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CENTRE FOR SCIENCE AND RESEARCH

Medical Device Cream SPF100+ - *in vitro* safety profile, UV-induced CPD formation and spectroscopic evaluation of solar spectrum protection

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✓Ability of the SPF 100+ cream to prevent CPD formation

- *In vitro* tests demonstrated that the medical device SPF100+ effectively inhibits the formation of premutagenic cyclobutene pyrimidine dimers (CPDs).

✓Transmittance of SPF 100+ cream samples in a range of 290 – 900 nm

- The SPF 100+ medical device provides comprehensive protection against a broad spectrum of wavelengths, including UVB, UVA, HEV, and Near-IR.

✓Evaluation of product safety

- MTT *in vitro* studies as well as Agarose overlay test did not reveal cytotoxic properties of tested SPF 100+ Medical Device cream (assay performed in accordance with ISO 10993 standard).
- *Ex vivo* tests on an epidermal skin model did not reveal any irritant potential of tested product. The evaluation of sensitization was conducted based on the analysis of the interleukin-18 concentration in the medium collected following the treatment of the medical device SPF 100+.
- The mean concentration of IL-18 was determined to be 93 pg/mL. Consequently, the product can be classified as non-sensitizing based on this assessment. The negative control, NC-PBS, yielded a concentration of 215.67 pg/mL.

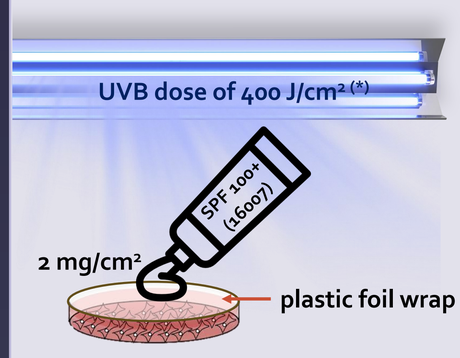
Introduction

Exposure to solar radiation is recognized as a factor for cancer initiation and progression. Genotoxicity of UVB is due to its direct absorption by DNA resulting in the formation of cyclobutene pyrimidine dimers (CPDs), pyrimidine photoproducts (6-4 PP) and Dewar valence isomer of the 6-4 PP. CPDs are the most abundant, most mutagenic and their recognition and repair by the nucleotide excision repair pathway (NER) is slow. The following study examined the efficacy of Medical Device Cream SPF 100+ (16007) in preventing CPD formation in fibroblasts following UVB irradiation. Additionally, the spectroscopic properties of the cream were investigated across the wavelength range of 290–900 nm. The safety of the product was evaluated through *in vitro* and *ex vivo* studies.

Pyrimidine dimer

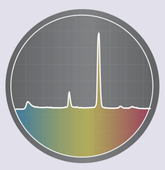
Materials and Methods

1. CPD formation



- In a preliminary study, it was determined that the plastic wrap did not impede the passage of UVB radiation.
- Confluent L929 cell dishes (in quadruplicate) were covered with plastic wrap and coated with an SPF 100+ cream, with the amount equating to 2 mg/cm².
- After single dose radiation, cells were lysed and total DNA was extracted using QUIAGEN DNeasy Blood & Tissue Kit to evaluate the CPD concentration with OxiSelect™ UV-Induced DNA Damage ELISA Kit (CPD Quantitation).

2. Spectroscopic assay



- A spectral scan was conducted using a BioTek Epoch Microplate Reader, in a range of 290–900 nm with an increment of 5 nm. The samples were distributed across 96-well UV-Star® microplates, the amount equating to 2 mg/cm². The absorbance values were determined, and the transmittance was calculated.

3. An evaluation of the product safety

- The safety of a product was evaluated in accordance with ISO 10993 by performing *in vitro* MTT cytotoxicity test and Agarose Overlay assay (in quadruplicate) whereas irritation and sensitization potential was tested on Epiderm model. The medium was collected to perform an ELISA for the presence of interleukin IL-18.

Results

1. CPD formation

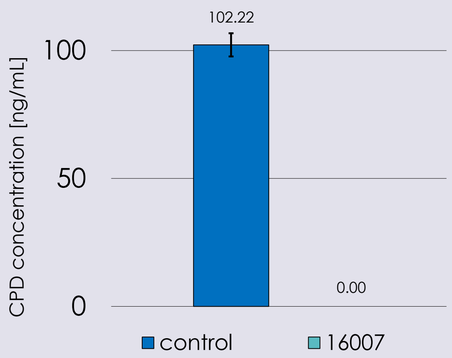


Figure 1. Concentration of CPD in DNA extracted from L929 cells after single dose irradiation of 400 J/cm².

- Dose of 400 J/cm² did not affect cell viability.
- The control consisted of foil-wrapped confluent cell dishes that were not coated with SPF 100+ cream.
- No CPDs were detected in samples coated with SPF 100+ cream.

2. Spectroscopic assay

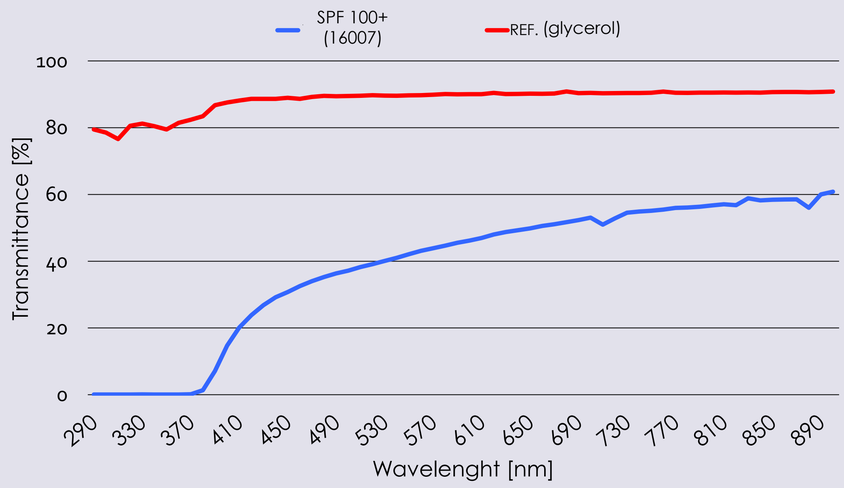


Figure 2. Spectral scan of tested sample SPF 100+ Medical Device Cream. Glycerol was used as reference due to its high transmittance potential.

Mean transmittance values for SPF 100+ Medical Device Cream:

Type of solar radiation	Wavelength range [nm]	Mean transmittance of tested SPF 100+ cream	Mean transmittance of glycerol
UVB	290 – 320	0.06%	78.8%
UVA	330 – 400	3%	82.8%
UV-vis	410 – 760	43.2%	89.8%
Near-IR	770 – 900	57.7%	90.6%

3. Safety evaluation of tested medical device

a. MTT cytotoxicity

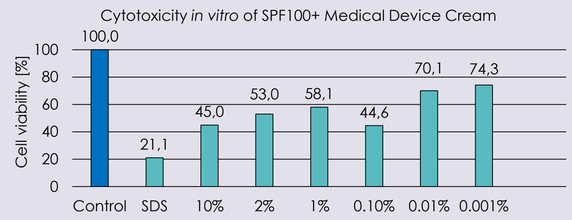
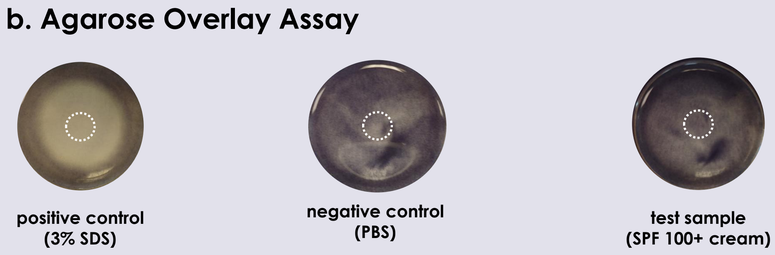


Figure 3. Cytotoxicity of SPF 100+ Medical Device Cream (16007) on L929 cells. Viability <70% of the control (PBS) – cytotoxic potential. Ref – 0.5% SDS. The tested product was non-cytotoxic at the concentration of at least and 0.01% (cells viability: 70.1%).

b. Agarose Overlay Assay



positive control (3% SDS) | **negative control (PBS)** | **test sample (SPF 100+ cream)**

Figure 4. Cytotoxicity of SPF 100+ Medical Device Cream (16007) in L929 cells investigated through Agarose Overlay Assay. Circle with dashed line corresponds to the place where cellulose disc was located on which product was applied. Cytotoxicity of a product was excluded due to lack of lysis zone in cell monolayer below the paper disc where tested product was applied.

c. Irritation and sensitization potential

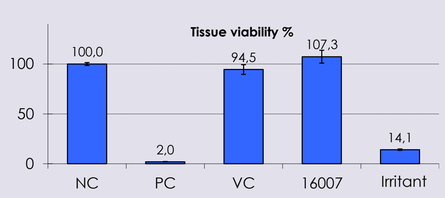


Figure 5. Skin irritation potential of SPF 100+ Medical Device Cream (16007) on EpiDerm model. PC – positive control (5% SDS); NC – negative control (PBS); Irritant – blend of parabens (methylparaben, ethylparaben, propylparaben, butylparaben); VC – vehicle (0.9% sodium chloride solution). Tissue viability ≤ 50% of the NC — irritant. Tissue viability ≥ 50% of the NC — non-irritant. **The study product is non-irritating (tissue viability – 107.3%).**

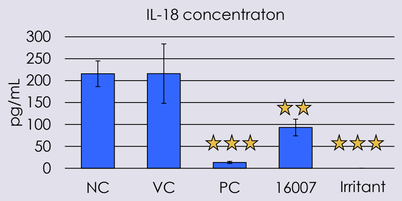


Figure 6. Expression of proinflammatory interleukins in culture medium of EpiDerm after treatment with SPF100+ Medical Device Cream.
Abbreviations as on Fig. 5. ☆corresponds to p value (** <0.002, *** <0.001).
The tested product can be classified as non-sensitizing.

References

(*) Drigeard Desgarnier MC, Rochette PJ. Enhancement of UVB-induced DNA damage repair after a chronic low-dose UVB pre-stimulation. DNA Repair (Amst). 2018 Mar;63:56-62. doi: 10.1016/j.dnarep.2018.01.008.