated in Figure 5b, which showed that the fluorescence area disappearing speed of dandruff-affected scalp was faster than that of healthy scalp.

A typical demonstration of fluorescence diminishing during regular shampoo washing (3 times per week) after DC staining was illustrated in Figure 6. The fluorescence had disappeared on dandruff-affected scalp 10 days after DC labelling (Fig 6(a)), while it was still present on healthy scalp (Fig 6(b)). This demonstrated that the difference in cell renewal rate between healthy scalp and dandruff-affected scalp could be determined by this method.

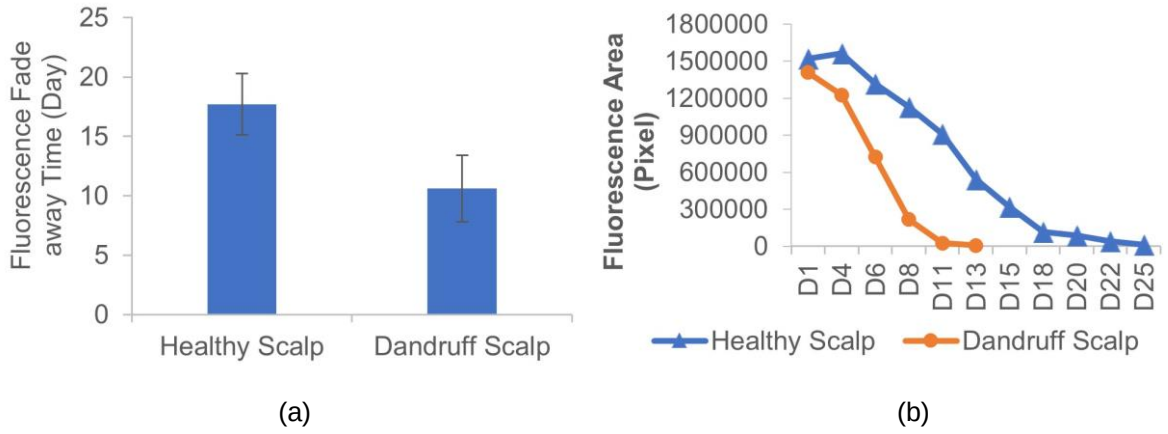


Figure 5. The comparison of fluorescence fadeaway time (days) (a) and the fluorescence area dynamics (b) between healthy scalp and dandruff affected scalp.

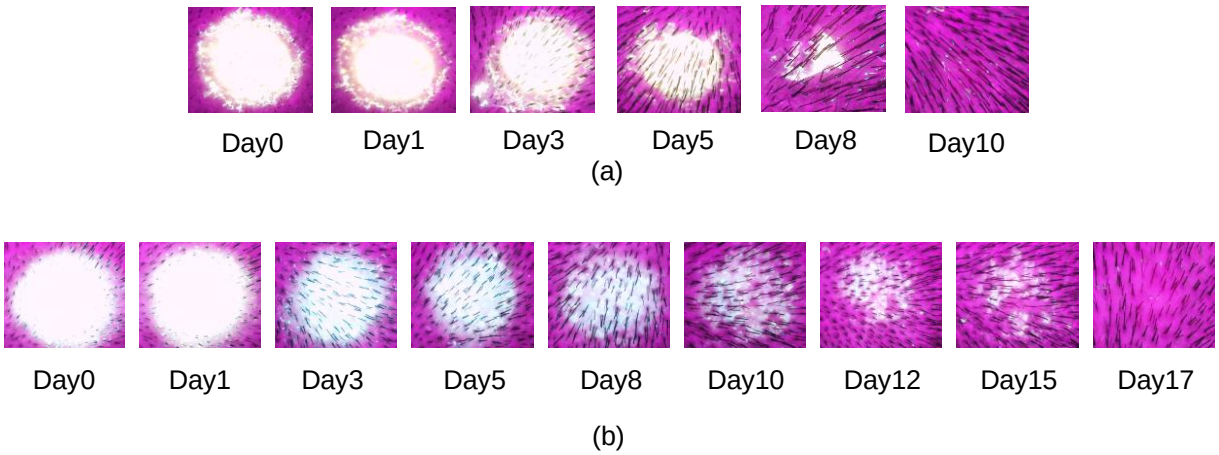


Figure 6. Illustration of fluorescence diminishing period after DC staining on (a) dandruff affected scalp and (b) healthy scalp.

4. Discussion

The results of this study demonstrate that the 5% DC patch is feasible to apply on the scalp to determine the turnover rate of scalp stratum corneum. The difference observed between healthy scalp and dandruff-affected scalp aligns well with previous investigations that revealed the SC turnover rate of dandruff-affected scalp is around 2 times faster than that of healthy scalp [14].

Visual observation is widely applied to evaluate dandruff severity, sometimes together with photographic methods. The dandruff scoring method remains a gold standard in dandruff severity evaluation, despite its dependence on expertise. These methods focus more on final appearance investigation of dandruff, regardless of period investigation of cell renewal. This study aimed to offer a new dimension to visualize the period of desquamation with fluorescence labeled by DC to quantify the SC turnover rate of the scalp. Compared to biopsies method labeled with tritiated thymidine, DC patch application is a noninvasive and non-radioactive method. Higher SC turnover rate is one of the symptoms of dandruff-affected scalp [14].

The 5% DC patch is widely applied to determine the desquamation rate of SC, while the literature on DC application dealing with the determination of scalp SC turnover rate is scarce. Most of the literature [13,15-17] has investigated the cell renewal rate on facial skin, forearm, and other regions of the human body. Our study represents a new attempt to apply a 5% DC patch on the scalp together with a photographic method to determine the SC turnover rate on the scalp. A dermascope with ultraviolet light mode was applied to photograph the DC fluorescence in this study. Compared to the digital camera photographic method, the dermascope offers a more convenient and direct way to take fluorescence photographs for quantitative and qualitative aspects of cell renewal on the scalp.

Generally, 24-hour occlusive treatment of 5% DC patch [13,15-17] is used on facial skin or forearm, while overnight treatment presents a significant challenge in guaranteeing the fluorescence effect during sleep for subjects. Especially in hot and high humidity environments, sweat and sebum secretion on the scalp can have a substantial impact on the staining effect of DC. In this study, a 12-hour application of 5% DC patch in a controlled environment was conducted to ensure better fluorescence effect. Previous studies have revealed that longer occlusive treatment of 5% DC patch stains more layers [16]. Other evidence has demonstrated that 16h exposure is sufficient to stain the full depth of stratum corneum [18].

This study explored the SC turnover rate difference between healthy and dandruff-affected groups. The controlled standard environment provided better control over the entire test design. The discrimination between dandruff-affected and healthy groups was well differentiated, which demonstrates that a 12-hour application of 5% DC patch is sufficient to distinguish group differences under well-controlled conditions with limited sample size. With shortened and controlled application, test efficiency and quality can be improved. However, it is still worth investigating the stain layer difference between two groups when extending the application time.

This study demonstrates that the DC labeling method is a promising approach to visualize the cell renewal rate on the scalp and distinguish the difference between healthy scalp and

dandruff-affected scalp. This work may pave the way for anti-dandruff research and treatment strategies.

5. Conclusion

Our research has demonstrated that the dansyl chloride method can be effectively applied to determine the stratum corneum turnover time of the scalp through quantitative analysis of the fluorescence area after staining. The developed method can be utilized to study the scalp turnover rate differences between various scalp conditions and healthy scalp. This approach may pave the way for further research in areas such as anti-dandruff treatments and related therapeutic strategies.

6. Acknowledgments

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7. Conflict of Interest

All authors are employed by L'Oréal.

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