Keratinocytes' viability evaluation after UVB exposure using a fluorescent dye on the Sapphire Biomolecular Imager

In collaboration with the González Maglio Lab, University of Buenos Aires, Argentina

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Introduction

Fluorescent probes and dyes allow the evaluation of cellular functions such as viability, calcium flux, reactive oxygen species (ROS) production, or mitochondrial function. Modifications in fluorescence intensity reflect changes in cellular functions, such as the ability to reduce dyes such as resazurin due to NADPH dehydrogenase (or NADH dehydrogenase) activity.[1] This note presents the use of a resazurin-based dye to evaluate cellular viability in ultraviolet B (UVB) exposed keratinocytes and to determine the protective effect of a cosmetic.

UVB radiation promotes skin cancer development after long-term exposure. It also produces damage after a single exposure, such as sunburn, characterized by local inflammation and epidermal cell death. At the cellular level, ROS production, mitochondrial damage, and cell death occur in irradiated keratinocytes.[2] Macroscopically, this is observed as red and swollen skin (erythema). Sun protection factor (SPF) of a given cosmetic is defined, according to the FDA, as "a measure of how much solar energy (UV radiation) is required to produce sunburn on protected skin (i.e., in the presence of sunscreen) relative to the amount of solar energy required to produce sunburn on unprotected skin."[3]

SPF is determined in the healthy skin of human volunteers, who are exposed to increasing UVB doses in two portions of the back: one unprotected and the other protected with a specific amount of the cosmetic under study. Twenty-four hours after irradiation, the minimal dose capable of producing erythema (MED) on protected and unprotected skin is registered. The ratio between $MED_{protected}/MED_{unprotected}$ represents the SPF.[4]

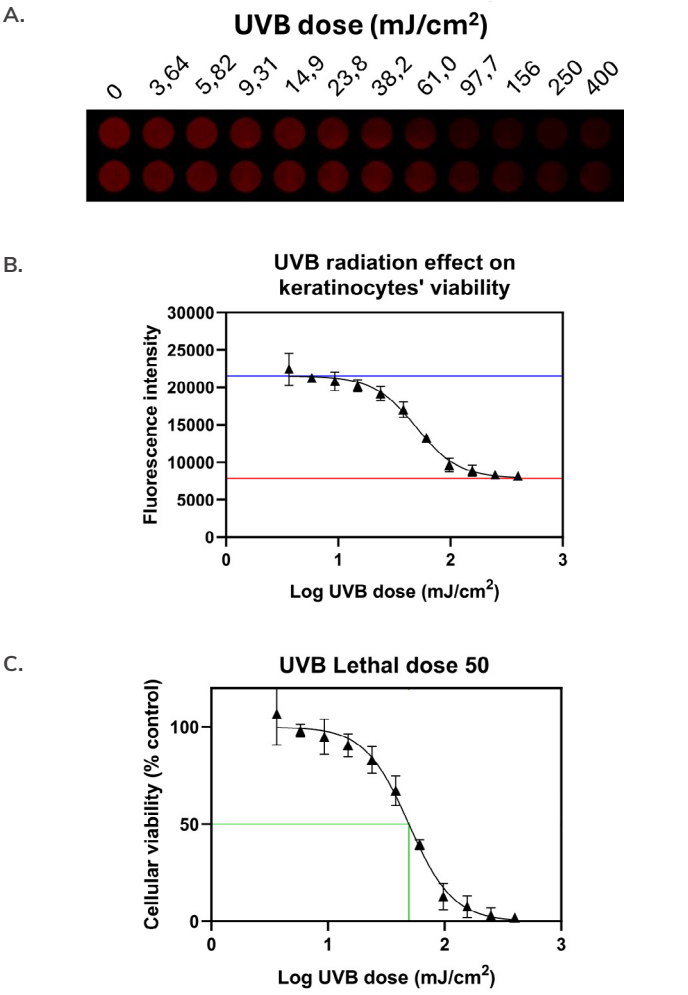


Figure 1. Cell viability quantification in UVB-exposed keratinocytes. A) Fluorescence image of cellular viability evaluation in 96-well plate obtained with Azure Sapphire Biomolecular Imager, using excitation and emission wavelengths of 520/565 nm. B) Fluorescence analysis was performed with ImageJ software. The curve shows the fluorescence quantification of the plate presented in A). C) Representation of the results as a viability percentage. Lethal dose 50 (LD50) is highlighted.

Materials and Methods

The human keratinocyte cell line (HaCaT) was used for the experiments. HaCaT cells were cultured in Dubelcco's modified Eagle's medium, high glucose (DMEM-HG; Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and an antibiotic solution (PSG; Gibco-BRL). Cells were irradiated using an 8W UVM-28 Mid-Range Wave (302 nm) lamp from Ultraviolet Products (UVP, Upland, CA, USA). Alamar blue (Thermo Fischer Scientific) was used to evaluate cellular viability 24 hours after irradiation.

Experimental procedures include the following steps:

1. HaCaT cells were seeded on 96-flat clear plastic well plates at a density of $5x10^5$ cells/ml in complete medium. Cells were cultured for 24h at 37°C to promote cell adherence to the surface, 5% CO_2.

2. Cells were washed with phosphate-buffered saline (PBS) and irradiated with the UVB lamp, using 11 radiation doses, from 3 to 400 mJ/cm², and including an unirradiated control.

3. The wells were filled with 100 µl of buffer (unprotected) or a cosmetic photoprotector solubilized in buffer (protected) before irradiation for the photoprotection experiments.

4. After exposure, the wells were washed and filled with 50 µl cell culture medium. The plate was left for 21h at 37°C, 5% CO_2.

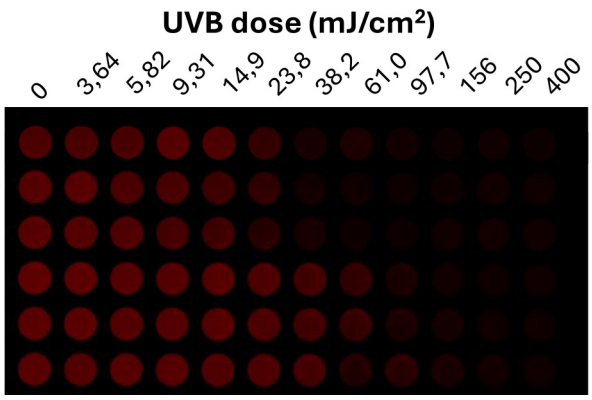
5. Without removing the culture medium, 50 µl of diluted Alamar blue (1/5 in culture medium) was added to each well. The plate was maintained at 37°C for 3 hours.

6. The plate was scanned on the Azure Sapphire Biomolecular Imager at the highest speed using the AzureSpectra 550 channel. The intensity was set to L2, and the pixel size was 100 µm.

7. Images were analyzed using ImageJ to quantify the fluorescence intensity in each well.

Results

A.



B.

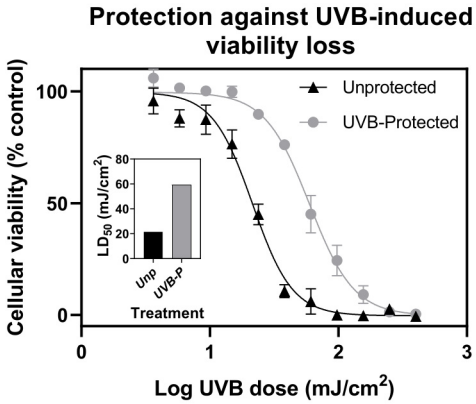


Figure 2. Evaluation of photoprotection in UVB-exposed keratinocytes. A) Fluorescence image of cellular viability evaluation in 96-well plate obtained with Azure Sapphire Biomolecular Imager, using excitation and emission wavelengths of 520/565 nm. The upper three rows were unprotected and the lower three were protected. B) Fluorescence analysis performed with ImageJ software. Curves show the quantification of the plate presented in A). The insert in the figure shows the comparison of the LD50 for protected and unprotected cells.

Conclusion

The Sapphire Biomolecular Imager allows the detection of fluorescence emitted by adherent cells grown in clear plastic well plates incubated with resazurin-based dyes. It was demonstrated that the fluorescence intensity detected in the wells using Alamar blue decreased as the radiation dose increased, indicating a significant loss of cellular viability. These results can be analyzed to obtain a quantitative evaluation of photoprotection for cosmetics products.

Acknowledgements

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References

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