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## ***Precision Assessment of the 28-Day Skin Turnover Cycle and Epidermal Regeneration Effect of *Iris germanica* Root Extract***

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### **1. Introduction**

The turnover rate in the stratum corneum is well-known to be approximately 28 days and this cycle tends to slow down with age [1,2]. However, common methods for evaluating turnover rates measure changes in fluorescence or color of the outermost epidermis and are typically limited to observations within 3 weeks [3,4]. These techniques are limited to observing the surface layer of skin, making it fail to account for the full 28-day cycle. A recently introduced non-toxic method measures epidermal turnover by tracking the fading of UVA-induced pigmentation over time, without using radiolabeled substances [5]. However, its limitation lies in evaluating turnover solely based on melanin changes, without assessing other UVA-induced skin alterations.

Our previous study highlighted the skin benefits of *Iris germanica* root extract (IRE) obtained from the rhizome of *Iris germanica* L., such as wound healing, antioxidants, and skin rejuvenation [6]. In this study, we developed a novel evaluation method designed to align with the actual 28-day turnover cycle of human skin, enabling a more precise and comprehensive assessment of epidermal renewal. Furthermore, we investigated the effects of IRE activating the regeneration of older keratinocytes proven in vitro. Our findings revealed that IRE significantly promotes keratinocyte differentiation and regeneration in vivo, thereby accelerating the turnover cycle. These results suggest that IRE effectively restores the delayed turnover rate in aging skin to a healthy 28-day cycle, making it as an innovative solution in well-aging and advanced skincare regimens.

### **2. Materials and Methods**

#### *2.1. UVA Irradiation and Evaluation of Skin Turnover*

This study was conducted to evaluate skin turnover following UVA exposure in Asian women aged 20 to 59 years. All procedures were carried out at the P&K Skin Research Center. To minimize environmental variability, all measurements were performed in a temperature and humidity controlled room, both before and after UVA irradiation.

A preliminary study was first conducted on five participants to determine the appropriate irradiation dose and evaluation intervals for assessing skin turnover. UVA irradiation was applied using a Multiport Solar Simulator, 601-300W (Solar Light, USA) at varying doses ranging from 10 J/cm<sup>2</sup> to 40 J/cm<sup>2</sup>. A defined area on the right upper arm, free from any pigmentation or visible skin damage, was selected and exposed to UVA at a consistent light intensity.

Melanin and erythema indices were assessed using the Mexameter MX18 (Courage+Khazaka electronic GmbH, Germany) on both the product applied and untreated areas. Measurements were taken at the following time points: before UVA exposure, 24 hours after irradiation, and on days 7, 14, 21, 28, and 35 after IRE serum application.

Based on the pilot study results and previously published literature, the main study enrolled 24 Asian female subjects aged 20 to 59 years with Fitzpatrick skin types II and IV. In this study, UVA irradiation was performed at a dose of 15 J/cm<sup>2</sup>. Skin pigmentation and erythema were measured using the Mexameter at baseline (prior to irradiation), 24 hours post-irradiation (Day 1), and on days 14 (Day 14), 21 (Day 21), and 28 (Day 28) following IRE serum application.

To document visual changes, standardized photographs of the same test site on the right upper arm were captured using the VISIA-CR imaging system (Canfield Imaging Systems, USA). All images were taken using the Standard 2 mode to ensure consistency and reproducibility across time points.

## 2.2. Statistical Analysis

Normality was assessed using SPSS to evaluate differences before and after product use, as well as between the test product and the untreated control. For within-group comparisons, if the data satisfied the assumption of normality, repeated measures ANOVA was performed, followed by post-hoc analysis using the Bonferroni method. If the data did not meet the normality assumption, the Friedman test was used, and post-hoc analysis was conducted using the Wilcoxon signed-rank test with Bonferroni correction.

For between-group comparisons, the paired t-test was used for normally distributed data, while the Wilcoxon signed-rank test was applied for non-normally distributed data.

To determine the skin turnover cycle, a linear trend line was generated based on the change in values between the pre-UV irradiation state and the number of days of product use. The number of days required for the skin to return to the pre-irradiation state was then estimated from this trend.

## 3. Results

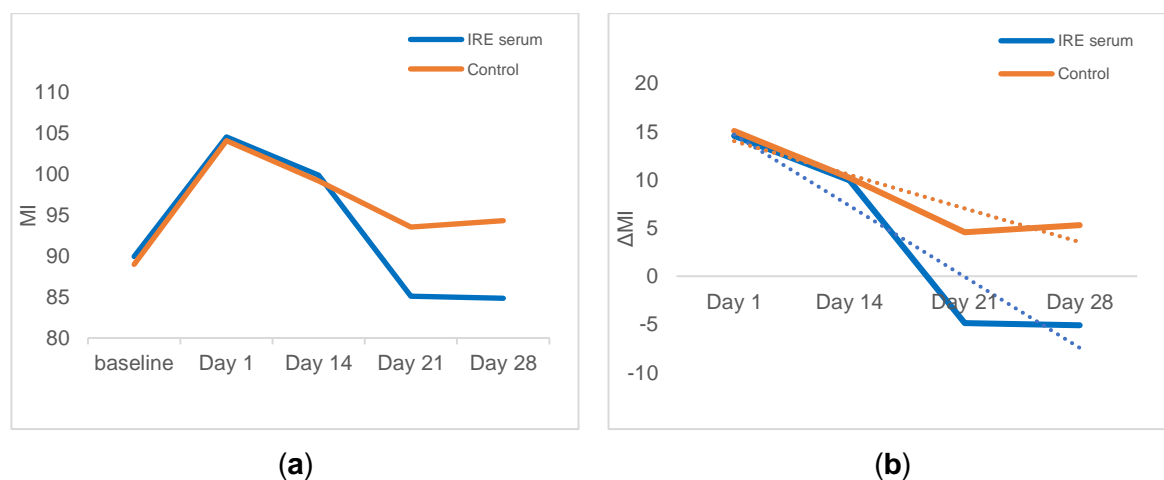
### 3.1. Melanin-Based Epidermal Turnover Assessment

Following UVA irradiation, a marked increase in the melanin index (MI) was observed at Day 1 (24 hours post-irradiation) in both the IRE serum-treated and untreated control sites. Over the study period, the IRE serum-treated site exhibited a rapid decline in MI values, returning toward baseline (before UVA irradiation) more quickly than the control (Figure 1a). The delta

melanin index ( $\Delta MI$ ) was calculated as the difference from baseline. As shown in Figure 1b,  $\Delta MI$  values decreased consistently over time, with a steeper trendline observed in the IRE serum-treated site, indicating an accelerated turnover rate. Turnover completion days, defined as the time point at which  $\Delta MI$  reached zero, were estimated using a linear trend line. The IRE serum-treated site completed skin turnover at 21.0 days, 19.2 days faster than the 40.2 days required in the control site (Table 1).

Classification	(Mean $\pm$ SD)	
	MI	
	IRE serum-applied	Non-applied
Before UVA irradiation (baseline)	89.917 $\pm$ 19.634	88.958 $\pm$ 20.479
24 hours post-irradiation (Day 1)	104.472 $\pm$ 18.226	104.014 $\pm$ 20.808
After 14 days of use (Day 14)	99.833 $\pm$ 19.064	99.139 $\pm$ 19.574
After 21 days of use (Day 21)	85.056 $\pm$ 14.665	93.514 $\pm$ 18.822
After 28 days of use (Day 28)	84.833 $\pm$ 13.577	94.264 $\pm$ 18.120
Skin turnover (Days)	21.0	40.2

**Table 1.** Summary of melanin index (MI) values and estimated turnover completion day ( $\Delta MI = 0$ ) for IRE serum-treated and control sites. Data are presented as mean  $\pm$  SD for baseline and Days 1, 14, 21, and 28. Skin turnover days were calculated using a linear trend line of  $\Delta MI$  values.



**Figure 1.** Time-course changes in melanin index (MI) over 28 days: (a) Changes in MI values over 28 days; (b) Delta MI ( $\Delta MI$ ), calculated as the difference from baseline at each time point. Dotted lines represent linear trendlines.

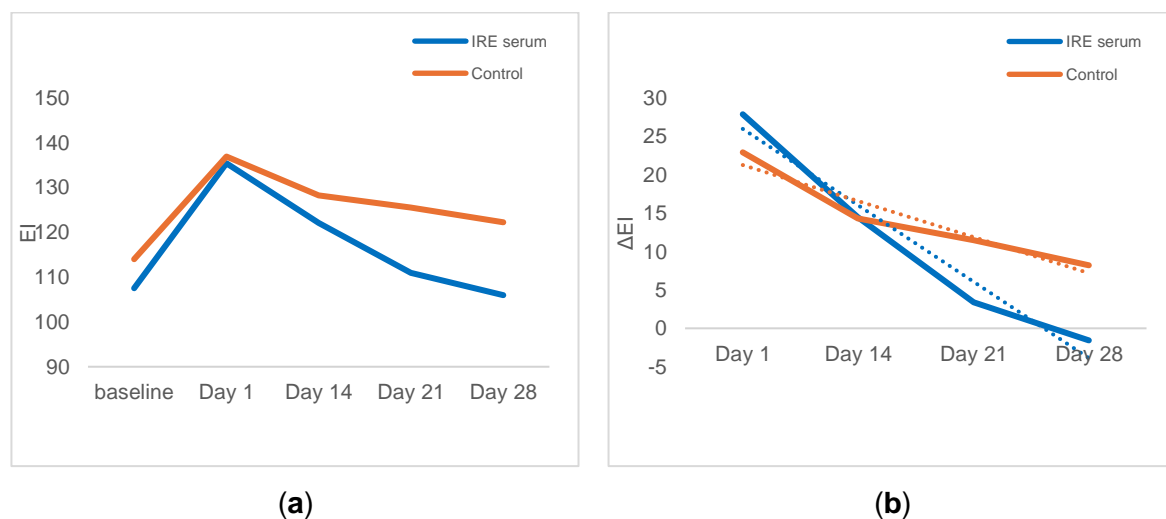
### 3.2. Erythema-Based Epidermal Turnover Assessment

After UVA irradiation, erythema index (EI) values showed a sharp increase at Day 1 (24 hours post-irradiation). From Day 14 onward, the IRE serum-treated site exhibited a rapid decline in EI values, returning toward baseline (before UVA irradiation) more quickly than the control (Figure 2a). As shown in Figure 2b,  $\Delta EI$  values gradually declined over time. The steeper decrease in the IRE serum-treated site indicates faster recovery from UVA-induced erythema, suggesting improved epidermal skin turnover. Based on the  $\Delta EI$  trendlines, the IRE serum-

treated site completed skin turnover at 25.7 days, 21.4 days faster than the 47.1 days required in the control site (Table 2).

Classification	EI (Mean $\pm$ SD)	
	IRE serum applied	Non-applied
Before UVA irradiation (baseline)	107.542 $\pm$ 29.923	114.000 $\pm$ 31.207
24 hours post-irradiation (Day 1)	135.417 $\pm$ 29.994	136.917 $\pm$ 29.762
After 14 days of use (Day 14)	122.055 $\pm$ 24.500	128.278 $\pm$ 27.443
After 21 days of use (Day 21)	110.917 $\pm$ 23.887	125.500 $\pm$ 26.301
After 28 days of use (Day 28)	105.972 $\pm$ 24.712	122.208 $\pm$ 24.610
Skin turnover (Days)	25.7	47.1

**Table 2.** Summary of erythema index (EI) values and estimated turnover completion day ( $\Delta EI = 0$ ) for IRE se-rum-treated and control sites. Data are presented as mean  $\pm$  SD for baseline and Days 1, 14, 21, and 28. Skin turnover days were calculated using a linear trend line of  $\Delta EI$  values.



**Figure 2.** Time-course changes in erythema index (EI) over 28 days: (a) Changes in EI values over 28 days; (b) Delta EI ( $\Delta EI$ ), calculated as the difference from baseline at each time point. Dotted lines represent linear trendlines.

#### 4. Discussion

In this study, we optimized UVA irradiation conditions at a dose of 15 J/cm<sup>2</sup> to induce measurable changes in skin pigmentation and erythema within the known skin turnover cycle of approximately 28 days. This method effectively triggered both melanin production and erythema, while also allowing enough time for full recovery from damage, thereby enabling observation of the complete skin turnover process.

It was essential to assess both melanin and erythema indices to comprehensively evaluate the skin turnover cycle. Melanin index (MI) tend to drop sharply between Day14 and Day21 during the recovery phase, showing a steep decline that varies in timing and intensity between individuals. This variability makes it difficult to generalize results or draw a consistent

trendline based solely on MI. In contrast, erythema typically decreases more gradually and exhibits a relatively uniform pattern across individuals, thereby positioning the erythema index (EI) as a more stable and reliable marker. Therefore, relying on MI alone could lead to inaccurate interpretations of skin turnover cycle, whereas incorporating EI as a complementary marker allows for a more balanced and accurate assessment. By evaluating both parameters together, we could overcome the limitations inherent in each and draw a more reliable and representative conclusion regarding the skin's turnover process.

MI returned to baseline within 21 days in the IRE serum-treated group. This is notably faster than in the untreated control group, where complete resolution of melanin took approximately 40.2 days—indicating that the IRE serum accelerated the skin turnover cycle by 19.2 days. Similarly, erythema subsided in 25.7 days in the IRE serum-treated group, compared to 47.1 days in the control group, reflecting a 21.4-day reduction in recovery time. These findings suggest that the IRE serum significantly promotes skin regeneration. Given that the average age of participants was 48.6 years—an age at which the skin turnover rate naturally slows—the observed normalization of turnover to approximately 28 days in the serum-treated group implies a restorative effect of the serum on aging skin. By effectively accelerating the regeneration process, the IRE serum may help restore a more youthful skin renewal cycle, which could have implications for broader anti-aging and skin recovery applications.

## 5. Conclusion

Using an optimized precision assessment method incorporating both melanin and erythema indices, we effectively evaluated the skin turnover cycle. Application of the IRE serum accelerated recovery by 47% for melanin and 45% for erythema, demonstrating its potential to rejuvenate and normalize delayed turnover in aging skin by restoring a more youthful regenerative rhythm.

## 6. Reference

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