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“Evaluation of oxidative stress in skin: interest of a new technology for quantifying *in vivo* the impact of solar aggressions and the antioxidant activity of cosmetics”

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1. Introduction

Oxidative stress, driven by an imbalance between reactive oxygen species (ROS) and the skin's antioxidant (AOX) defenses, is a major contributor to skin aging and various dermatological disorders. Environmental aggressors such as UV radiation, blue light, and pollution — collectively known as the skin exposome — promote ROS production, damaging lipids, proteins, and DNA, and ultimately compromising skin integrity [1,2].

While the skin relies on enzymatic defenses like superoxide dismutase (SOD) and catalase (CAT) to maintain redox balance, chronic exposure to these stressors can overwhelm these protective systems, leading to functional and structural impairments [3,4].

Our laboratory has previously characterized this oxidative imbalance through *in vivo* studies, using non-invasive skin surface sampling techniques to quantify markers of enzymatic activity (SOD, CAT), lipid peroxidation (e.g., peroxidized squalene, malondialdehyde), and protein carbonylation. Although these methods provide valuable insights, they remain time-consuming, require costly laboratory equipment, and do not enable rapid or on-site evaluation.

In this context, Skin-Biosense emerges as an innovative electrochemical sensor designed to rapidly, simply, and non-invasively assess the skin's electrochemical state. Initially validated *in tubo* on cosmetic ingredients and formulations, the technology was successfully adapted to biological models such as reconstructed human epidermis (RHE) and skin explants, where it demonstrated relevance for studying oxidative stress related to photodamage and barrier

function. These developments represent a step toward clinical application, with promising first *in vivo* results in the context of barrier-related conditions [5].

The present study serves as clinical proof of concept for the application of Skin-Biosense in assessing solar-induced oxidative stress *in vivo*. While the original protocol included both UV and blue light exposures, the article focuses on the blue light model, which yielded the most consistent and interpretable data. This model was used to characterize the oxidative impact of blue light on the skin's electrochemical state, and to explore the potential of this technology for future evaluation of protective strategies.

2. Materials and Methods

A clinical study was conducted on 22 healthy volunteers (19 women and 2 men; mean age: 43 years old), phototypes II-III. Two test areas (4x4cm) were selected on the middle of the back of each subject:

- one untreated and unexposed (control),
- and one untreated but exposed to blue light.

The exposed area was irradiated with blue light (420 nm) at 80 J/cm² using a Waldmann UV 802 L lamp, equipped with Blue V neon lights.

Two types of samples were collected from each area:

1. Catalase Activity: Skin surface sampling was performed 24 hours after blue light exposure using swabs pre-soaked in a sampling solution. The swabs were rubbed on the skin for 90 seconds, then frozen in an aqueous medium. Catalase enzymatic activity was subsequently assessed using a fluorescence-based biochemical assay.
2. Skin-Biosense electrochemical profile: 24 hours after blue light exposure, a sampling patch consisting of a 1 cm-diameter cotton pad pre-soaked in phosphate buffer solution was applied to the skin for 60 seconds to collect electrochemical species. One patch was used per zone. The collected patches were then snap-frozen and stored at -80 °C until analysis.

The Skin-Biosense device is based on electrochemical sensing. After a calibration step, each frozen cotton pad was thawed and placed directly on the sensor.

During the measurement, a potential sweep was applied to the sensor, and the resulting current was recorded, producing a voltammogram (also referred to as an electrochemical profile). This profile shows oxidation peaks, each corresponding to the presence of cutaneous electroactive species in the sample, such as antioxidants.

Each peak was then integrated, and its area was converted into a quantitative score, reflecting the global antioxidant signature or electrochemical activity of the sample.

3. Results

Electrochemical profiles were successfully obtained from all collected samples, with 2 to 4 oxidation peaks detected per sample. The most common peaks were located at approximately 0.7 V, 0.8 V, 1.2 V, and 1.4 V. Ongoing internal studies aim to precisely identify the electroactive species corresponding to each peak. Mean profiles from control and exposed areas are shown in Figure 1.

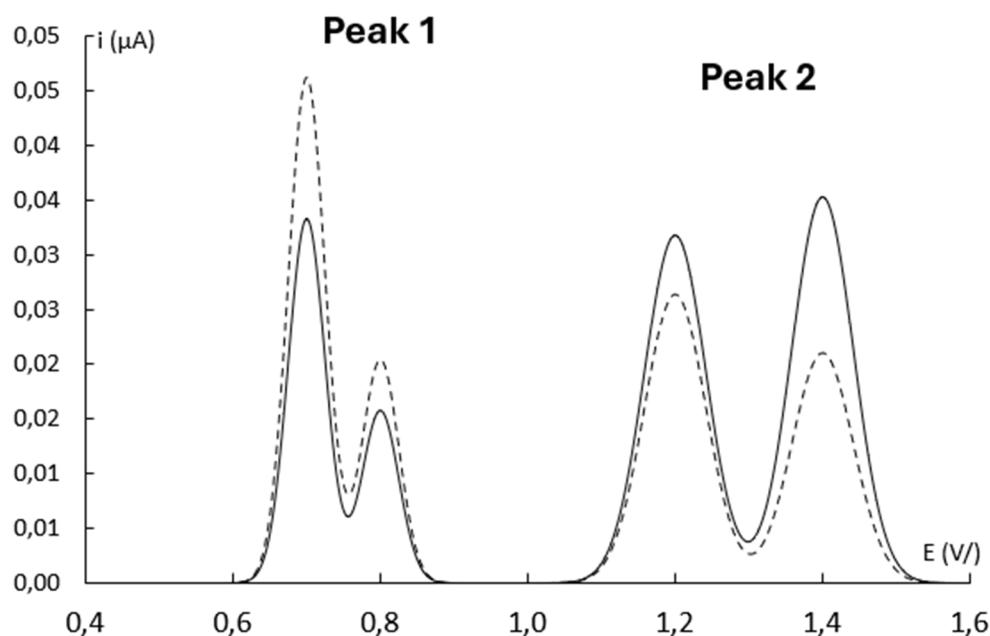


Figure 1. Mean electrochemical profiles obtained on patches with Skin-Biosense on unexposed (full line) and exposed (dashed line) areas.

An electrochemical profile was also observed in previous studies using RHE and skin explants, confirming the relevance of the sensor for oxidative stress assessment. Although the frequency, intensity and location of the peaks varied depending on the model, the existence of electrochemical profiles across model supports their biological significance and strengthens the translational potential of the technology.

For further analysis, the closely spaced peaks at 0.7 and 0.8 V were grouped as Peak 1, while those at 1.2 and 1.4 V were grouped as Peak 2, due to the difficulty of resolving them individually without advanced deconvolution techniques.

Interestingly, the peak at 0.8 V was absent in 14 out of 44 samples (32%), and the peak at 1.2 V was absent in 11 samples (25%), highlighting a significant inter-individual variability in skin electrochemical composition. This heterogeneity supports the relevance of the patch-based sampling method and confirms its ability to capture in a non-invasive way electrochemical signatures in clinical subjects.

When comparing the electrochemical scores of Peaks 1 and 2 (Figure 2), opposite trends were observed between the control and blue light-exposed zones: Peak 1 increased from 0.52 to

0.70, representing a 35% increase while Peak 2 decreased from 0.71 to 0.48, corresponding to a 32% decrease.

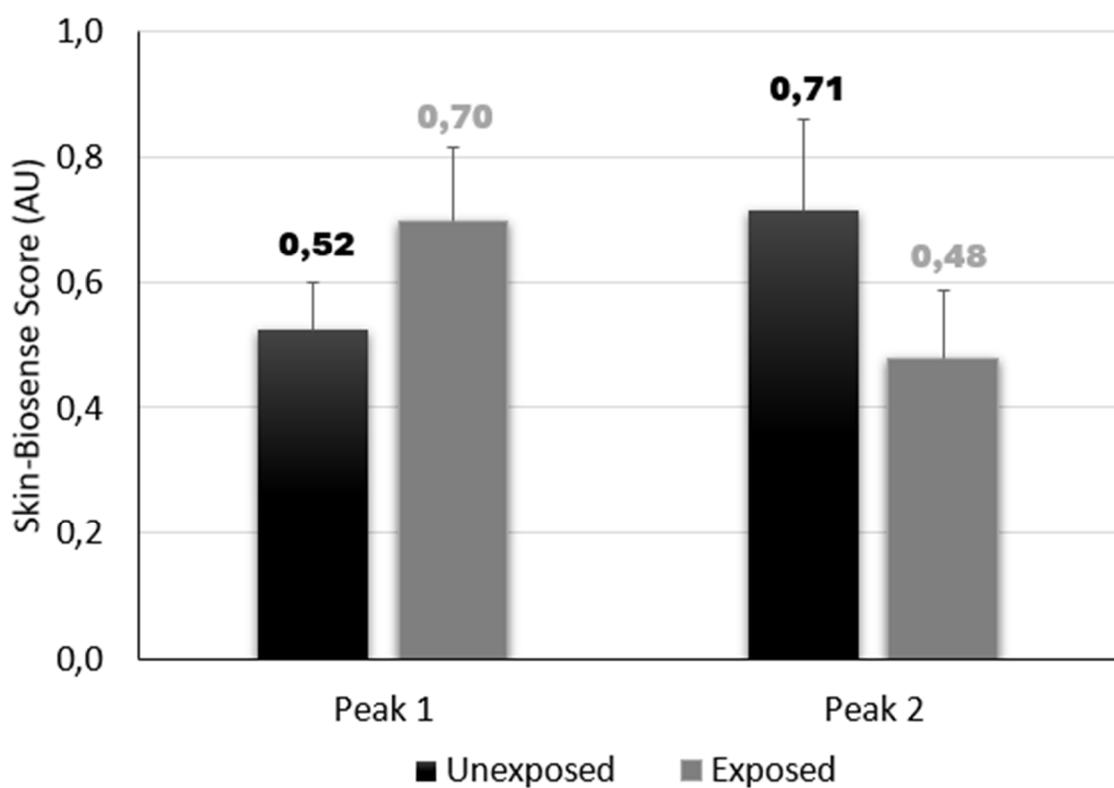


Figure 2. Mean Skin-Biosense score of Peak 1 and Peak 2 extracted from electrochemical profiles on unexposed and exposed areas.

These opposite variations suggest an adaptive skin response to blue light-induced oxidative stress. On one hand, the increase in Peak 1 may reflect the upregulation or accumulation of specific electroactive compounds aimed at counteracting oxidative damage. On the other hand, the decrease in Peak 2 suggests consumption or depletion of other antioxidant species that acted as a first line of defense.

This interpretation is supported by catalase activity measurements (Figure 3), which showed a 48% reduction in activity following blue light exposure.

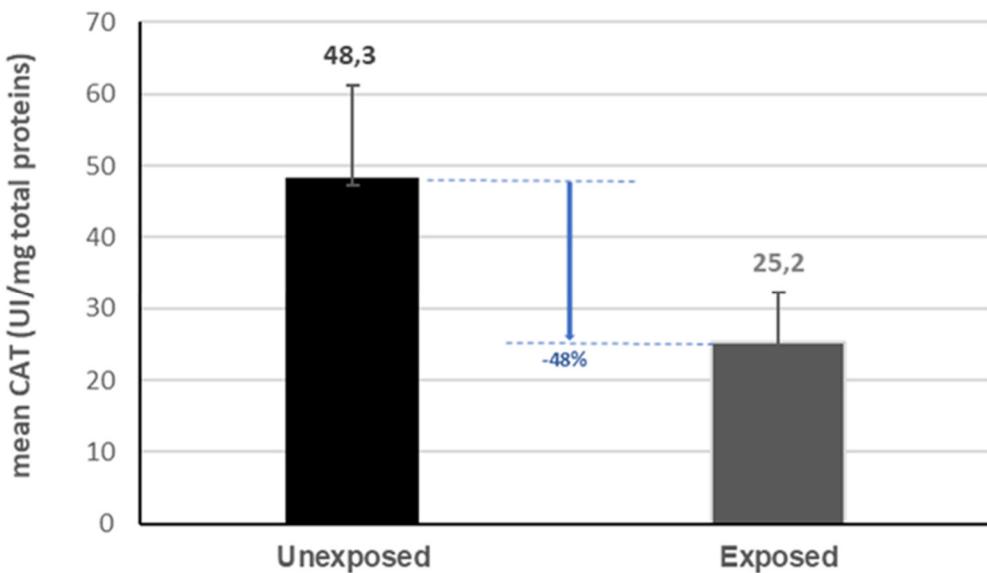


Figure 3. Mean Catalase activity determined on swab samples on unexposed and exposed areas.

Although catalase itself is not detected in the electrochemical profile, the parallel reduction of Peak 2 may indicate that the associated electrochemical species behave analogously, being consumed or downregulated as part of the oxidative stress response.

4. Discussion

This clinical proof-of-concept study demonstrated the potentialities of the Skin-Biosense electrochemical sensor as a non-invasive tool for the evaluation of oxidative stress *in vivo*. Thanks to its simple skin surface sampling protocol, electrochemical profiles were successfully collected 24 hours after blue light exposure. These profiles provided qualitative insights into the skin's electrochemical composition, revealing clear differences between exposed and unexposed areas. The observed variations—approximately $\pm 30\%$ in the main electrochemical signals—reflect either the degradation or mobilization of electroactive species in response to oxidative stress. These findings were further supported by catalase activity measurements, offering a complementary and converging readout.

Encouraged by these results, several perspectives emerge for future exploration. The sensor could be used to evaluate the preventive or curative efficacy of topical products designed to modulate oxidative stress. The model can be extended to other aggressors such as UV radiation and photo-pollution. Dynamic studies at earlier timepoints (e.g., 1–2 hours post-exposure), where skin responses are likely maximal, as well as dose-response evaluations of blue light, would further refine the model.

In addition, the current workflow involved freezing of collected samples, which may lead to partial loss of electrochemical signal. Given the simplicity of the technology, real-time, on-site

measurements should be prioritized in future studies to preserve signal integrity and enhance usability. Ultimately, the electrochemical profile could serve as a dynamic, non-invasive biomarker, with broad applications in dermatology, cosmetic science, and clinical research.

References

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