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"Advancing Skincare Science: Safety Evaluation of Spicule-Based Products Using Confocal Imaging"

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1. Introduction

The human skin is a complex and multilayered organ forming a critical component of the body's innate immune system [1]. The stratum corneum, the outermost layer of the epidermis, acts as a primary barrier, effectively blocking the penetration of external substances [2]. However, this protective function also presents a major obstacle to the transdermal delivery of cosmetic active ingredients [3]. To overcome this barrier, numerous physical and chemical enhancement techniques, such as electroporation and nanoparticles, have been developed [4-5]. However, each method carries limitations like invasiveness, patient compliance or delivery efficiency [6].

Microneedles, consisted of micron-sized projections that puncture the stratum corneum with minimal discomfort, have gained attention for their ability to deliver active compounds effectively while simultaneously triggering wound-healing responses [7-8]. This dual functionality makes microneedles particularly appealing in cosmetic applications [9]. However, conventional microneedle arrays are limited by their fixed application area and the transient nature of the microchannels they create [9-10]. These channels begin to close within minutes due to the skin's natural recovery mechanisms, significantly restricting the window for active ingredient absorption [6,11]. To overcome these limitations, spicules have emerged as a novel alternative. Spicules are natural or synthetic micro needle-like structures measuring 50–250 µm in size [12-13]. These can be applied in a dispersed manner, allowing broader and more flexible coverage of the skin [14].

Despite the growing use of spicules in cosmetic formulations, concerns still remain regarding their safety and long-term skin compatibility of spicules when applied to human skin. According to previous studies, spicules had penetrated up to the depth of stratum corneum and were gradually exfoliated through the natural desquamation process [12-13]. However, these findings are based on *in vitro* or animal models. The retention time of spicules potential accumulation effects in human subjects still remain unknown. Therefore, there is a critical need for the investigation of penetration, retention, and potential for irritation or inflammation of spicules in human skin to ensure the safe and effective application of spicule-based formulations in cosmetics. In addition, the safety and efficacy of spicules may vary depending on their

source material, shape, and length, which can influence their interaction with the skin [15-16]. Therefore, comprehensive clinical investigations that consider not only irritation and penetration but also product usability and cosmetic benefits are essential to ensure the safe and effective application of spicule-based formulations in cosmetics.

In this study, we first aimed to evaluate the efficacy of the spicule-containing formulation by assessing improvements in skin smoothness, pore area, and elasticity following product use. After confirming its efficacy, we subsequently investigated the safety of the formulation through a primary skin irritation test and in-use perception study which assessed subjective sensations such as stinging and itching. Finally, we observed the penetration and retention of spicules in human skin over a seven-day period reflectance using confocal microscopy.

Importantly, the study included formulations containing spicules of different compositions and length, and evaluated both short-term and long-term effects. Spicule retention was also assessed using Confocal Laser Scanning Microscope (CLSM), enabling multi-dimensional analysis of their behavior in human skin [17-18]. Taken together, this study offers a comprehensive clinical evaluation of spicules from multiple perspectives, including efficacy, safety, usability, and penetration.

2. Materials and Methods

2.1. Efficacy Evaluation

To evaluate the skin improvement effects of spicule-based products, two types of efficacy tests were conducted. A short-term test assessed the immediate effects of a single application, whereas a long-term test evaluated cumulative effects after repeated use.

2.1.1. Short-term study

In the short-term study, five healthy participants (1 males, 4 females; mean age: 29.8 years) applied both Test Product 1 (0.5% hydrolyzed sponge cream) and Test Product 2 (0.5% calcium aluminum borosilicate cream) once to the face, with each product applied to opposite sides (left and right) for intra-individual comparison. Skin texture and pore condition were evaluated before and after application using Antera 3D (Miravex, Ireland), a high-resolution optical imaging system that reconstructs 3D skin surface topography. With an analysis area of 56 × 56 mm^2, the Antera 3D system uses multidirectional, seven-wavelength LED lighting to capture and analyze skin topography and pigment concentration. Quantitative parameters included Roughness a (arithmetic average roughness, AU), Roughness q (root mean square roughness, AU), and pore affected area (mm^2). The pore affected area represents the total pore area automatically detected within the designated analysis region on the test site. These metrics collectively reflect surface texture and pore appearance.

2.1.2. Long-term study

In the long-term study, five different participants (2 males, 3 females; mean age: 32.6 years) applied Test Product 3 (1% calcium aluminum borosilicate cream) to the face twice daily for four weeks. For safety considerations, the spicule concentration was limited to 1%, a relatively low level, to minimize potential irritation during prolonged use. Skin elasticity was assessed at baseline, Week 2, and Week 4 using a Cutometer (MPA 580; Courage + Khazaka Electronic, Germany). Cutometer applies negative pressure of 450 mbar to draw the skin vertically through the aperture, then measures its displacement through optical means. A measurement session was completed by three cycles of 2-s suction, followed by 2-s relaxation. The overall elasticity was determined using the R2 parameter.

2.2. Safety Evaluation

2.2.1. Primary Irritation Test

A 24-hour closed patch test was conducted on 35 healthy participants (1 male, 34 females; mean age: 43.2 years) to assess the irritation potential of Test Product 4 (7% calcium aluminum borosilicate lotion) and Test Product 5 (0.5% hydrolyzed sponge essence). Each product (20 µL) was loaded into an IQ Ultimate chamber (Chemotechnique Diagnostics, Sweden), applied to the upper back, and secured using 3M Micropore tape. The chambers were removed after 24 hours.

Skin reactions were visually evaluated by trained investigators at 1 hour and 24 hours post-removal. Assessments were performed according to the Frosch & Kligman method and CTFA guidelines, using a modified Draize scoring system. The irritation index was calculated as the average score across all participants for each product. This test was conducted following the COLIPA guideline for skin compatibility assessment [19].

2.2.2. In-use Perception Test

An in-use perception test was conducted to evaluate subjective skin sensations associated with the application of spicule-containing formulations. Two test products (Test Product 6 and Test Product 7) were prepared as essence-impregnated swabs, each containing 0.3% hydrolyzed sponge. While the spicule concentration was identical in both products, they differed in spicule length. Test Product 6 contained intact spicules with an approximate diameter of 10 µm and a length of 220–250 µm. In contrast, Test Product 7 used the same material that had undergone a grinding process to reduce spicule length, resulting in shorter spicules measuring approximately 50–70 µm in length, with similar diameters (~10 µm). This size reduction was intended to minimize potential sensory irritation during application.

The test was performed on 10 healthy participants (5 males, 5 females; mean age: 30.5 years). Each product was applied once to opposite sides of the face (left and right cheeks) to allow intra-individual comparison. Subjective skin sensations were assessed at four time points: during application, immediately after application, 5 minutes post-application, and 10 minutes post-application. Participants rated their perception of stinging and itching sensations using a 6-point scale: 0 = no sensation, 1 = very slight, 2 = slight, 3 = moderate, 4 = strong, 5 = very strong. The evaluation was designed to capture both the intensity and temporal progression of sensory responses following a single application of each product with differing spicule sizes.

2.2.3. Confocal Imaging-Based Safety Evaluation

To investigate the skin penetration behavior of spicules, in vivo confocal imaging studies were conducted using Test Product 4 (7% calcium aluminum borosilicate lotion). Two healthy female participants (in their 20s and 30s) applied Test Product 4 once daily for three consecutive days to the mid-forearm in an appropriate amount. No further application was made after Day 3. Imaging was performed using a Vivascope 1500 (Lucid Inc., USA), a non-invasive CLSM designed for real-time, high-resolution imaging of skin structures at the cellular level. Optical sectioning was achieved at depths ranging from 0 to 70 µm, in 10 µm intervals. Spicule presence and location were evaluated at five time points: before application, immediately after the first application, Day 3 (before and after washing), and Day 7 (follow-up).

2.3. Statistical Analysis

All statistical analyses were performed using SPSS Statistics software (version 28.0.1.1; IBM Corp., Armonk, NY, USA). Given the small sample size (n = 5), non-parametric methods were selected to evaluate changes in skin parameters for efficacy evaluation. In the short-term

study, differences before and after a single application were analyzed using the Wilcoxon signed-rank test. In the long-term study, changes across baseline, Week 2, and Week 4 were analyzed using the Friedman test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Efficacy Evaluation

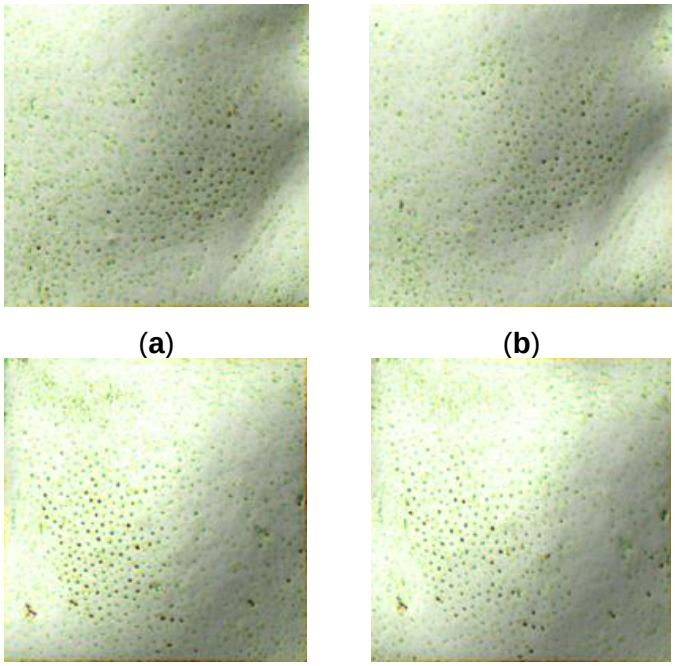
Both short-term and long-term efficacy assessments demonstrated measurable improvements in skin condition following application of spicule-based products.

In the short-term study, Test Product 1 and Test Product 2 were applied once. Both products showed numerical reductions in Ra, Rq and pore affected area after application. Although the changes did not reach statistical significance (p = 0.0625, Wilcoxon signed-rank test), a trend toward improvement was observed. The small sample size (n = 5) may have influenced the statistical outcome. Test Product 2 exhibited greater improvements across all parameters compared to Test Product 1 (Table 1).

Table 1. Changes in skin roughness and pore affected area after a single application of Test Product 1 and Test Product 2 (n = 5).

Product	Skin evaluation index		Before	After
Test Product 1	Ra	Mean	3.042	2.865
		% Change	–5.82%	
		p-value	0.0625	
	Rq	Mean	3.922	3.709
		% Change	–5.43%	
		p-value	0.0625	
	Pore area	Mean	32.975	27.237
		% Change	–17.40%	
		p-value	0.0625	
Test Product 2	Ra	Mean	3.126	2.912
		% Change	–6.85%	
		p-value	0.0625	
	Rq	Mean	3.748	3.445
		% Change	–8.08%	
		p-value	0.0625	
	Pore area	Mean	38.083	29.681
		% Change	–22.06%	
		p-value	0.0625	

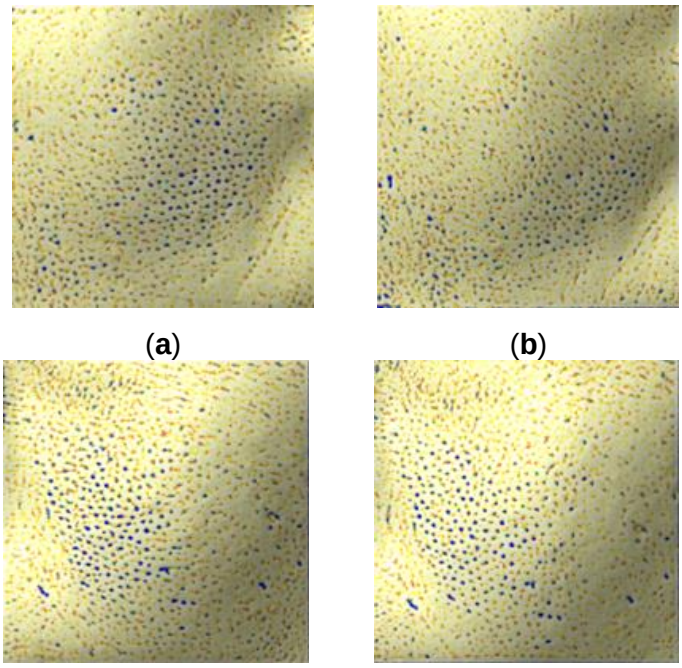
In addition to quantitative analysis, visual comparisons of skin texture and pore condition were conducted using Antera 3D imaging. Representative images before and after product application are shown in Figure 1 (skin texture) and Figure 2 (pore appearance).



(**a**) (**b**)

(**c**) (**d**)

Figure 1. Representative skin texture images captured using Antera 3D: (a) Test Product 1 – Before application; (b) Test Product 1 – After application; (c) Test Product 2 – Before application; (d) Test Product 2 – After application.



(**a**) (**b**)

(**c**) (**d**)

Figure 2. Representative pore appearance images captured using Antera 3D: (a) Test Product 1 – Before application; (b) Test Product 1 – After application; (c) Test Product 2 – Before application; (d) Test Product 2 – After application.

In the long-term study, participants applied Test Product 3 twice daily for four weeks. Skin elasticity, measured via the R2 parameter, progressively increased over time. Statistical analysis was performed using the Friedman test, and a statistically significant improvement

was observed (p = 0.0224). A 10.64% increase was recorded at Week 4 compared to baseline (Table 2).

Table 2. Changes in skin elasticity (R2) over four weeks of Test Product 3 use (n = 5)

Skin evaluation index		Test Product 3		
		Baseline	**Week 2**	**Week 4**
R2	Mean	74.79	81.48±	82.75
	% Change	-	+8.95%	+10.64%
	p-value	0.0224 *		

* Probability *p* (Friedman test, Significant: **p*<0.05)

3.2. Safety Evaluation

Both Test Product 4 and Test Product 5 were evaluated for skin irritation potential using a 24-hour closed patch test on 35 participants. No visible adverse skin reactions were observed at either 1 hour or 24 hours post-removal for both products. The irritation index, calculated based on mean visual scores assessed by trained investigators, was 0.00 for both products, indicating that neither product induced any measurable irritation under occlusive conditions (Table 3).

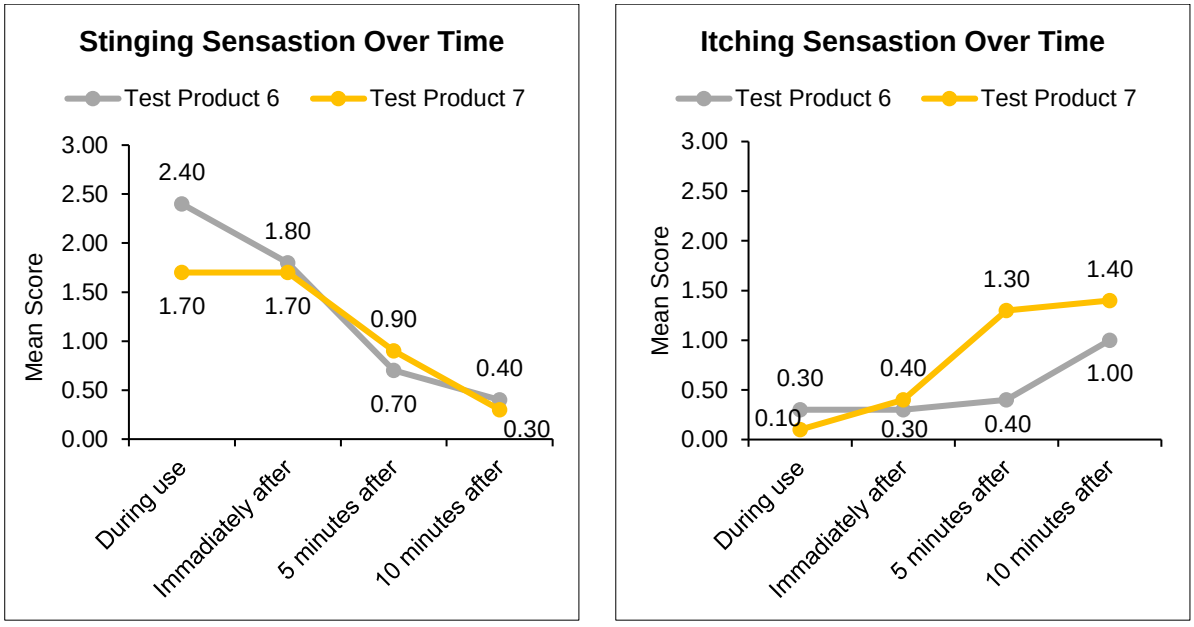
Table 3. Skin irritation index of Test Product 4 and Test Product 5 (n = 35)

Product	**Skin Irritation Index**	**Classification**
Test Product 4	0.00	Non-irritant
Test Product 5	0.00	Non-irritant

Subjective sensory responses were assessed following the application of two spicule-containing products with different spicule lengths. Test Product 6 contained longer spicules (220–250 µm), while Test Product 7 contained shorter spicules (50–70 µm). Participants (n = 10) evaluated stinging and itching sensations at four time points—during application, immediately after, 5 minutes after, and 10 minutes after application—using a 6-point scale (0 = none, 5 = very strong). The results are summarized in Figure 3.

The average stinging score was higher for Test Product 6 compared to Test Product 7 during application. However, from immediately after to 10 minutes post-application, no notable difference was observed between the two products. In contrast, the average itching score showed minimal difference between the two products during and immediately after application. From 5 minutes post-application onward, itching was more pronounced in Test Product 7 than in Test Product 6.

Overall, Test Product 7 showed a slightly higher total mean sensory score (0.91) than Test Product 6 (0.98), although the difference was minimal. Stinging was reported as the most prominent sensation for both products, especially during and immediately after application, while itching was generally mild. Both sensations declined substantially within 10 minutes after use, indicating transient and tolerable responses. All scores remained below 3.0, suggesting that both formulations were well tolerated regardless of spicule length.



(a) **(b)**

Figure 3. Changes in sensory responses over time following a single application of spicule-based formulations: (a) stinging sensation, (b) itching sensation.

Confocal imaging revealed the presence and retention of spicules on the skin surface and within the upper layers of the stratum corneum following product application. In the in vivo study using Test Product 4, spicules were observed immediately after the first application and remained visible within the superficial layers and follicular openings on Day 3. By Day 7, no spicules or fragments were detected, suggesting natural elimination through washing and epidermal turnover. No penetration beyond 70 µm was observed at any time point. Representative images captured by Vivascope 1500 are shown in Figure 4.

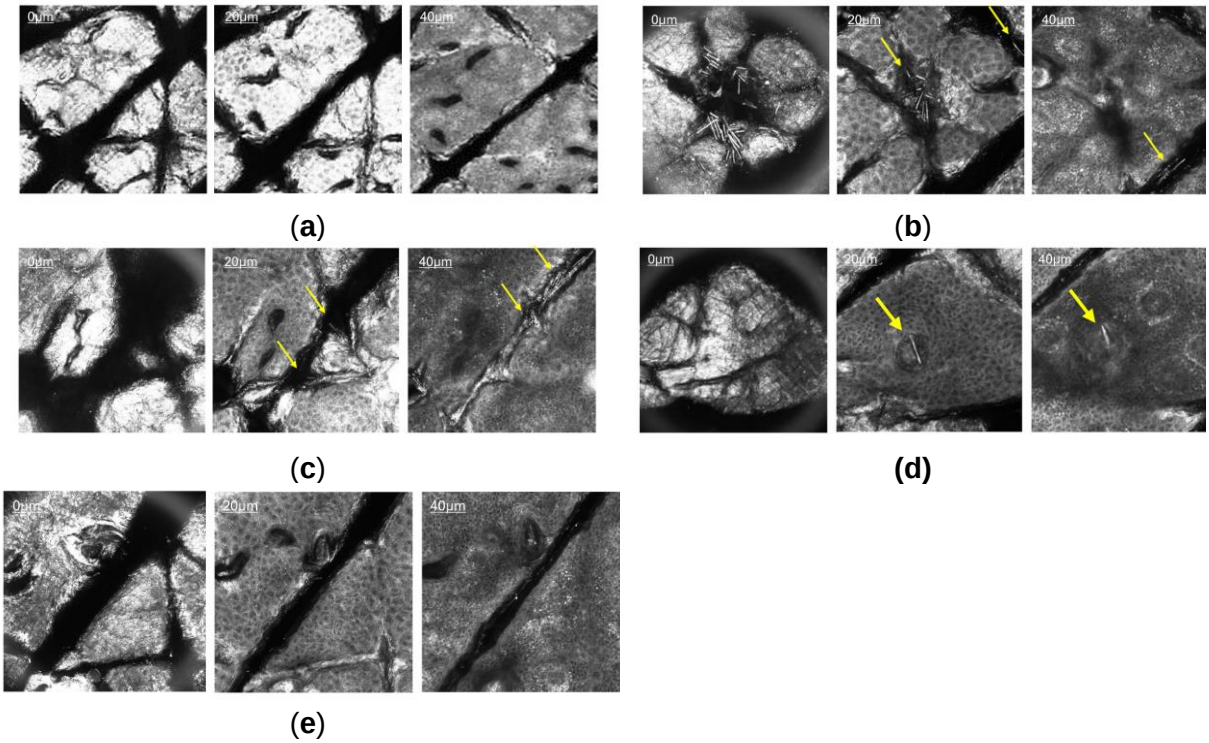


Figure 4. In vivo confocal images (Vivascope 1500) by skin depth of spicules after application of Test Product 4: (a) Before application; (b) Immediately after the first applicatoin; (c) Before washing on the third day; (d) After washing on the third day; (e) Four days after discontinuation.

4. Discussion

Spicules are primarily derived from marine sponges and are composed of either silica (SiO_2) or synthetic materials such as calcium aluminum borosilicate [20-21]. Recently, various types of spicule materials have been developed [20-21].

In this study, we evaluated the efficacy of spicule creams formulated with sponge extract and calcium aluminum borosilicate. Both products demonstrated immediate improvements in skin texture and pore appearance. Also, We observed a statistically significant improvement in skin elasticity after four weeks of using a cream containing 1% calcium aluminum borosilicate. These findings support previous studies reporting that spicule-based cosmetics can improve various skin parameters such as wrinkles, elasticity, and acne in healthy skin populations [15,22].

In terms of safety, primary irritation tests were performed using products with different spicule concentrations and materials. Patch tests were conducted with commercially available products 7% calcium aluminum borosilicate lotion and 0.5% hydrolyzed sponge extract essence and both were classified as Non-irritant. However, it is important to note that the tests were conducted only on healthy subjects, excluding those with sensitive or compromised skin barriers.

Besides conducting a primary irritation test to assess contact-induced irritation, we carried out a subjective evaluation survey focusing on the sensory responses of participants during the application of spicule products. In this study, we conducted a comparative test with the expectation that the perceived level of stinging and itching would differ according to the size of the spicules. While some subjective sensations, including stinging and itching, were reported during the in-use perception tests, these symptoms were transient, showed minimal variation with spicule size, and were not correlated with objective signs such as erythema or edema. Therefore, these findings suggest that contact irritation and sensory perception of irritation may not necessarily correspond.

Interestingly, different temporal patterns were observed between stinging and itching sensations. Stinging was primarily reported during application and quickly subsided within 10 minutes. In contrast, itching tended to emerge later, becoming more prominent 5 to 10 minutes after application, regardless of spicule size. This phenomenon may be attributed to mechanical stimulation of sensory nerve endings in the upper epidermis or follicular structures [23], where spicules are temporarily retained. Although no observable irritation was present, these findings suggest that the physical presence of spicules alone may elicit transient sensory responses.

Importantly, using confocal laser scanning microscopy (CLSM), we directly visualized the behavior of spicules in human skin. Spicules remained confined within the epidermis, did not penetrate beyond 70 μm, and disappeared after approximately one week, suggesting they are naturally eliminated through the skin turnover process without systemic absorption. By utilizing CLSM, we observed the dynamic behavior of actual spicules. This study provides meaningful findings that can enhance and supplement existing safety evaluation methods. We confirmed that spicules are not absorbed transdermally, thereby providing additional safety data for spicule-containing products in the cosmetic market. However, we recognize that variations may occur depending on the product formulation, the specifications of the spicules, and the characteristics of the consumers. Therefore, additional study is needed because safety results may vary depending on various factors.

5. Conclusion

Various studies have been conducted to promote transdermal absorption and improve skin condition by applying physical friction to spikule cosmetics. However, research regarding their safety remains insufficient. To date, no studies have directly visualized the penetration pathways of spicules within human skin. This is the first study that directly visualizes the real-time transcutaneous penetration and dynamic retention behavior of spicules in human skin using CLSM. This study clearly revealed that spicules do not undergo transdermal absorption and are instead eliminated through the natural skin turnover process. In conclusion, while previous studies have confirmed that spicules can offer various skin benefits in individuals with healthy skin, it is important to exercise caution when applying them to individuals with sensitive skin or weakened skin barriers. We intend to conduct long-term studies involving a broader range of subjects.

6. References

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