AN IMAGING-BASED METHOD TO EVALUATE ANTI-ACNE EFFICACY AT EARLY STAGE

LI Shuting[1]*, ZHOU Zhijun (Amanda) [1], LIU Xin (Sean) [1], WANG Hequn (Tracy) [1], FLAMENT Frédéric[2], STEEL Andrew[1]

1 Research and Innovation Department, L'Oréal China, 550 Jinyu Road, Pudong, Shanghai, China;

2 Research and Innovation Department, L'Oréal,188-200 Rue Paul Hochart, 94550 Chevilly-Larue, France

* LI Shuting, No. 550 Jinyu Road, Pudong, Shanghai, P.R.China 201206, 86 21 20611784, shuting.li@rd.loreal.com

Abstract

Background: Acne is a common skin condition with a high prevalence, worldwide. Current method to clinically evaluate treatment efficacy for individuals with acne prone skin relies on the observation of the evolution of the clinical inflammatory and non-inflammatory lesions. However, before the acne lesions emerge, to our knowledge, there is no way to define and characterize acne proneness through visualization. Hyper-keratinization of follicle is one of the main pathogenic factors of acne development, which happens underneath skin surface and even at the early stage before clinical lesions form. Reflectance confocal microscopy (RCM) with its nature of having high image contrast from keratin structure, opens a new window onto the in-vivo characterization of follicle and pore health at microstructure level and enables the possibilities to provide management plans and interventions for acne prone skin at early stage.

We aim to explore the structural differences between normal skin and acne prone skin with macroscopically normal appearance, thus to characterize the features of acne prone skin and to build new method to evaluate anti-acne efficacy.

Methods: 10 volunteers with acne-prone (AP) skin and 5 volunteers with less acne-prone (LAP) skin were recruited and completed this clinical trial. RCM was applied to explore and analyze the microstructure of hair follicles on normal-looking skin of both groups. Volunteers with AP skin were asked to use a serum product with peeling ingredients (glycolic acid, salicylic acid, capryloyl salicylic acid) for 4 weeks. Product efficacy on microstructure of hair follicle was evaluated after 4-week of application. Correlation among RCM measurement and self-claimed acne history were also analyzed.

Results: This study showed that the size of the follicles in AP skin range (80 to 230 um) is larger than LAP (50um to120 um) skin follicle's size at epidermis level. AP skin also found a higher ratio of hyper-keratinized hair follicle (51.39% vs.26.24%), higher ratio of thick keratinized border (25.48% vs.11.20%) more irregular shaped hair follicle (25.27% vs.16.00%), and more hair follicles with inner keratin contents (14.09% vs. 6.70%) than LAP skin. RCM measurement results also showed a high correlation with self-claimed historical acne frequency. It was also found that after 4-week treatment, the ratio of hyper-keratinized hair follicles reduced 12.25% ($p<0.05$) and the ratio of thick keratin border decreases 10.51% ($p<0.05$) for AP skin group.

Conclusions: A microscopic image-based method has been developed to characterize the hyper-keratinization of the follicles and acne proneness, which enables a new direction of clinical assessment on anti-acne and acne-prevention efficacy.

Key words: acne prone skin, hyper-keratinization, hair follicle, reflectance confocal microscopy (RCM), peeling ingredients.

Introduction

Acne Vulgaris is a common skin condition of pilosebaceous unit [1], which affects most adolescents and adults with a high prevalence in worldwide [2-4] and has negative impact on the quality of life (QOL) of acne patients. Four well-known pathogenetic factors of acnes include the increased sebum production and over-activated sebaceous gland, hyperkeratosis of ingrainfundibulum and sebaceous duct, P.acnes colonization, and inflammation [5,6]. Hyper-keratinization of hair follicle is one of the major pathogenic factors of acne development, but the hyper-keratinized hair follicles are usually unrecognized and could not be observed by naked eyes [6]. Previous studies have indicated that micro-comedones are the initial lesion in acne, and originate from hyper-keratinization of the infundibulum and sebaceous duct [6,7,8]. Previous work also showed that the biopsy sections of normal-looking skin in an acne-prone individual will frequently (28%) demonstrate histological features of microcomedones [9,10]. It is known that there are indeed invisible micro-comedones of normal looking skin and it is necessary to apply topical solutions not only on clinically apparent lesions but also on the whole face [6]. Moreover, micro-comedones significantly decrease during therapy but rebound almost immediately after discontinuation of a topical retinoid treatment solution. Therefore, a maintenance therapy to reduce

the potential for recurrence of visible lesions should be considered as a critical part of routine acne treatment [11].

Traditional clinical diagnosis method of acne problem relies on the clinical diagnosis of inflammatory and non-inflammatory lesions by dermatologist. Prior to the clinical acne lesions formation, the characterization of micro-comedone usually relies on the immunohistochemistry staining methodology, which is an invasive method that requires skin biopsy. There is not yet an effective non-invasive method to define and characterize microcomedones and acne proneness through visualization. Particularly, there is lack of non-invasive methodology to evaluate and demonstrate product performance on anti-acne and acne prevention at the early stage. To our best knowledge, reflectance confocal microscopy (RCM) with its nature of having high image contrast from keratin [12], can visualize the hyper-keratinization features of the follicles in acne patients with normal looking skin. This provides an opportunity to characterize the morphology of follicles and pore health at microstructure level noninvasively and in vivo. At the same time, it also enables new possibilities of acne management plans of for acne prone (AP) skin subjects at early stage.

In present work, we aim to explore the morphological differences of hair follicles between normal/healthy skin of less acne prone (LAP) volunteers and normal-looking skin of AP volunteers, to characterize the features of AP skin and to build new method to evaluate anti-acne efficacy at the early stage. Furthermore, we aim to investigate the correlation of the characteristics of hyper-keratinization of hair follicle with the frequency of acne and build the prediction model of acne occurrence.

Materials and Methods

To characterize the difference features of acne-prone skin and normal/healthy skin, 5 less acne prone (LAP) skin volunteers (Cell 1) 10 acne prone (AP)skin volunteers (Cell 2) and are enrolled in this study. AP and LAP skin type are screened by acne historical questionnaire based on the frequency and severity level of acne declared by volunteers and final classification are done by dermatologist based on the result of acne history check and on-site clinical evaluation. AP skin volunteers self-claimed that they have acne history in the adolescence and suffered from mild to moderate acne problem with higher frequency and more severe acne problem (Grade 2-3), and they

are often disturbed by acne problems. Whereas the LAP skin volunteers are self-declared without acne history and not disturbed by acne problems. Trans epidermal water loss (TEWL) of cheek area of both AP and LAP skin volunteers are measured by Vapormeter (Delfin Technologies, Finland).

Reflectance confocal microscopy (RCM) (Caliber.I.D Vivascope®1500 Multi-wavelength) is introduced to characterize the feature of morphology of hair follicle of normal-looking skin area (lower cheek area) of both AP and LAP skin volunteers. Block scan images on each relevant skin layer (stratum corneum, granules, spinosum, basal layer) are captured; single follicles are then focused with depth scan to visualize the 3D structure of hair follicle. Follicle size, ratio of hyper-keratinized follicle, ratio of follicles with thick keratinized border, ratio of irregular shaped follicle, ratio of follicle with inner keratin content are researched (Fig 1). Ratio of follicles present with specific features = (Number of follicles with specific features/total number of follicles) *100%. The differences of above attributes between AP skin group and LAP skin are analyzed and compared. In order to understand the correlation between morphological features of hair follicles and occurrence of acne, correlation analysis of between RCM measurement result and acne history check result are analyzed. After obtaining all the attributes and researching the correlation, key attributes correlated to the acne occurrence are included as the training data set through machine learning method to pre-diagnose the two types of skin: AP skin and LAP skin.

In this study, a 4-week treatment was only performed on the AP skin subjects. For the AP skin subjects, product efficacy on the improvement of hyper-keratinization of hair follicle are analyze after 4-week application of an anti-acne product with peeling ingredients (glycolic acid, salicylic acid, capryloyl salicylic acid). Product efficacy on microstructure (hyper-keratinization, shape, and diameter of hair follicle duct etc.) of hair follicle of AP skin was evaluated after 4-week of application (T4wk) by comparing with baseline (T0, before product application).

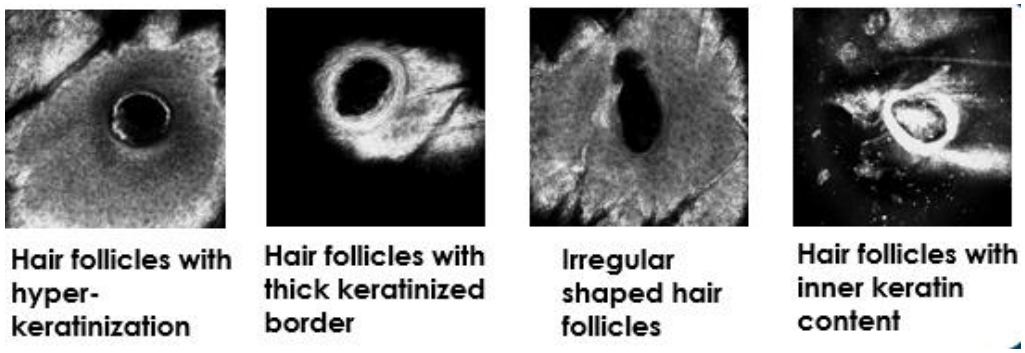


Fig 1. Illustration of different features of hair follicles in RCM imaging, from left to right shows hyper-keratinized follicle, follicles with thick keratinized border, ratio of irregular shaped follicle, follicle with inner keratin content

Statistical analysis was performed using SPSS (version 25.0, IBM). Continuous data with normal distribution were compared between two groups using the Student t test. Continuous data with non-normal distribution were analyzed using non-parametric tests. Repeated measured continuous data were analyzed using ANOVA or Wilcoxon rank sum test. Categorical data were expressed as frequencies and percentages and analyzed using the Chi-square test or Fisher's exact test. Two-sided $P<0.05$ was statistically significant. Pearson coefficients were computed between each RCM measurement results and self-claimed historical acne frequency and severity.

Results

Fifteen volunteers aged from 21 to 40 years old, are divided into 2 groups, AP skin and LAP skin. The demographic information is shown in below table. (Table 1)

Table 1 Demographic information of all 15 volunteers

Group	Sample size	Acne proneness	Age	Skin type			Acne frequency*				Acne severity*			
				Oily	*Combination*	*Dry*	*0*	*1*	*2*	*3*	*0*	*1*	*2*	*3*
Cell 1	5	LAP	27-40	0	2	3	5	0	0	0	1	4	0	0
Cell 2	10	AP	21-37	2	7	1	0	2	7	1	0	0	3	7

Note: Acne frequency level 0: over 3 months, 1: monthly, 2: biweekly, and 3: weekly; Acne severity degree 0: no acne lesions, 1: rare non-inflammatory acnes, 2: mild acnes with non-inflammatory lesions, 3: moderate acnes with many non-inflammatory lesions and some inflammatory lesions.

Among all the volunteers, 2 were with oily skin, 9 with combination skin, 4 with dry skin, and none of them are self-declared sensitive skin. In self-claimed acne history, result showed the acne

frequency level in AP skin group is higher than in the LAP skin group, 20% ,70% and 10% of volunteers in AP skin group are acne frequency monthly, biweekly, and weekly, 100% of volunteers in LAP skin group have acne frequency over 3 months. Result of acne severity degree showed AP skin have more serious acne than LAP skin, 70% and 30% of the volunteers in AP skin have severity degree 3 and severity degree 2, 20% and 80% of volunteers of LAP skin have severity degree 1 and severity degree 0.

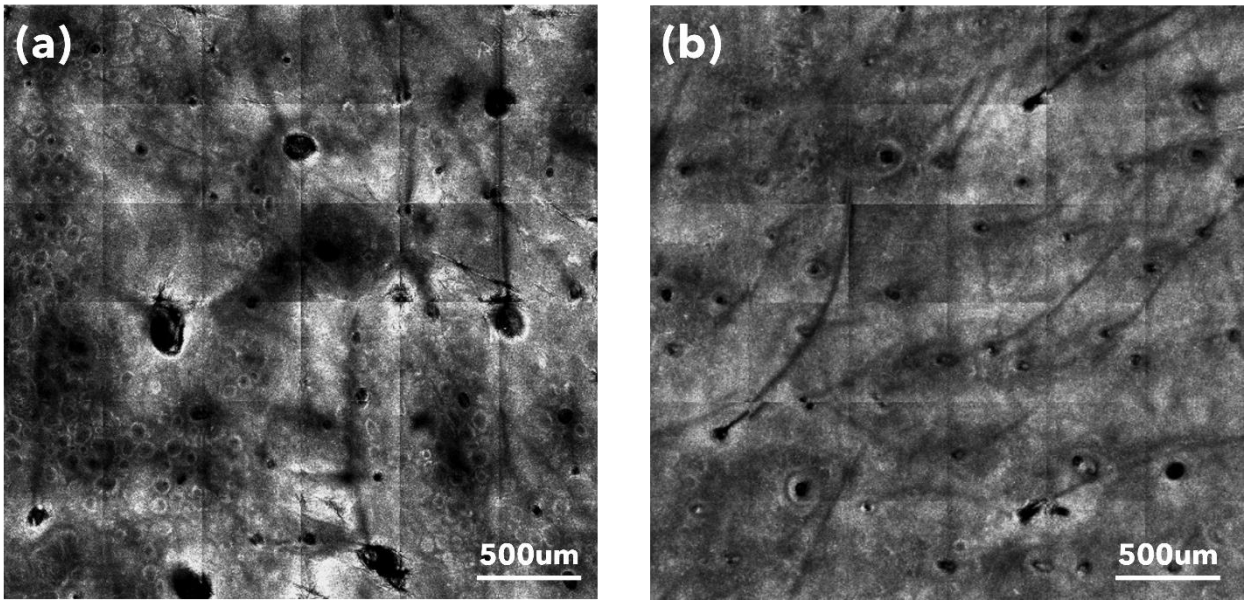


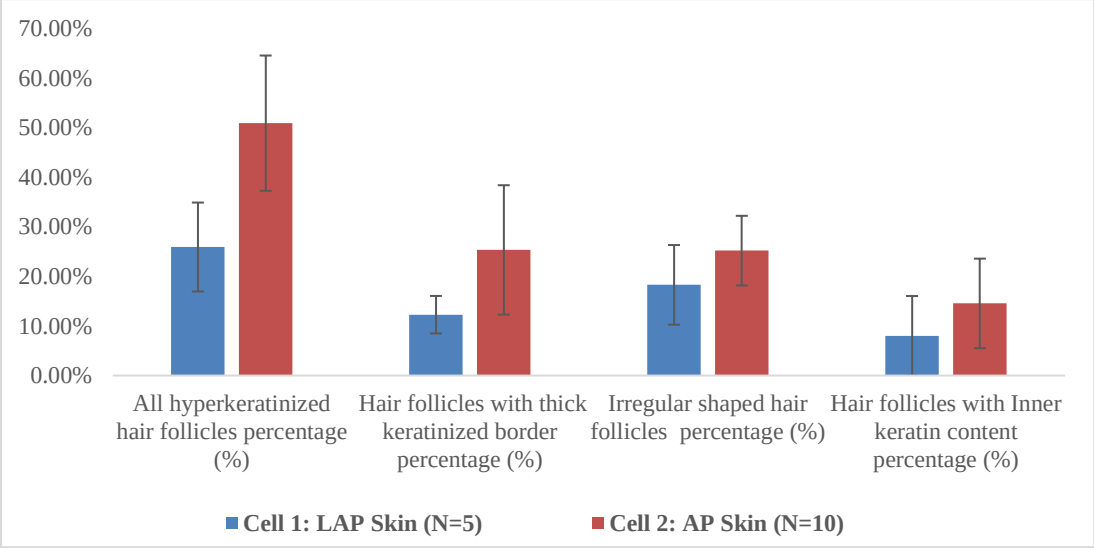
Fig. 2 *Comparison of RCM images of hair follicles on scanned facial areas between 2 groups (a) volunteer from AP skin group (Cell 2) (b)volunteer from LAP skin group (Cell 1)*

RCM measurement showed that the size of the follicles in AP skin range (80 to 230 um) is larger than LAP skin (50 to120 um) skin follicle's size at epidermis level (Fig.2). As shown in Fig.3-A, AP skin also found a higher rate of hyper-keratinized hair follicle (51.39% vs.26.24%), higher rate of thick keratinized border (25.48% vs.11.20%) more irregular shaped hair follicle (25.27% vs.16.00%), and more hair follicles with inner keratin contents (14.09% vs. 6.70%) than LAP skin. AP skin is found with higher TEWL (Trans Epidermal Water Loss, 12.9 g/m^2h vs.11.0 g/m^2h).

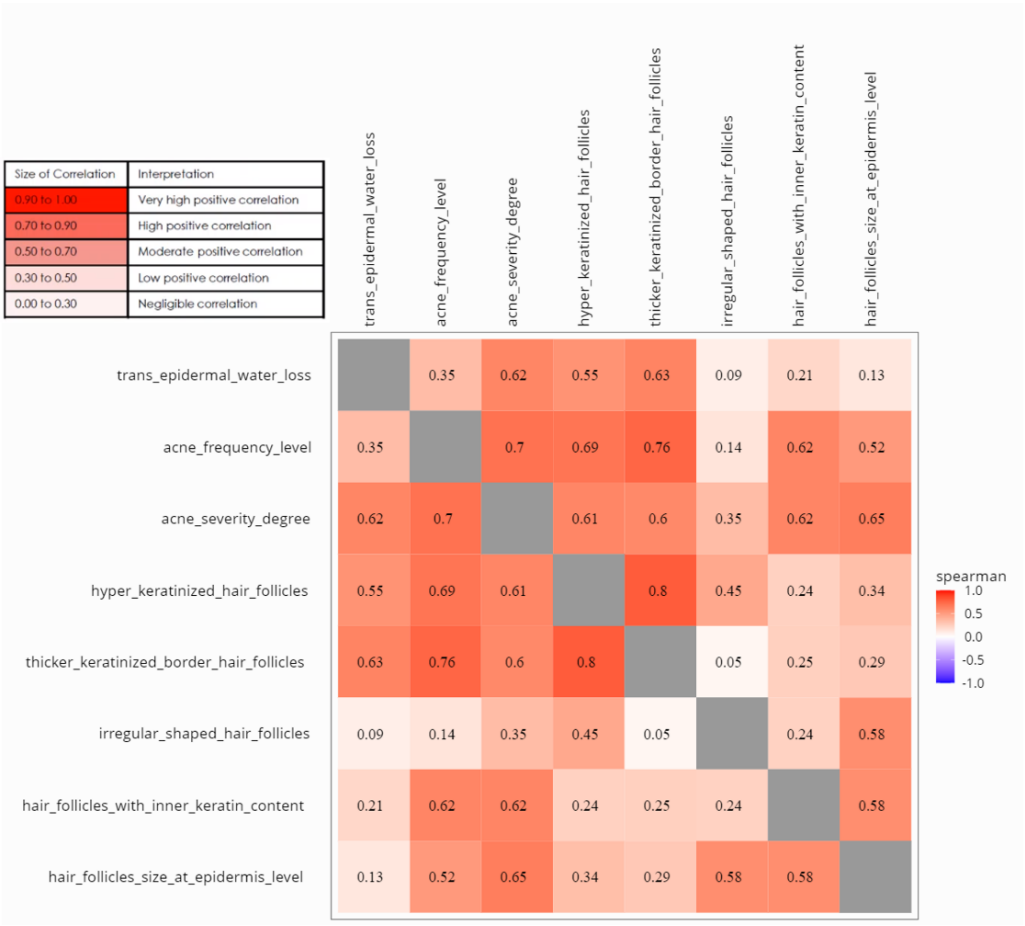
Correlation matrix is shown as Fig. 3-B. RCM measurement results showed correlation with self-claimed historical acne frequency and severity. Acne frequency level showed a high positive correlation with thick keratinized border (0.76), moderate positive correlation with hyper-keratinized hair follicles (0.69) and hair follicles with inner keratin content (0.62). Acne severity

degree showed moderate positive correlation with bigger hair follicles size (0.65), hair follicles with inner keratin content (0.62), hyper-keratinized hair follicles (0.61), and thicker keratinized border (0.60).

A



B



C

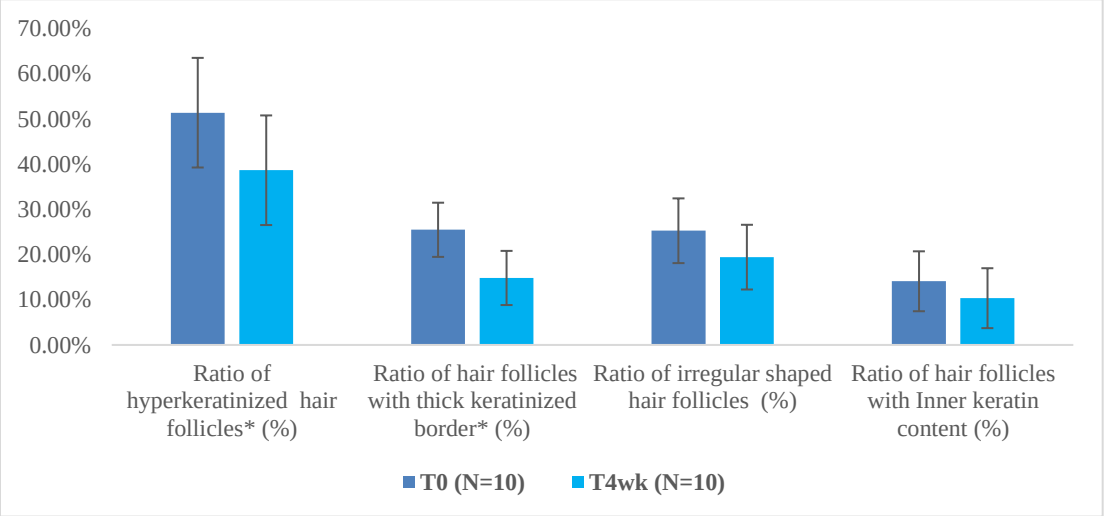


Fig. 3 *(A) Comparison of hair follicle characteristics measured by RCM between LAP skin group (Cell 1) and AP skin group (Cell 2); (B)Correlation among TEWL, self-claimed acne history (acne frequency level, acne severity degree) and RCM measurement (hyper-keratinized hair follicles, thicker keratinized border hair follicles, irregular shaped hair follicles, hair follicles with inner keratin content, hair follicles size at epidermis level); (C)RCM measurement result comparison between initial data (T0) and after 4-week treatment (T4wk) of AP skin. Note: * P<0.05.*

It was also found that after 4-week treatment of AP skin group, the degree of hyper-keratinization around hair follicles tracked was effectively reduced, as shown in the scheme of Fig. 4.

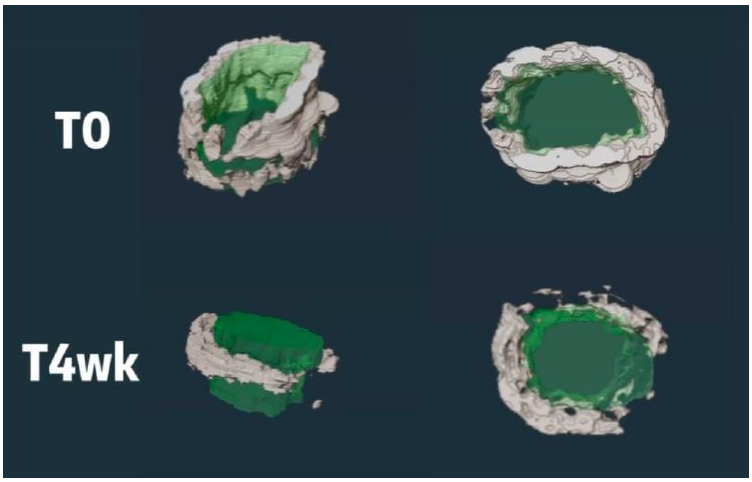


Fig. 4 *Comparison of hyper-keratinization around hair follicles tracked before (T0) and after4-week treatment (T4wk)*

The size of hair follicles in epidermis level decreases 26.03% ($p<0.05$). The ratio of hyper-keratinized hair follicles reduces 12.75% ($p<0.05$) and the ratio of thick keratin border decreases 10.66% ($p<0.05$), the ratio of irregular shaped hair follicle and the ratio of hair follicles with inner keratin contents decreases without statistical significance, as shown in Fig. 3-C.

Discussion.

Based on the correlation analysis between RCM parameters and acne history parameters, we further explored the possibility to identify key influencing factors for both "acne proneness" classification and "acne frequency" classification via decision tree machine learning method [13]. Among all the parameters assessed, we found "ratio of hyper-keratinized hair follicle" in RCM imaging is the most influencing factor to acne proneness and also correlate to acne frequency. We also found other influencing factors to acne frequency, i.e. "ratio of thicken hair follicle boarder", "ratio of hair follicle with inner keratin content". Based on the factors we identified, decision tree machine learning models were built to predict classification of "AP skin" vs "LAP skin" (Fig.5-A) as well as the "acne frequency" (Fig.5-B). The accuracy for "acne proneness" classification model is 100% by a 70%-30% training-test sampling. Due to the limited total sample size (N=15) the model's accuracy need further validation once we obtain more sample data. The threshold may vary after more samples are introduce to the machine learning model training.

Here we propose the solution by building such a prediction model to resolves the previous mentioned challenges people often met during making the classification of 'AP skin' vs 'LAP skin', which normally need a face-to-face diagnosis with dermatologist along with a self-claim acne history record from the patient. In addition, there is also considerations on the bias and difficulty for a patient to recall their acne history for a specific period of timeframe. Our research proposed a fully image-based methodology to evaluate acne proneness and anti-acne efficacy by assessing the hair follicle morphology in RCM images. Also, from the application point of view, a device or a system (Fig 6) can be further developed to automatically provide result not only for clinical studies but also for consumers in needs to choose the right product based on their current acne status and also for those seeking for cosmetic product to prevent acne occurrence at early stage.

In the clinical study, we found that the key influencing factor RCM parameters identified by can be improve significantly by applying a serum product with peeling ingredients (glycolic acid, salicylic acid, capryloyl salicylic acid) for 4 weeks which indicate an anti-acne efficacy. Same association also reported to have clinical efficacy on reduce number of non-inflammatory and

inflammatory acne [14]. One idea to emerge from above studies, cosmetic product with the same or similar peeling ingredient association can be considered not only as a treatment for mild to moderate to acne problem individuals, but also potentially can be a maintenance treatment to reduce the potential for recurrence of visible lesions for those individuals seeking for acne skin management solution in a longer time period.

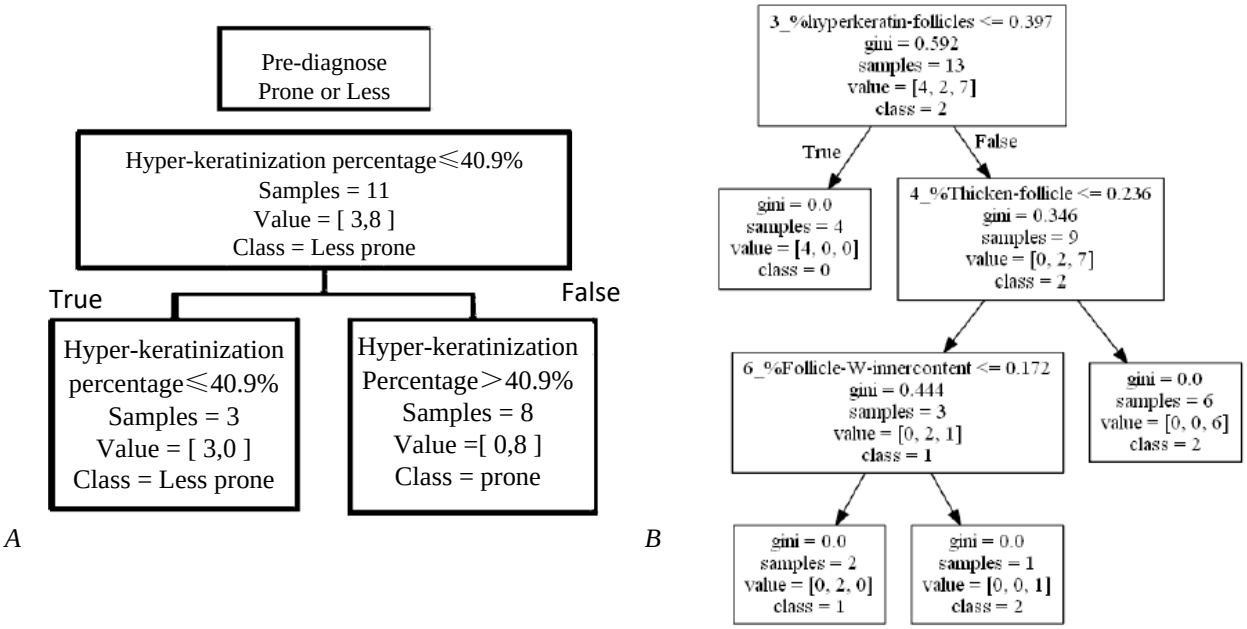


Fig 5. *(A)Decision tree for acne proneness classification, left branch is 'LAP skin', right branch is 'acne prone skin'; (B)Decision tree for acne frequency classification, Class 0 ="acne happen less than once every 3 month", Class 1 = "acne happens once every month"; Class2="acne happens every 2 weeks".*

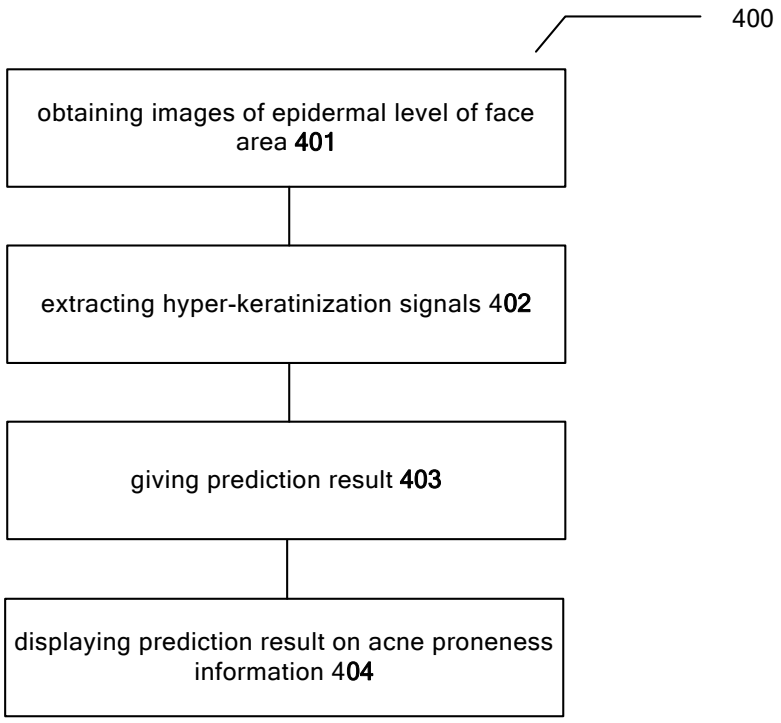


Fig 6. *Example for how the acne proneness classification system works.*

Conclusion.

Analyzing the hyper-keratinization related parameters of the follicles through RCM imaging is an effective new method to characterize acne proneness. The proposed machine learning based method can help diagnose and classify the acne proneness and provide alternative options and complementary information to traditional dermatologist-based diagnosis. Peeling ingredient association is proven to improve the follicle hyper-keratinization status and to ultimately reduce the acne proneness and acne frequency. We envision the proposed method also provides new directions for anti-acne clinical assessment and for acne-prevention through evaluating the hyperactivation of key biological processes of follicles at early stage.

Acknowledgments. The authors wish to thank Chengda Ye, Jing Li, Yueqing Niu for their strong support and discussion in completion of this paper.

Conflict of Interest Statement. This work is financially supported by L'Oréal.

References.

[1] Christine S. K. Fuchs， Amanda J.B.Andersen， Marco Ardigo et al. Acne vulgaris severity graded by In Vivo Reflectance Confocal Microscopy and Optical Coherence Tomography. Laser in Surgery and Medicine. 2019; 51:104-113.

[2] Wolkenstein, P., Machovcová, A., Szepietowski, J. C. et al.. Acne prevalence and associations with lifestyle: a cross-sectional online survey of adolescents/young adults in 7 European countries. Journal of the European Academy of Dermatology and Venereology. 2017; 32(2), 298–306.

[3] Uslu, G., Şendur, N., Uslu, M., Şavk, E. et al.. Acne: prevalence, perceptions and effects on psychological health among adolescents in Aydin, Turkey. Journal of the European Academy of Dermatology and Venereology. 2002; 22(4), 462–469.

[4] Yiwei Shen, Tinglin Wang, Cheng Zhou et al. Prevalence of Acne Vulgaris in Chinese Adolescents and adults:A Community-based Study of 17,345 Subjects in Six Cities. Acta Dermato-Venereologica. 2012;92(1), 40-44.

[5] Manfredini, M., Mazzaglia, G., Ciardo, S. et al. Acne: in vivo morphologic study of lesions and surrounding skin by means of reflectance confocal microscopy. Journal of the European Academy of Dermatology and Venereology. 2014; 29(5), 933–939.

[6] Strauss JS, Kligman AM. Pathogenesis and Treatment of Acne and Rosacea.

[7] John S. Strauss, Albert M. Kligman. The pathologic dynamics of acne vulgaris. Arch Dermatol. 1960;82(5)， 779-790.

[8] Knaggs HE, Holland DB, Morris C, et al. Quantification of cellular proliferation in acne using the monoclonal antibody Ki-67. Journal of Investigative Dermatology. 1994; 102, 89–92.

[9] William J. Cunliffe, D.B. Holliand, A. Jeremy. Comedone Formation: Etiology, Clinical Presentation, andTreatment. Clinics in Dermatology. 2004; 22, 367–374.

[10] Cunliffe WJ, Holland DB, Clark SM, et al. Comedogenesis: some new aetiological, clinical and therapeutic strategies. British Journal of Dermatology. 2000; 142 (6):1084 – 91.

[11] A. Nast,B. Dréno,V. Bettoli, et al. European Evidence-based (S3) Guidelines for the Treatment of Acne. Journal of the European Academy of Dermatology and Venereology.

2012; 26 (Suppl. 1), 1–29.

[12] Muguet Guenot, L., Vourc’h Jourdain, M., Saint-Jean, M. et al. . Confocal microscopy in adult women with acne. International Journal of Dermatology. 2018; 57(3), 278–283.

[13] Beyeler M. Machine Learning for OpenCV[M]. 1. Packt Publishing, 2017.

[14] LI Shu-ting; HE Xiao-feng; ZHANG Zhong-xing; Julie CRIBIER; Sabina MERALLI-BALLOU; Andrew STEEL; JU Qiang; NIU Yue-qing. Clinical efficacy of a topical formula containing glycolic acid,salicylic acid,lipohydroxy acid,and niacinamide in patients with mild to moderate acne[J]. Journal of Clinical Dermatology, 2022(3):175-179.