Temperature dependence of ultraweak photon emission in fibroblastic differentiation after irradiation with artificial sunlight

Hugo J Niggli*
BioFoton AG, P.O. Box 28, CH-1731 Ependes, Switzerland

Yield of ultraweak photon emission in a cell culture model for biophotonic measurements using fibroblastic differentiation depended on the temperature of photonic measurement. The ultraweak photon emission of medium was significantly higher at 37°C than at 25°C and after UVB-irradiation this difference was even more pronounced. While with cells in the medium no temperature dependence could be determined in nonirradiated samples, after UVB-irradiation of cells an increase of biophotonic emission was observed in postmitotic fibroblasts. While after several UVB exposures normal cells begin to absorb the ultraviolet light, cells from patients with the disease Xeroderma Pigmentosum loose this capability. In view that fibroblasts play an essential role in skin aging, skin carcinogenesis and wound healing, the biophotonic model using the fibroblastic differentiation system provides to be a new and powerful non-invasive tool for the development of skin science.

Keywords: Differentiation skin cell, Fibroblast, Human cells, Photodimer, Photon emission, Temperature dependent emission, Ultraviolet stimulation

Spontaneous ultraweak photon emission (PE) has been extensively described in yeast, plant and animal cells1-8. Because it was observed that nuclei play an important role in the emission of this very weak light7,8, the experiments were extended to cultured normal and DNA-excision-repair-deficient XPA cells9. These results revealed an important difference between normal and XPA cells and it was proposed that XPA cells are unable to store ultraweak photons which are efficiently trapped in normal cells. Therefore there may exist an unknown type of intracellular photobiostimulation4.

Bayreuther and co-workers9-11 showed biochemical and morphological evidence for the fibroblast differentiation system in vitro. These authors have seen that normal human skin fibroblasts in culture spontaneously differentiate along the cell lineage of mitotic (MF) and postmitotic fibroblasts (PMF). Additionally, they developed methods to shorten the transition period and to increase the frequency of distinct postmitotic cell types using chemical agents such as antitumor antibiotics collectively termed mitosunes. Treatment of fibroblasts with mitomycin C induces characteristic morphological changes in these cells and brings about specific shifts in the [35S]-methionine

polypeptide pattern of total cellular proteins supporting the notion that MMC accelerates the differentiation pathway from mitotic to postmitotic fibroblasts in vitro from about one year down to several weeks9,10. It was in defined stages of this fibroblast differentiation system found that UV-light elevates photon emission in MMC-induced postmitotic XPA-fibroblasts compared to mitotic XPA-cells4. Induction region of ultraweak photons is in the UVA region between 330-380 nm1. Nevertheless, it has remained an open question how temperature influence this very weak light in fibroblastic differentiation.

In order to find answers to this question, spontaneous ultraweak photon emission studies were performed in fibroblastic cells and these ultraweak radiation processes were studied in mitotic and postmitotic stages of normal and DNA-repair-deficient XPA fibroblasts at 25° and 37°C.

Materials and Methods
Cell culturing — Skin fibroblasts from a normal individual (GM 38) and from a xeroderma pigmentosum patient of complementation group A, XP12BE (GM05509A) were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ, USA). XPA cells derived from a 10-year old female (CRL 1223), were purchased from the American type culture collection. Cells were cultivated in tissue culture plastic flasks (surface 75 cm²; Gibco, Basel, Switzerland) in 15 ml of Dulbecco's modified Eagle's medium

*Present address:
Rte. d' Essert 27, CH-1733 Treveyx,
Phone/Fax: 0041-26-4131445
E-mail: biofoton@swissonline.ch
(DMEM; Gibco, Basel, Switzerland) supplemented with 10% fetal calf serum and 100 U/ml penicillin/streptomycin as previously described. They were routinely passaged 1:2 each 7 days as described. Postmitotic XPA cells were prepared by treatment with mitomycin C.

Preparation of cells for ultra-weak photon measurements — Medium was removed from the tissue culture flasks prior to irradiation, the cells were trypsized and the action of trypsin was stopped by the addition of 50 μl of a 5% aqueous solution of soybean trypsin inhibitor to each flask. For the ultraweak photon emission determinations in mitotic and postmitotic fibroblasts, the contents of 20 and 100 flasks (surface, 75 cm²; Gibco, Basel, Switzerland), respectively, were pooled to yield up to 3x10⁶ cells. The cells were always centrifuged at approximately 1,000 rpm for 3 min and resuspended in 10 ml DMEM (Gibco, Basel, Switzerland) from which phenol red was omitted.

Irradiation facilities — All experiments were performed with at least 7x10⁶ cells/sample with the buffer solutions described above. The cells and the control buffers were irradiated in 10 ml portions at a cell density of 0.7-3x10⁶ cells/ml. For UV-experiments an unfiltered UV-source from OSRAM (Ultra-Vitalux) was used. The Oslram Ultra-Vitalux is an intermediate pressure, UVC filtered, mercury vapor lamp, which has a mercury vapor line spectrum and was extensively described before. This lamp emits 0.5 and 2.6% of the total irradiance in the UVB and UVA range, respectively. The fluence rate of 3 W/m² at 297 nm was determined with a IL 770 A germicidal-erythemal radiometer equipped with a SEE400 photodetector containing a NS 297 interference filter with half power points between 290-301 nm. During individual experiments the fluence rate was stable. The cells were irradiated on a rotating platform as previously described earlier.

Ultraweak photon detections — For ultraweak photon emission measurements, the cell suspension in a volume of 10 ml was transferred immediately after UV-irradiation and/or trypsinisation to a quartz sample glass (2.2x2.2x3.8 cm; thickness 0.15 cm). Detection and registration of light-induced emitted photons was described earlier. The test sample was kept in a dark chamber in front of a single photon counting device equipped with an EMI 9558 QA photomultiplier tube (diameter of the cathode 48 mm, cooled to -25°C). This high-sensitivity photon counting device, described in detail by Popp and co-workers, measured photon fluxes as low as 10⁻¹¹ W in the range between 220 and 850 nm. Briefly, signal amplification is about 10⁶-10⁷, dark count ranges between 10-15 counts/sec (cps). Maximum efficiency of the S20-cathode is 20-30% at 200-350 nm, decaying almost linearly down to 0% at a wavelength of 870 nm. Mean quantum efficiency in the entire spectral range is about 10%. The integral intensity values within each interval of 40 ms were stored and processed by an interfaced computer.

Results

Ultraweak photon emission in Dulbecco’s modified Eagle’s medium (DMEM) — Figure 1 shows ultraweak photon emission determinations at 25°C and 37°C in DMEM, from which phenol red has been omitted, with UVB-irradiations up to 750 J/m². The ultraweak photon emission both of unirradiated control medium and UVB irradiated samples was at least 50% lower at 25°C than at 37°C. After three successive irradiations of 250 J/m² UVB, this quantitatively change of the ultraviolet light-induced ultraweak photon emission intensity was even more pronounced.

Biophotonic emission in mitotic and postmitotic human skin fibroblasts in comparison to XPA-cells — Figure 2 depicts spontaneous ultraweak photon emission in distinct differentiation stages of normal fibroblasts (GM38) both at 25°C and 37°C. There is no discernible difference found between untreated mitotic fibroblasts and mitomycin C-induced postmitotic cultures up to three weeks following MMC-treatment at 25°C. A small decrease immediately after MMC-treatment could be observed. At 37°C a small, statistically not significant increase was found in postmitotic cells.

![Fig. 1 — Spontaneous ultraweak photon emission in DMEM medium at 25°C and 37°C following irradiation with UVB-light from an artificial sunlight source. The time interval for biophotonic measurements was 40 ms and the number of determinations (n) was 256.](image-url)
Figure 3 shows ultraweak photon emission in mitotic and postmitotic excision repair deficient XPA cells both at 25°C and 37°C. There is a significant increase at both temperatures for the biophotonic emission in postmitotic cells. Most interestingly, the difference is at 25°C even more pronounced than at 37°C.

Photon emission in human skin fibroblasts following successive UVB-irradiations of 250 J/m²—In UVB-irradiated (250 J/m²) normal fibroblasts an increase of ultraweak photon emission in postmitotic cells was observed after irradiation at 25°C (Fig. 4). Similar results were found at 37°C (data not shown). While after 250 J/m² UVB the increase both in normal and XPA cells was 100%, three successive irradiations with the same UVB fluence to a total of 750 J/m² showed distinct differences for these two cell types (Fig. 5). While normal cells showed a decrease down to 60%, repair deficient XPA cells showed an increase up to 145%.

Discussion
The observation of an almost 2-fold increase of ultraweak photons in UVB exposed medium by raising the temperature from 25°C to 37°C (Fig. 1) supports the temperature effect of pyrimidine dimer efficiency published almost 20 years ago. There it was shown that more DNA-photoproducts with UVB-irradiation are formed by raising the temperature. In view of a simple photochemical effect as basis for this low radiation it would be expected that this increased ultraweak photon emission with raised temperature should also be observed in cells. It is intriguing that such a temperature effect in normal cells for
ultraweak photon emission could not be measured (Fig. 2) although electronically excited molecules are the origin of this very weak radiation. Normal cells tend to absorb significantly ultraweak photons after artificial sunlight-irradiation. As seen in Fig. 5 normal cells tend indeed to store UV-photons following several successive irradiations. From the results presented here it can be concluded that normal cells absorb ultraweak photons by an unknown photochemical process. The finding of a significant increase at both temperatures for the biophotonic emission in postmitotic cells originated from a patient with the disease Xeroderma Pigmentosum, as shown in Fig. 3 as well as after several UVB-exposures in the same cells depicted in Fig. 5, is confirmed our reported experiments of induced emission of ultraweak photons followed by UV-irradiation in these repair deficient cells. Mitomycin C-induced postmitotic normal fibroblasts retain the capacity to repair pyrimidine photodimers formed after UV-irradiation. Most interestingly in MMC-treated mitotic and postmitotic normal and XP-deficient human skin fibroblasts about 3-4 times higher levels of glutathione, an important intracellular scavenging system were discovered, pointing to the fact that direct oxidative stress is not responsible for this effect because most of the excited molecules return in picoseconds to the ground state, as most recently shown cultured cells are mostly induced in biophotonic emission by light in the UVA range of 330-380 nm.

A new hypothesis is proposed on the basis that light itself is responsible for this effect (Scheme 1). As mentioned above, spontaneous ultraweak photon emission (PE) has been extensively described in yeast, plant and animal cells. Tilbury discussed in the multi-author review of van Wijk that ultraweak photon emission has been detected in both the visible and ultraviolet region. Radiation in the visible region appears to be due to excited carbonyl groups and/or excited singlet oxygen dimers arising from lipid peroxidation, which in turn are associated with an increase in various reactive oxygen species such as the superoxide anion, hydrogen peroxide, hydroxyl radical and singlet oxygen. There is also substantial evidence for DNA playing a key role in these emissions. This macromolecule may especially be involved in the emission of ultraweak photons by the cell in the UV-region of the spectrum. In our report ten years ago, experiments with cultured human cells in which normal and DNA-excision-repair-deficient XP cells were UV-irradiated in medium and balanced salt solution (BSS) were assessed for ultraweak photon emission, there was evidence of induced-photon emission from normal cells in BSS but clear evidence of a UV fluence-dependent emission in XP cells in medium and in BSS. Overall, these results revealed an important difference between normal and XP cells and it was proposed that XP cells are unable to store ultraweak photons which are efficiently absorbed in normal cells and perhaps used to regulate metabolic activity. In the present study, we confirm these results and, moreover, we found in defined stages of the fibroblast differentiation system, which has been extensively described in detail by Bayreuther and co-workers, that UV-light elevates spontaneous photon emission in MMC-induced postmitotic XP-fibroblasts up to a factor of at least 2 compared to normal postmitotic cells as presented in Figs 4 and 5. As mentioned above, the wavelength range of these induced ultraweak photons in these repair deficient cells is in the UVA region (330-380 nm). Plant et al. have shown that circadian oscillators are present throughout the body of Drosophila which are switched on most probably by light. The present findings confirm the recent results of Cohen and Popp which found specific biological rhythms for the ultraweak photon emission in the hands and forehead of the human body. In this respect Miyamoto and Sancar discovered that plant and mammalian cells have blue-light sensitive proteins inducing the circadian clock in mammals. It is very likely, however, that not only proteins but also nucleic acids, as depicted in Scheme 1, act as chromophores for sunlight. An important part may be involved via the UV light-induced photochemical reaction of dimer formation in a absorption process of light energy into DNA with subsequent activation of biochemical reac-

**Scheme 1—Proposed mechanism of pyrimidine dimers as a**
**absorption system in DNA in order to convert light energy into**
**biochemical signals.**
tions. This would be similar to the mechanism of the light-driven photoisomerization of the chromophore retinal discovered 25 years ago in bacteriorhodopsin and subsequently confirmed in the visualisation pathway of the eye. The last hypothesis would support that dimer formation and excision could be an expression of epidermal communication and not only a sign of UV damage as previously presented. Eller et al. reported that UV-induced pyrimidine photodimers are stimulating melanogenesis. This discovery is further corroborated by the observation that the maximal or light-driven photodimerization of the chromophore deficient XPA-cells. Therefore these findings confirm that photodimers initiate biochemical processes and serve as molecular signals. In this respect, nucleic acids may act as chromophores in the genetic material. Photon release within the cell could be via free radical reactions as proposed by Voeikov and an important part of this energy may be pumped via excimer reaction in a kind of light absorption process into DNA. In conclusion, the present biophysical data obtained in human cells following irradiation at different temperatures indeed imply that there is a powerful light absorbing system in cells in order to convert light energy into biochemical signals.

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