Reflectance spectroscopy for evaluating hair follicle cycle

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ABSTRACT

Hair follicle, as a mini-organ with perpetually cycling of telogen, anagen and catagen, provides a valuable experimental model for studying hair and organ regeneration. The transition of hair follicle from telogen to anagen is a significant sign for successful regeneration. So far discrimination of the hair follicle stage is mostly based on canonical histological examination and empirical speculation based on skin color. Hardly a method has been proposed to quantitatively evaluate the hair follicle stage. In this work, a commercial optical fiber spectrometer was applied to monitor diffuse reflectance of mouse skin with hair follicle cycling, and then the change of reflectance was obtained. Histological examination was used to verify the hair follicle stage. In comparison with the histological examination, the skin diffuse reflectance was relatively high for mouse with telogen hair follicles; it decreased once hair follicles transited to anagen stage; then it increased reversely at catagen stage. This study provided a new method to quantitatively evaluate the hair follicle stage, and should be valuable for the basic and therapeutic investigations on hair regeneration.

Keywords: hair follicle cycle, hair regeneration, diffuse reflectance spectra, histological examination

1. INTRODUCTION

Hair follicle study is of great importance for the treatment of hair loss disease, and the inducing of telogen to anagen transition is one of most important ways to induce hair regeneration [1-4]. In addition, the hair follicle affords a valuable model for regenerative investigation due to its regular remodeling through cycling phases of telogen (hair rest), anagen (hair growth) and catagen (hair involution) [5-6]. In fact, the regenerative capacity of hair follicle is closely related to its cycling stage [7]. Besides, it also varies between different individuals, and even desynchronizes in different regions of one individual [8]. However, the precise discrimination of the hair follicle stage is mostly based on canonical histological examination, which is invasive and needs to remove samples off the body [9]. Another way depends on observing the change of skin color, which is empirical and equivocal [9]. In order to assess the *in vivo* hair follicle stages non-invasively and objectively, there is of great need for reliable and minimally invasive methods for depicting hair regeneration process.

Colorimetry has been used to measure *de novo* hair regeneration based on terms of light intensity (L^*), green to red (a^*) and blue to yellow (b^*) color bands [10]. But it needed to remove the skin sample off the experiment animals. In addition, it was a global assessment and cannot afford inter-individual variation. Point scanning confocal microscopy was used to image the entire intact hair follicle, but it could only focus on one *ex vivo* hair follicle, with too limited views [11].

The diffuse reflectance spectroscopy has been successfully applied to investigate *in vivo* bio-tissues [12-17]. The composition of reflectance spectrum carries information of the tissue structure, distribution and concentration of chromospheres such as melanin and hemoglobin [12,14,15]. Besides, the reflectance spectroscopy documents information of topical skin area, which should afford promising tool to distinguish inter-individual variation [13,18].

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In this study, a commercial optical fiber spectrometer was applied to monitor diffuse reflectance of mouse skin, and the change of reflectance was quantified. Then the hair follicle stage was defined based on the change of reflectance. Besides, macroscopic appearance and histological examination were also examined to classify the stage of hair follicle.

2. MATERIALS AND METHODS

2.1 Animal model and experimental protocol

This study was approved by Huazhong University of Science and Technology Institutional Review Board for Animal Study. Three-week old, female C57BL/6 mice (n=30) were obtained from Experimental Animal Center of Wuhan University (Wuhan, China) and were fed under standard rodent feeding condition. Hair follicles of the mice were just at the beginning of a new anagen stage [9]. Before experiment, animals were intraperitoneally anesthetized using a mixture of chloral hydrate (0.02 g/ml) mixed with ethylurethanm (0.1 g/ml). The dorsal hair was shaved cleanly by razor blade, with two bilateral areas in dorsal skin. Then the mice were used for discrimination of hair follicle stage for the next 3 weeks. The macroscopical change of mouse was photographed every day, and the skin reflectance spectrum was also measured.

2.2 VIS–NIR Fiber Spectrometer

In this study, a VIS–NIR fiber spectrometer (USB-4000, Ocean Optics, Florida, USA) was used to measure the diffuse reflectance spectra of in vivo mouse skin. The system is composed of light source (HL-2000), a reflection probe (R400-7), and spectrometer as well as computer software. Before the experiment, the system was preheated for about 30 minutes and then standardized by reflectance standard made up of barium sulfate. During the experiment, the probe was vertical and soft touched to in vivo dorsal skin and then the data was saved. For each area, the reflectance spectrum was measured for three times, and then averaged as final measuring value.

2.3 Histological Examination

In order to canonically distinguish the hair follicle stage, histological examination was performed. Skin biopsies of mice skin were taken at different days between ages of three to six weeks old. Three mice were sacrificed at each time point and the dorsal skin were detached. The skin samples were then fixed with 4% neutral formaldehyde and then were dehydrated using graded alcohol. After paraffin embedding, the samples were sliced with thickness of 4 to 5 μm and then stained with hematoxylin and eosin (HE). Finally, each slice was imaged by a microscopy (Nikon Ni-E, Nikon, Japan) equipped with a color digital industrial camera (DS-Fi2, Nikon, Japan).

2.4 Quantitative Analysis

In order to quantitatively investigate the change of reflectance of mousedorsal skin with hair follicle cycle, the relative change in reflectance ΔR_{rel} at 481 nm was obtained based on formulas (1). Here, R_0 is the initial values of reflectance, and R_T is the reflectance of different days in a hair follicle cycle.

$$\Delta R_{\text{rel}} = \frac{\Delta R}{R_0} = \frac{R_T - R_0}{R_0} \tag{1}$$

3. RESULTS AND ANALYSIS

3.1 Change of mouse dorsal skin with hair follicle cycle

Periodicity change of mouse dorsal skin with hair follicle progressing is shown in Figure 1. It can be seen that the color of dorsal skin keeps pink from day 0 to 5; and as time goes on, the color of skin turns to light blue (D8) and dark blue (D11). Then the skin color shallows back to light blue (D18), gray (D21) and finally pink (D23). An entire growth cycle is about 23 days.

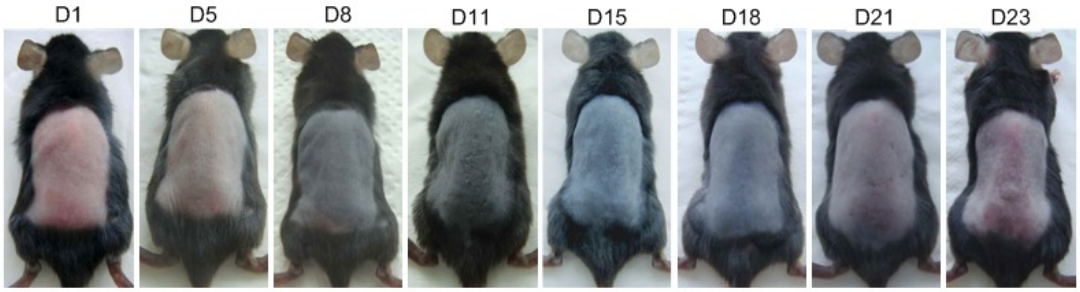

Fig. 1. Visualized change of mouse dorsal skin with hair follicle cycle. D means Day, different days after the anagen stage beginning.

3.2 Typical change of reflectance spectrum of mouse skin with hair follicle cycle

Figure 2 shows typical change of reflectance spectra of mouse skin with hair follicle cycle, three color represent the trend of decrease, increase and recovery of reflectance spectrum at different hair follicle stage, respectively. At Day 0-1, the reflectance spectra are relatively high; and then the spectrum decreases lower and lower with the anagen stage progressing. 11 days after the anagen beginning, the reflectance spectra decreases most. Then the reflectance spectrum increases higher and higher from day 15 to 21. 23 days later, the reflectance spectrum goes back to highest level, and to be similar with that of Day 0.

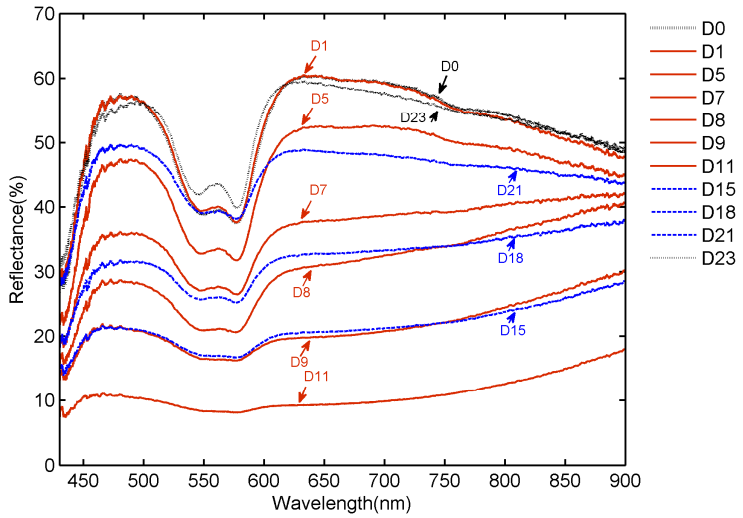

Fig. 2. Typical change of diffuse reflectance spectra of mouse skin with hair follicle cycle. Text arrow indicate different days (D) after the anagen stage beginning. The tendency of decrease, increase and recovery of reflectance spectrum at different hair follicle stage are colored as red, blue and gray, respectively.

3.3 Relative change of skin reflectance with hair follicle cycle

Further, the relative change of skin reflectance at different days of hair follicle cycle was quantified, as figure 3 shows. It can be found that there is small change at the first day of the anagen. Then the reflectance decreases by 13% at day 5, and further decreases at the following 6 days, finally reduces by up to 80%. Then once the hair follicle returns to catagen stage, the relative change of reflectance was gradually decreased, 23 days later, there is almost no change of reflectance compared with day 0.

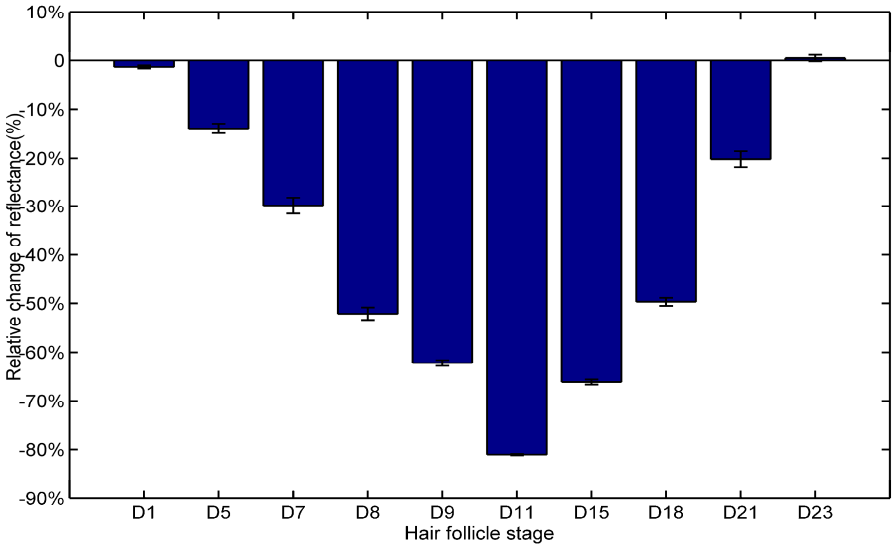

Fig.3 Relative change of skin reflectance at different days of hair follicle cycle. Data shows as mean±SD (n=6).

3.4 Histological examination of hair follicle

The canonical histological examination of hair follicle at different days are shown as figure 4. At day 1, the hair follicle is short and entirely located in the skin dermis, with a round, ball-like hair bulb. Then 5 days later, the keratinocyte strands between dermal papilla and club hair are seemed thickened. 8 days later, the hair follicle grows down to the adipose tissue, and hair bulb is inflated, with obvious dermal papilla rounded by thickened matrix cells. 11 days later, the hair follicle is elongated and reached the bottom of thickened adipose tissue, and hair shaft is visible. Then from day 15, hair follicle keeps long, but the hair bulb begins to shrink. Further, the hair follicle gets shorter and shorter, with thinner and slimsier structure. Finally, 23 days later, the entire hair follicle is re-located in the dermis, with a round, ball-like hair bulb.

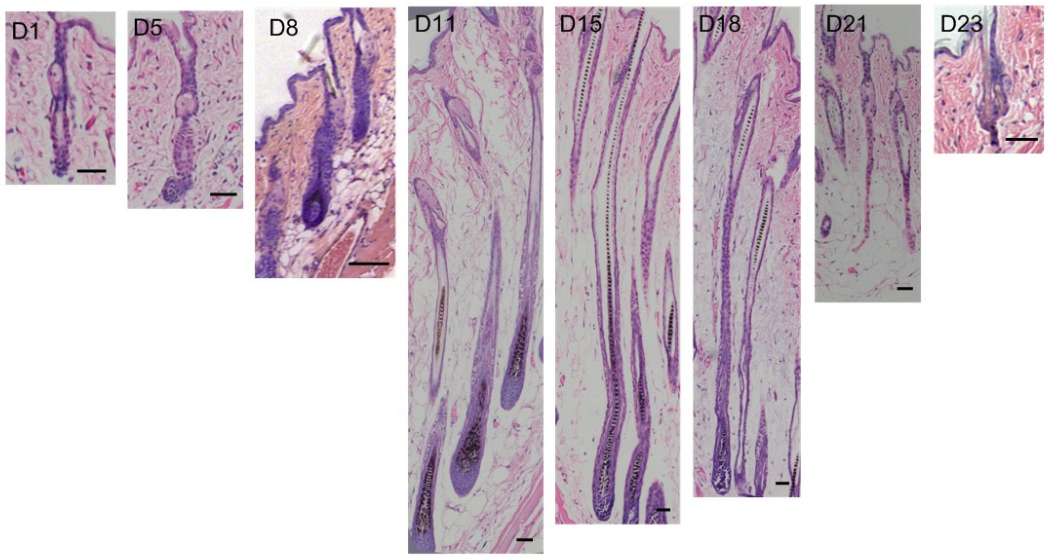

Fig.4. Profiles of hair follicle at different days of hair follicle cycle, examined by HE staining. Scale bar: 100 μm.

4. DISCUSSION

The transition of hair follicle from telogen to anagen is a significant sign for successful regeneration, but it is difficult to distinguish the beginning of anagen from the previous telogen by empirical speculation based on skin color. Canonical histological examination was considered as gold-standard, but it is invasive and time-consuming. In this study, we tried to characterize the hair follicle stage based on reflectance spectroscopy, and verified by histological examination.

3-weeks old mouse were used in this study until they grow to 6-weeks old. The mice were at telogen stage at 3-weeks old, and then would transmit to anagen stage at the first day of the forth week [9]. We firstly recorded the macroscopical change of skin color in mouse. Results showed that at the first 5 days, there was hardly visible change in the skin color (Fig. 1), and then skin turned to light blue, dark blue and then went back to light blue and pink. The change of the skin color reflected the hair follicle stage to some extent; pink-to-dark change mean telogen-to-anagen transition, dark-to-light mean anagen-to-catagen transition, and light-to-pink mean catagen-to-telogen transition [9].

However, it was empirical and subjective. For example, the skin color remains largely unchanged at the first 5 days (Fig. 1), and it could not distinguish the anagen progressing and initialization. Instead, the measurement of reflectance spectrum with hair follicle cycle could show the difference between day 1 and day 5, there was a decrease of about 10% in the reflectance (Fig. 3). Besides, the change of reflectance made the hair follicle cycle to be quantifiable. In the first 11 days, the skin reflectance decreased by from 2% to 80%, and subsequently recovered in the following 12 days. The former decrease reflected the progressing of anagen stage, and the latter recovery reflected the catagen to telogen stages. By such a quantified comparison with the initial state (Day 0), the precise hair follicle stage could be told easily.

Compared with the morphological phenomenon, it could be speculated that the change of reflectance was due to the change of skin color. As we know, melanocytes are located in the basal layer of the epidermis and hair follicle matrix cells. They would begin to produce melanin pigments for hair once the hair follicle enters anagen stage, then die and disappear when hair follicle regress and rest [19], just as reflected in figure 4. In fact, the whole dorsal skin of mouse would turn to black, instead of the hair follicle location, one reason is mouse possessing dense hair coat, and another might due to skin epidermal melanocytes function. And the relationship between the two kinds of melanocytes should deserve further investigation.

Besides, the probe of reflectance spectroscopy is small, not like the global assessment of Colorimetry [10], it could be used to identify subtle change in small areas of individual, which could be useful for detection of inter-individual variation. Besides, it affords in vivo information, and could be used for long-term tracing of treatment. Compared with the existing methods, this work achieved precise measurement of *in vivo* model, and avoided the contingency of a single measurement.

5. CONCLUSION

This work evaluated the hair follicle stage based on reflectance spectroscopy, and compared with classical histological examination. Results showed that the skin diffuse reflectance was relatively high for mouse with telogen hair follicles; it decreased once hair follicles transited to anagen stage; then it increased reversely at catagen stage. Quantification of the reflectance revealed a maximum decrease (80%) when hair follicle got to the late anagen stage (Day 11). It can be concluded that the reflectance spectroscopy was valuable and credible to evaluate the hair follicle stage. And this work should provide a new method for evaluation hair regeneration.

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