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Full transcriptomics, a powerful tool for ingredient innovation and repurposing

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1. Introduction

The cosmetic industry is currently navigating a rapidly evolving landscape shaped by heightened consumer expectations, increasing demand for multifunctional and sustainable ingredients, and increasingly stringent regulatory requirements. While these regulations play a vital role in ensuring product safety and transparency, they simultaneously constrain innovation by complicating the development of novel actives. In this context, the repurposing of well-characterized ingredients—assigning them new applications based on novel mechanisms of action—has emerged as a promising strategy to accelerate innovation without compromising regulatory compliance.

Historically, the bioactivity of cosmetic ingredients has been assessed through targeted approaches focusing on a limited set of biomarkers. Although these methods are useful to confirm known effects, they are often insufficient to capture the full complexity of cellular responses, especially for multifunctional compounds. This limitation underlines the need for holistic and integrative approaches capable of exploring the global impact of active ingredients on cellular physiology.

In recent years, full transcriptomics, an untargeted high-throughput omics technology, has become increasingly relevant for the mechanistic characterization of bioactive compounds. By enabling simultaneous quantification of the expression levels of thousands of genes, transcriptomics offers a systems-level perspective on cellular responses, and allows for the identification of both expected and novel signaling pathways [1]. When paired with bioinformatic tools such as pathway enrichment and upstream regulator analysis, this

approach provides unprecedented insights into the complex molecular interactions involved in skin physiology.

Despite its potential, the use of transcriptomics in cosmetic science remains limited, primarily due to the complexity of data analysis and interpretation, which requires interdisciplinary expertise in molecular biology, data science, and dermo-cosmetology. Nevertheless, its capacity to uncover unexpected biological effects has already been demonstrated in dermatological and cosmetic research [2,3].

Among the active compounds of interest in dermocosmetic research are silicium-based derivatives (SiDs), which are widely used for their structural role in extracellular matrix homeostasis and their reputed anti-aging properties. While several studies have highlighted the importance of silicium in connective tissue biology and mineralization [4,5], the full spectrum of their effects on dermal cells remains largely unexplored. The present study aims to address this knowledge gap by applying full transcriptomic analysis to human dermal fibroblasts exposed to a cosmetic-grade SiD. Our objective is to investigate the broader transcriptional response elicited by this compound, and to assess the value of transcriptomics as a strategic tool for mechanistic exploration and claim extension in the field of ingredient repurposing.

2. Materials and methods

Cell culture: Primary fibroblasts (PF2) were isolated from hospital-derived waste tissue by out-growth of fibroblasts from explanted tissue pieces. PF2 are a pool of fibroblasts from mammoplasties of 5 female donors aged 21 to 51. Cells were then expanded up to passage 8 (Qima, Gençay, France). PF2 were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) supplemented with L-glutamine (L-Glu) and antibiotics. Cells were seeded in 24-well plates and cultured in a culture medium at 37°C in a 5% CO₂ humidified atmosphere for 24 h, followed by a further 24 h in a test medium (DMEM + 1% FBS). The medium was then replaced by a test medium containing or not (control) SiD at 20, 100 or 200 µM. The cells were incubated under these conditions for 24 h. All these conditions were performed in triplicate (n=3). At the end of incubation, culture supernatants were removed, and cells were rinsed with PBS solution. Plates were then immediately frozen at -80°C.

RNA isolation: RNA from each sample was extracted using Tri Pure Isolation Reagent® (Roche Diagnostics GmbH, Mannheim, Germany) according to the supplier's protocol. RNA quality was assessed by capillary electrophoresis (Bioanalyzer 2100, Agilent, Santa Clara, USA). Complementary DNA was synthesized by reverse transcription of total RNA in the

presence of oligo(dT) and the Transcriptor Reverse Transcriptase enzyme (Roche). qPCR reactions were performed on Light Cycler (Roche Molecular Systems, Meylan, France) by Qima (Gencay, France). Four housekeeping genes were used to determine the relative mRNA levels: *RPS28* (Ribosomal protein S28), *GAPDH* (Glyceraldehyde-3-phosphate dehydrogenase), *ACTB* (Actin beta) et *B2M* (Beta-2-microglobulin).

Total RNA sequencing analysis: The Illumina NEBNext® Ultra™ II Directional mRNA Library Prep Kit (New England BioLabs, Evry, France) was used to prepare RNA sequencing (RNA-seq) libraries with 100 ng of input RNA. Paired-end RNA-seq was carried out on an Illumina NextSeq sequencing instrument (Helixio, Clermont-Ferrand, France). The RNA-seq read pairs were then mapped to the reference human GRCh38 genome using STAR Software (Spliced Transcripts Alignment to a Reference, <https://github.com/alexdobin/STAR>). Statistical analyses were performed using R software (Free Software Foundation, Inc., Boston, MA, USA) and R package edgeR (<https://bioconductor.org/>). Genes were considered differentially expressed if they had an adjusted p-value lower than 0.05 (Benjamini-Hochberg correction).

Biological pathway analysis: The Ingenuity Pathways Analysis software (IPA, Qiagen, Redwood City, USA) was used to analyze the biological pathways involved in the differential gene expression dataset. IPA allows the identification of metabolic pathways predicted from an expression gene list.

Statistical analysis: For histograms, the data was presented as mean ± SEM. GraphPad Prism (version 10.4.0, GraphPad software LLC, San Diego, CA, USA) was used to assess significant differences using an ANOVA or Student t-test. A statistically significant value of $p < 0.05$ was used.

3. Results

Gene expression modulation by SiD

Transcriptomic profiling revealed a concentration-dependent modulation of gene expression following treatment with the siliconium-derived compound (SiD).

As shown in Figure 1A, up to 1500 genes were modulated at the highest dose (200 µM), based on an FDR threshold of <0.05 . Among these, a substantial subset exhibited a fold change greater than 1.5 (Figure 1B), underscoring a robust transcriptional response to SiD exposure.

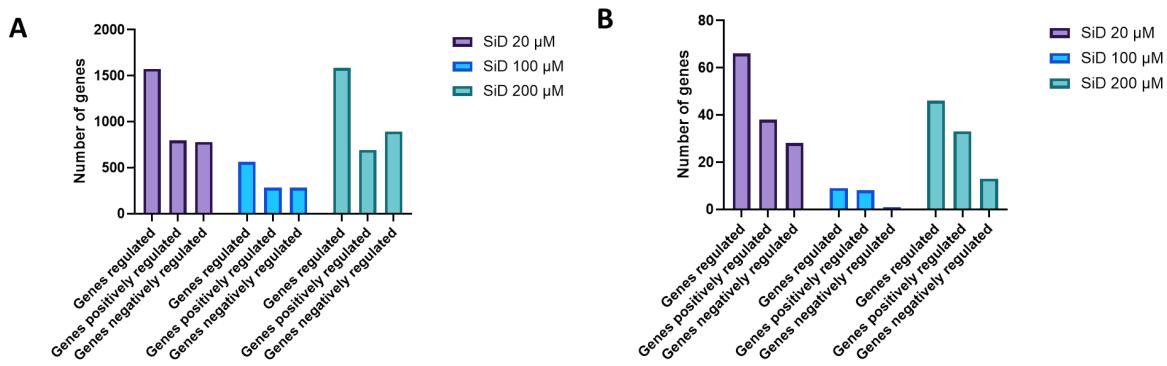


Figure 1. Number of genes regulated by growing concentrations of SiD with a False Discovery Rate (FDR) < 0.05 (A). Among these, number of genes with a fold change > 1.5 (B).

Regulation of genes involved in matrix organization by SiD

Further analysis focused on genes implicated in extracellular matrix (ECM) organization and remodeling. SiD significantly upregulated the expression of enzymes and structural components such as lysyl oxidase (LOX), lysyl hydroxylase (LH or PLOD3), hyaluronan synthase 2 (HAS2), and various proteoglycans (Figure 2A). Additionally, antioxidant enzymes were found to be modulated by SiD treatment. Notably, SiD significantly increased the expression of *HMOX1* and *SOD2*, two genes that are downstream targets of the Nrf2 signaling pathway, a key regulator of cellular redox homeostasis (Figure 2B).

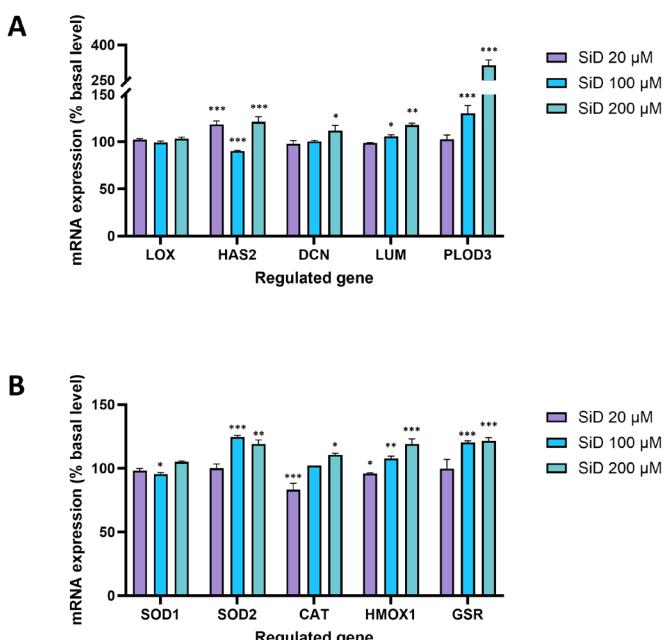


Figure 2. Differential gene expression induced by SiD related to extracellular matrix organization (A) and antioxidant response (B). mRNA levels were quantified by RT-qPCR. The bars indicate means + standard error. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001 using Students' T-test.

Enrichment of biological pathways by SiD

Ingenuity pathway analysis (IPA) of DEGs at increasing concentrations of SiD (20 µM, 100 µM, and 200 µM) revealed significant enrichment of multiple canonical signaling pathways (Figure 3).

At all tested concentrations, SiD robustly activated pathways related to ECM organization and cell adhesion, such as integrin signaling, actin cytoskeleton signaling, and remodeling of epithelial adherent junctions. Notably, at 200 µM, there was a marked downregulation of glycosaminoglycan metabolism pathways, pointing to potential stimulation of ECM components such as proteoglycans. The activation of the selenoamino acid metabolism pathway by SiD suggests a potential enhancement of cellular antioxidant defense through increased synthesis of selenoproteins. Interestingly, EIF2 Signaling was significantly activated at 200 µM of SiD, suggesting a potential involvement of the EIF2AK4/GCN2 kinase, a known sensor of nutrient and oxidative stress.

4. Discussion

This study used full transcriptomic profiling to investigate the molecular responses of human dermal fibroblasts to a siliconium-derivative (SiD). Our results showed a concentration-dependent modulation of gene expression, with up to 1500 genes differentially expressed at the highest tested concentration. As expected, genes involved in extracellular matrix (ECM) organization, such as *LOX*, *LH*, and *HAS2*, were upregulated, supporting the established structural role of SiD [6-8].

The enrichment analysis adds an important dimension to these findings. At low concentration (20 µM), SiD prominently activated pathways involved in cell adhesion and cytoskeletal dynamics, including remodeling of epithelial adherent junctions and actin cytoskeleton signaling. This suggests early involvement in structural support and intercellular connectivity. At moderate and high concentrations (100 and 200 µM), transcriptomic signatures indicated strong activation of dermatan sulfate biosynthesis and a decrease in glycosaminoglycan metabolism and degradation, pointing to a stimulation of proteoglycan synthesis that contributes to dermal hydration and structural integrity [9,10].

In addition to its effects on ECM regulation and cytoskeletal dynamics, SiD also triggered the activation of the Nrf2 signaling pathway and the selenoamino acid metabolism.

Nrf2 is a central regulator of cellular antioxidant defense, and its activation, evidenced by the upregulation of downstream targets such as *HMOX1* and *SOD2*, points to an enhanced cellular capacity to counteract oxidative stress [11,12].

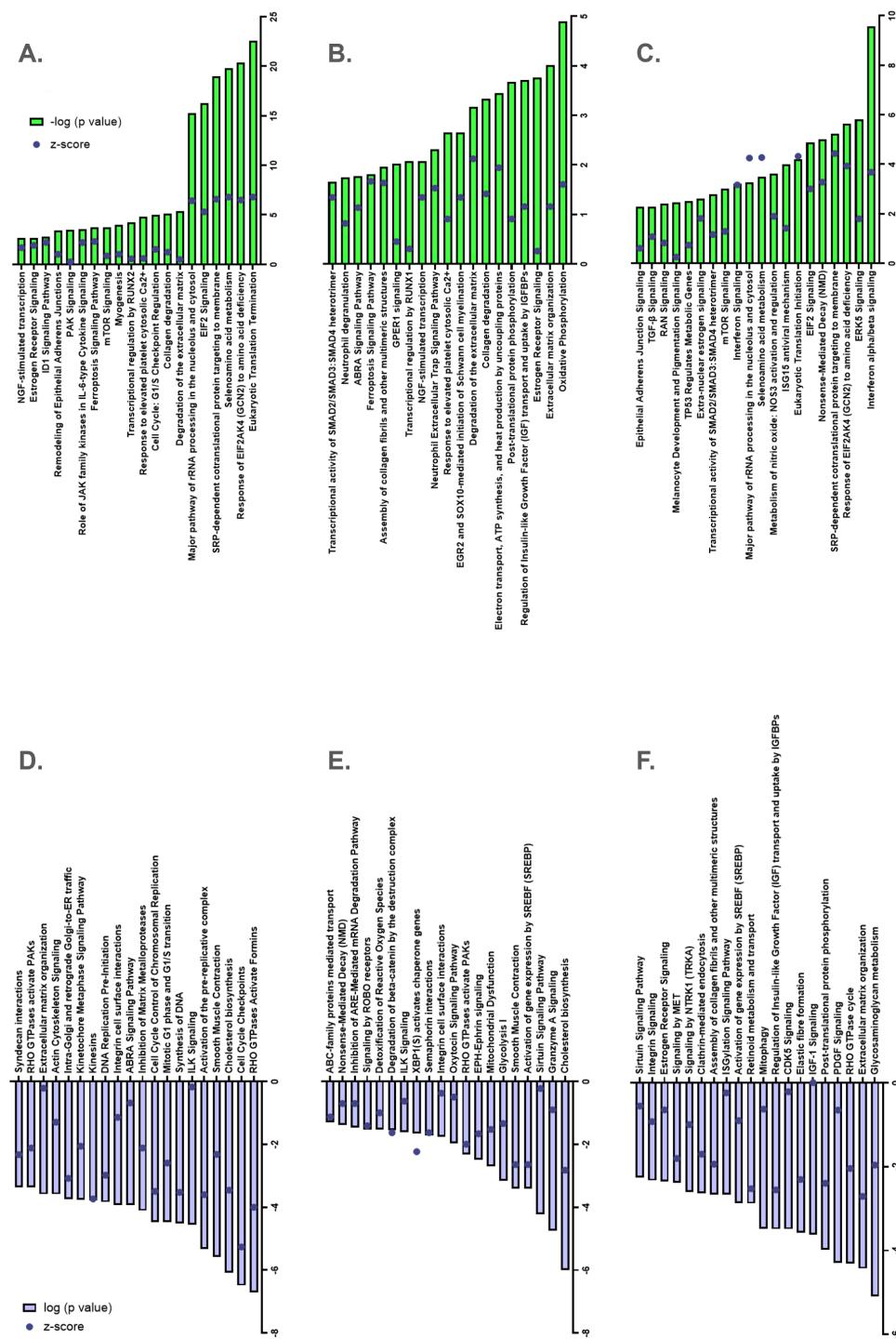


Figure 3: Enrichment analysis summarizing most representative up-regulated and down-regulated pathways by SiD at 20 μ M (A, D), 100 μ M (B, E) and 200 μ M (C, F) generated by Ingenuity Pathway Analysis (IPA). The Activation z-score predicts the activation state of the upstream regulator, using the molecule expression or protein phosphorylation patterns of the molecules downstream of an upstream regulator. An absolute z-score of ≥ 2 is considered significant.

The selenoamino acid metabolism pathway is essential for the biosynthesis of selenoproteins that are key players in maintaining redox homeostasis [13].

The concurrent induction of these two pathways suggests that SiD acts as a hormetic, capable of triggering adaptive responses that reinforce cellular resilience [14]. From a cosmetic perspective, this hormetic activation profile is particularly valuable, as it aligns with current strategies aimed at promoting skin longevity, detoxification, and protection against environmental aggressors such as pollution and UV-induced oxidative damage [15,16].

Surprisingly, transcriptomic analysis revealed the activation of signaling pathways associated with autophagy and cellular stress response, in particular the EIF2A/GCN2 pathway [17]. Activation of this pathway was particularly evident at 200 µM of SiD, suggesting the involvement of the EIF2AK4/GCN2 stress sensor. This kinase is known to regulate translation in response to nutrient deprivation or oxidative stress and has been implicated in the modulation of lifespan in *Caenorhabditis elegans* [18]. Its activation hints at a broader biological potential of SiD, possibly in skin resilience and longevity maintenance.

These findings illustrate the power of omics technologies, particularly full transcriptomics, to uncover both anticipated and novel biological activities of cosmetic ingredients. Such comprehensive, hypothesis-free approaches offer significant advantages over conventional targeted methods by providing a global view of cellular responses [19]. Many cosmetic ingredients are used based on observed effects—improved skin texture, hydration, or firmness—without a full understanding of their underlying mechanisms. Transcriptomic analysis bridges this gap by revealing molecular pathways that validate or explain these empirical observations. In our study, the upregulation of ECM genes confirms the structural role of SiD, while the modulation of EIF2AK4/GCN2/autophagy pathway suggests additional functions that were not previously attributed to this ingredient.

A central value of transcriptomics lies in its ability to identify new molecular targets, opening avenues for repurposing existing ingredients. For regulatory and cost-related reasons, reformulating or extending claims for well-established compounds is often more feasible than developing entirely new molecules [19]. By mapping global gene expression changes, researchers can uncover previously unrecognized activities and thus reposition ingredients for new cosmetic applications [19]. The case of SiD in this study exemplifies this approach: while it is traditionally used for ECM support, the newly observed involvement in stress adaptation and longevity/autophagy points toward potential benefits in skin protection, detoxification, or anti-aging strategies that go beyond structural claims.

Beyond their discovery potential, omics technologies support innovation at multiple levels. They allow for mechanism-based claim substantiation, biomarker identification for efficacy monitoring, formulation optimization through molecular synergy, and ethical and regulatory

alignment via reduction of *in vivo* testing requirements. Moreover, as multi-omics integration becomes increasingly accessible—combining transcriptomics with proteomics, metabolomics, or epigenomics—cosmetic researchers can gain deeper mechanistic insights and move toward systems-level understanding of skin biology.

5. Conclusion

Full transcriptomics represents a transformative tool in cosmetic science. It enables the identification of active mechanisms underlying ingredient efficacy, supports the discovery of new applications, and enhances product development by grounding innovation in robust biological evidence. The findings presented here with SiD provide a compelling example of how such approaches can be leveraged for the scientific repositioning of well-characterized ingredients, advancing both the mechanistic understanding and strategic potential of cosmetic actives.

6. References

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