IFSCC 2025 full paper (IFSCC2025-893)

"Effects of depth differences in skin internal markers on skin external markers"

Hyeyeon Yang*[1], Sung-Ha Park[1], Ji-Eun Woo[1], Byoung-Jun Park[1]

[1] Skin&Natural products Lab, Kolmar Korea Co., Ltd., Seoul, Korea, South

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1. Introduction

The skin is a large, complex organ with multiple layers that differ in structure and function. It forms the outermost layer of the body and serves as a barrier between the external environment and the body's interior.[1] Over time, the skin undergoes functional and structural changes,[2] and the causes of this aging can be broadly categorized into external and internal factors.[3-5]

Exogenous aging refers to aging caused by external environmental factors, such as UV radiation, infrared radiation, and air pollution. The most prominent form of exogenous aging is photoaging, which is caused by UV radiation. Symptoms of photoaging include decreased skin elasticity, increased wrinkles, and pigmentation.[6] Recent studies have shown that wrinkles, pigmentation, and sagging are more pronounced in older individuals due to their prolonged exposure to sunlight over time.[7]

Endogenous aging refers to the natural changes in tissue that occur over time due to genetic factors and the aging process.[4, 8] Its main characteristics include increased skin surface roughness, wrinkle formation, and subepidermal atrophy.[9] Specifically, as we age, the amount of hyaluronic acid (HA) in the epidermis decreases,[10] which can lead to reduced skin hydration, elasticity, and smoothness.[11] Simultaneously, the dermis experiences a decline in collagen and elastic fibers, diminishing the skin's elasticity and resilience, which further contributes to wrinkle formation.[12]

Skin aging progresses gradually from within, starting with invisible internal changes that eventually manifest as visible features on the skin's surface, such as wrinkles and sagging. These characteristics are considered classic markers of aging and typically become more pronounced as individuals grow older.[13]

Aging exhibits different patterns depending on gender. Collagen in the dermis decreases more rapidly in women with age, resulting in thinner skin compared to men.[14] Dermal elastin also shows a more pronounced decrease in women compared to men,[15] and skin elasticity decreases by 1.5% per year in the first 5 years after menopause.[16, 17]

Until recently, anti-aging treatments have primarily focused on managing visible signs of aging, such as wrinkles and sagging around the eyes. However, in recent years, there has been a growing interest in "early anti-aging," which aims to prevent and manage aging proactively, in

addition to improving already aged skin. Despite this shift, there is still no definitive marker to identify internal skin aging before visible signs appear on the skin's surface.
In this study, we investigated the correlation between visible external skin markers and invisible internal skin markers. Our goal was to identify markers that can detect early signs of skin aging occurring beneath the surface, even when no visible signs are apparent on the skin's surface.

2. Materials and Methods

2.1. Participants

Sixty-five Korean women aged 20–65 years participated in this study. The study was approved by the Institutional Ethics Committee (approval number: 12207777-A-N-01-DICN22280) and conducted in accordance with the basic principles of the Declaration of Helsinki. Informed consent was obtained from all participants after providing a complete explanation of the protocol.

2.2. Skin Biophysical Measurements

This study was conducted after a 20-minute stabilization period following facial cleansing, under controlled temperature (22°C ± 2°C) and humidity (50% ± 5%) conditions. The test site was selected as the crow's feet area and all skin biophysical markers were measured using noninvasive methods.
Skin biophysical markers were classified into external skin markers, which represent visible skin surface conditions, and internal skin markers, which represent invisible internal conditions.

External skin markers include wrinkles and sagging.
Skin wrinkles were measured using PRIMOS®CR (GFMesstechnik GmbH, Germany), and the representative parameters Ra, Rt, and Rz were analyzed.
The skin sagging angle was measured using F-ray® (Beyoung, Korea) and analyzed using Image-pro® 10 (Media Cybernetics, USA) on facial contour images.

Internal skin markers include skin hydration, elasticity, thickness. All internal skin markers were measured at various depths to understand their characteristics at different layers of the skin.

Skin hydration was measured at four depths using each probe of the Moisturemeter D® (Delfin, Finland). The 0.5 mm and 1.5 mm depths were considered epidermal, while the 2.5 mm and 5 mm depths were considered dermal.
Skin elasticity was evaluated using the Dermal Torque Meter DTM310® (Dia-Stron, USA) with 1 mm and 3 mm probes to assess skin elasticity at superficial and deep levels, respectively. The 1 mm depth measurement represented the elasticity of the epidermis, while the 3 mm depth measurement reflected the elasticity of the full skin thickness, including both the epidermis and dermis.
Skin thickness were measured using a DUB® Skin Scanner (50 MHz) (tpm GmbH, Germany). Skin thickness was evaluated separately for the epidermis and dermis.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS® software 24.0 for Windows (IBM SPSS, USA). Spearman's correlation coefficient (ρ, also signified by rs) measures the strength and direction of the association between two ranked variables. Spearman's coefficients were calculated,

and a correlation was considered statistically significant if the absolute value of the correlation coefficient was between 0.3 and 0.8, and the p-value was less than 0.05. The sign of the coefficient represents the direction of the relationship. If two variables tend to increase or decrease together, the coefficient is positive, and the line representing the correlation slopes upward. Conversely, If one variable increases while the other tends to decrease, the coefficient is negative, and the line representing the correlation slopes downward.

3. Results

To explore the factors influencing skin aging, we investigated the relationship between external skin markers(ESM) and internal skin markers(ISM) at different skin depths. ISM were analyzed based on measurement depth, with 0.5–1.5 mm representing the epidermis and 2.5 mm or deeper representing the dermis.

Furthermore, depth-specific differences in ISM were assessed to determine their contribution to ESM. Groups were classified based on the magnitude of ISM differences by skin depth, and their correlations with ESM were compared.

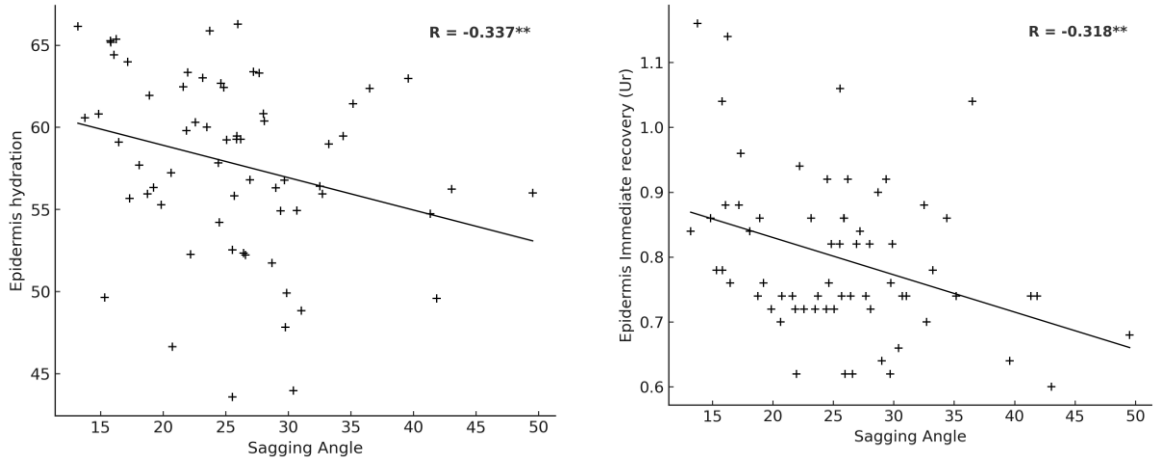
3.1. Correlation Between External Skin Markers (ESM) and Internal Skin Markers (ISM)

The correlation between ESM and ISM in the epidermal layer was analyzed, as shown in Table 1. The skin sagging showed a significant correlation with hydration and specific elasticity parameters, while no correlation was observed with epidermal thickness. Specifically, the skin sagging angle was negatively correlated with hydration at 0.5 mm depth and with Ur (immediate recovery) values at a depth of 1 mm (Figure 1). Conversely, wrinkle parameters (Ra, Rt, Rz) showed no significant correlations with ISM in the epidermis layer. Among ESM, skin sagging is significantly influenced by ISM within the epidermal layer. Specifically, a reduction in epidermal hydration and immediate recovery (Ur) is correlated with increased severity of sagging.

Table 1. Correlation between external skin markers and internal skin markers in the epidermal by Spearman's correlation coefficient.

	Depth	**Parameter**	**Spearman correlation coefficient**			
			Sagging	**Ra**	**Rt**	**Rz**
Hydration	0.5 mm	Skin moisture Content	−0.337**	−0.093	−0.06	−0.091
	1.5 mm		−0.256*	−0.141	−0.106	−0.139
Elasticity	1 mm	Ue (Immediate extensibility)	−0.225	−0.308*	−0.283*	−0.288*
		Uv (Additional extensibility)	0.003	0.176	0.203	0.179
		Ur (Immediate recovery)	−0.318**	−0.288*	−0.274*	−0.260*
		Uf (Total extensibility)	−0.149	−0.207	−0.174	−0.189
		R7 (Skin elasticity)	−0.138	−0.101	−0.117	−0.088
		R5 (Elastic recovery)	0.04	0.146	0.122	0.145
Epidermal Thickness			-0.045	−0.046	0.077	0.123

* Statistical significance (p value < 0.05), ** Statistical significance (p value < 0.01)



(a) (b)

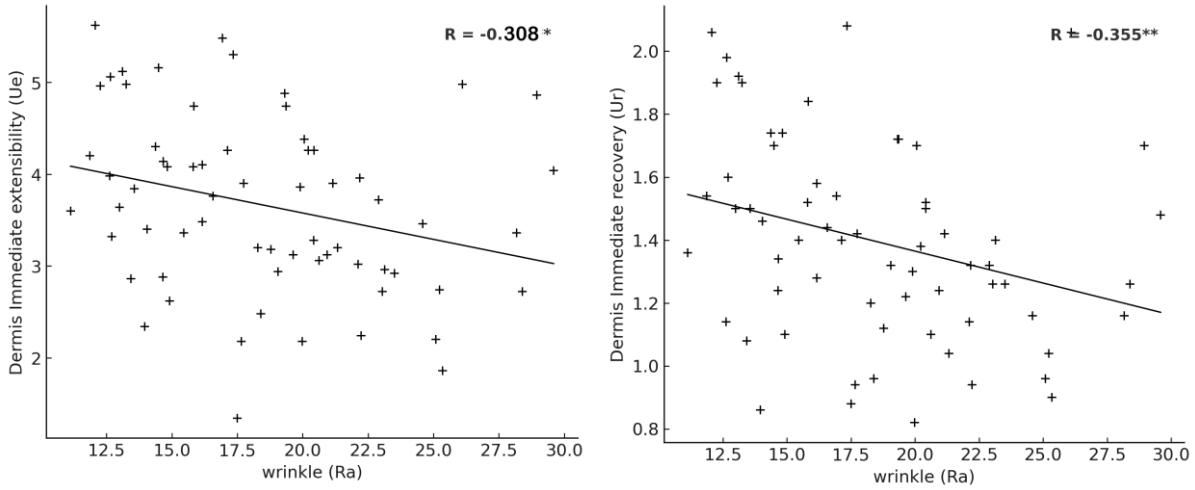
Figure 1. Correlation between skin sagging and epidermal depth markers : (a) skin epidermal hydration; (b) epidermal Ur (immediate recovery).

The correlation between ESM and ISM in the dermal layer was analyzed, as shown in Table 2. wrinkle parameters (Ra, Rt, Rz) were negatively correlated with Ue (immediate extensibility) and Ur (immediate recovery) values at a depth of 3 mm (Figure 2). No significant correlations were observed for dermal hydration, density and thickness. Additionally, the skin sagging angle showed no significant correlations with ISM in the dermal layer. Among ESM, wrinkles are significantly influenced by ISM within the dermal layer. Specifically, a reduction in dermal immediate extensibility (Ue) and immediate recovery (Ur) is correlated with increased severity of wrinkles.

Table 2. Correlation between external skin markers and internal skin markers in the dermal by Spearman's correlation coefficient.

	Depth	**Parameter**	**Spearman correlation coefficient**			
			Sagging	**Ra**	**Rt**	**Rz**
Hydration	2.5 mm	Skin moisture Content	−0.242	−0.146	−0.109	−0.147
	5 mm		−0.270*	−0.162	−0.133	−0.166
Elasticity	3 mm	Ue (Immediate extensibility)	−0.132	−0.332**	−0.324**	−0.316*
		Uv (Additional extensibility)	0.059	−0.118	−0.112	−0.113
		Ur (Immediate recovery)	−0.228	−0.355**	−0.342**	−0.336**
		Uf (Total extensibility)	−0.072	−0.289*	−0.280*	−0.274*
		R7 (Skin elasticity)	−0.281*	−0.176	−0.16	−0.166
		R5 (Elastic recovery)	−0.125	0.017	0.022	0.022
	Dermal Density		-0.088	−0.088	−0.001	0.033
	Dermal Thickness		0.072	0.072	0.141	0.145

* Statistical significance (p value < 0.05), ** Statistical significance (p value < 0.01)



(a) (b)

Figure 2. Correlation between skin wrinkles and dermal depth internal skin markers : (a) Immediate extensibility Ue; (b) Immediate recovery Ur

3.2 Impact of Depth-Dependent Imbalances in ISM on ESM

To explore the impact of depth-dependent ISM imbalances on ESM, groups were classified based on differences in Ur/Ue (R5), a measure of net elasticity, between the epidermis (1 mm) and dermis (3 mm). K-Means clustering (k=2) was employed to minimize within-cluster variance of R5 depth difference values, using standard Euclidean distance without a predefined cutoff. Cluster centers were iteratively updated until convergence.

Two distinct groups emerged: one with large depth-dependent elasticity differences ("Large Difference") and another with small differences ("Small Difference"). The 'Large Difference' group exhibited a similar median compared to the 'Small Difference' group but demonstrated a broader distribution and higher upper values (Table 3, Figure 3). This indicates that a subset of individuals within the 'Large Difference' group exhibited significantly severe wrinkle patterns. These findings imply that while Ur/Ue (R5) depth disparity is not a universal predictor of external aging severity, subgroups with greater interlayer elasticity imbalances may display more pronounced wrinkle-related aging features. This highlights the importance of considering vertical elasticity differences in early-stage aging diagnostics.

Table 3. K-means clustering quantitative statistics

wrinkle parameter	Group	N	Mean	Median	STD Dev	IQR
Ra	Small	41	18.47	18.78	4.09	6.33
	Large	24	18.72	17.32	5.71	9.38
Rt	Small	41	149.22	148.83	30.87	47.65
	Large	24	152.41	136.11	41.11	60.37
Rz	Small	41	105.51	107.36	20.23	34.04
	Large	24	107.31	98.34	28.34	47.50

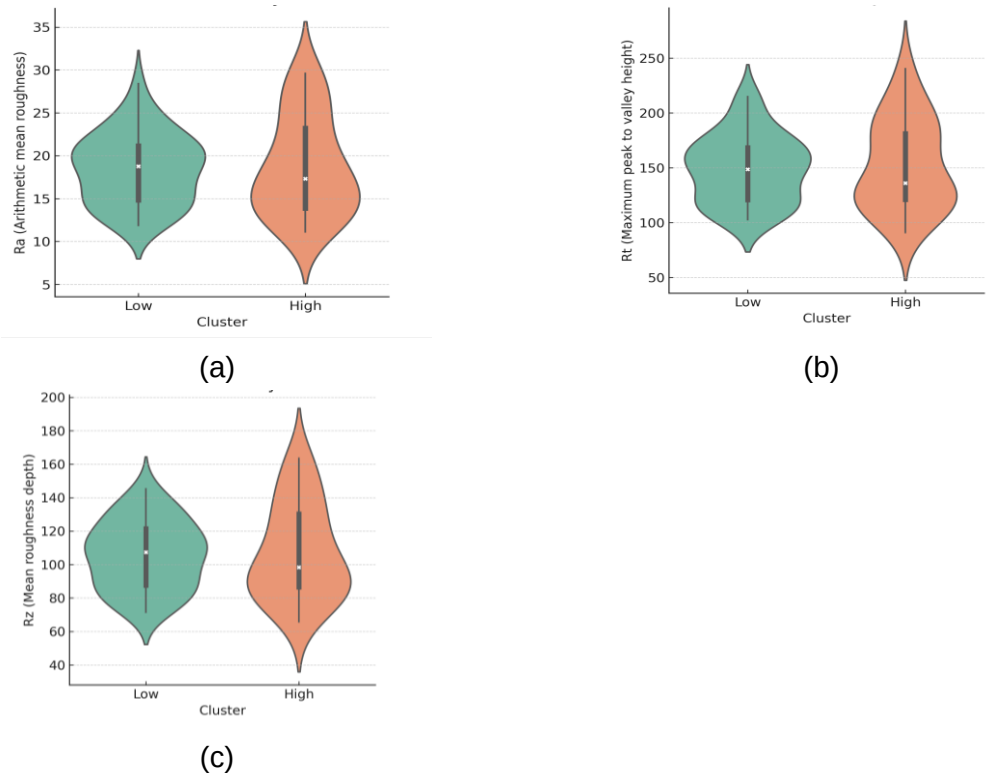


Figure 3. Distribution of wrinkle parameters by Cluster: (a) distribution of Ra; (b) distribution of Rt; (c) distribution of Rz.

4. Discussion

There are various factors that contribute to skin aging, with chronological age being the most significant. Typical signs of skin aging include increased roughness and sagging,[18-20] decreased brightness (L value),[21, 22] and reduced elasticity and elastic recovery.[23, 24] Recent studies have also explored correlations between skin characteristics to identify additional factors influencing skin aging.[25] However, a limitation of these studies is that they often consider the skin as a single layer, focusing only on surface characteristics.

This study aimed to elucidate the effects of depth-specific variations in internal skin markers on external aging features. By categorizing internal skin markers based on measurement depth and analyzing their correlations with external skin markers such as wrinkles and sagging, the study offers new insights into the early detection of skin aging beyond conventional surface-focused assessments.

Unlike previous studies that primarily regarded the skin as a single-layered structure or focused solely on surface-level characteristics, this study underscores the importance of depth-specific variations in internal markers and their influence on external aging markers. By employing a layered analytical approach, this research provides a novel perspective on skin aging, advancing beyond traditional surface-focused methodologies.

The analysis revealed significant negative correlations between wrinkle parameters (Ra, Rt, Rz) and dermal elasticity markers (Ue, Ur), indicating that reduced elasticity in deeper layers plays a key role in wrinkle formation. Conversely, the skin sagging angle was associated with epidermal hydration and superficial elasticity (Ur), suggesting that surface-level deterioration also contributes to external signs of aging. Furthermore, clustering analysis based on depth differences in Ur/Ue ratios demonstrated that individuals with larger interlayer disparities tended to exhibit more severe wrinkles. These findings underscore the importance of a layer-specific interpretation of skin aging, emphasizing that interlayer imbalances, rather than uniform decline, may contribute to visible aging symptoms.

However, a limitation of this study is its cross-sectional design, which does not allow for longitudinal tracking of how individual skin conditions evolve over time. Future studies should incorporate longitudinal designs to better capture aging dynamics at the individual level. Furthermore, as this study included only Korean female participants, the findings may not be generalizable to other sexes or ethnic groups. Thus, future research should include more diverse populations to enhance the external validity of the results.

While the current study focused on quantitative correlations between internal markers and external features, a more comprehensive understanding of skin aging would benefit from an analysis of biochemical components such as collagen, elastin, and hyaluronic acid. Particularly, the Dermal-Epidermal Junction (DEJ)—a key structure for mechanical stability and signal transmission between the epidermis and dermis—should be examined in relation to aging. Studying age-related changes in DEJ-associated proteins (e.g., laminin, integrins, fibronectin) may help clarify how the degradation of this interface contributes to external signs of aging.

By integrating such layer-specific biological data with external skin metrics, the development of AI-based aging prediction models could open new avenues for early diagnosis and personalized anti-aging strategies**.**

5. Conclusion

This study underscores the critical role of depth-specific internal skin markers in elucidating the mechanisms underlying skin aging. By establishing significant correlations between dermal elasticity and wrinkle formation, as well as epidermal hydration and skin sagging, this research provides compelling evidence that the aging process varies across distinct skin layers. Furthermore, the identified interlayer elasticity imbalances may serve as a promising early diagnostic marker for predicting visible aging features before their full manifestation.

The findings presented in this study contribute to a more nuanced understanding of skin aging, emphasizing the necessity of adopting a multi-layered approach in both investigative and clinical contexts. By highlighting the dynamic interplay between internal and external markers, this study offers an innovative perspective on proactive skin aging management.

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