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Laser-Ultraviolet-A induced ultra weak photon emission in human skin cells: A biophotonic comparison between keratinocytes and fibroblasts

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Photons participate in many atomic and molecular interactions and processes. Recent biophysical research has discovered an ultraweak radiation in biological tissues. It is now recognized that plants, animal and human cells emit this very weak biophotonic emission which can be readily measured with a sensitive photomultiplier system. UVA laser induced biophotonic emission of cultured cells was used in this report with the intention to detect biophysical changes between young and adult fibroblasts as well as between fibroblasts and keratinocytes. With suspension densities ranging from 1-8x106 cells/ml, it was evident that an increase of the UVA-laser-light induced photon emission intensity could be observed in young as well as adult fibroblastic cells. By the use of this method to determine ultraweak light emission, photons in cell suspensions in low volumes (100 µl) could be detected, in contrast to previous procedures using quantities up to 10 ml. Moreover, the analysis has been further refined by turning off the photomultiplier system electronically during irradiation leading to the first measurements of induced light emission in the cells after less than 10 µs instead of more than 100 milliseconds. These significant changes lead to an improvement factor up to 10^6 in comparison to classical detection procedures. In addition, different skin cells as fibroblasts and keratinocytes stemming from the same donor were measured using this new highly sensitive method in order to find new biophysical insight of light pathways. This is important in view to develop new strategies in biophotonics especially for use in alternative therapies.

Keywords: Fibroblast, Human, Photon, Skin, UVA-laser-induced, Ultraviolet, Ultraweak

Photobiological research on plant, animal and human cells in the last 30 years has demonstrated the existence of ultraweak photons in biological tissue which can be detected with highly sensitive photomultiplier systems[1-9]. In a report, published 15 years ago, experiments of UV-irradiated skin fibroblasts were performed on ultraweak photon emmission and it was recognized that repair deficient Xeroderma pigmentosum (XP) cells efficiently loose this very weak light in contrast to normal cells[6]. This important difference between normal and XP fibroblasts suggests that there is an effective intracellular mechanism of photon absorption in human cells. This process is most probably coupled with ultraweak delayed luminescence (DL). Popp and Yan[9] described this phenomenon a few years ago in biological systems in terms of coherent states. It has to be noted that the intensity of this ultraweak radiation is significantly lower than the known fluorescence or phosphorescence.

The cell nucleus seems very important as a source for ultraweak photon emission, a finding reported by Devraj *et.al*[10], more than a decade ago and confirmed in the meantime by Niggli[7] using DL. Further, it has been shown that in defined stages of the fibroblast differentiation system, which was described by Bayreuther and co-workers[11,12] that UV-light elevates this ultraweak photon emission in postmitotic XP-fibroblasts significantly compared to mitotic XP-cells[6]. A few years ago, we reported for fibroblasts that the most important induction spectral range for these very weak photons is the UVA region between 330-380 nm[8]. A similar observation was published by Albrecht-Buehler[13] in mammalian mitochondria after 365 nm excitation. Recently, Musumeci and co-workers developed a highly sensitive technique for UVA-laser-induced ultraweak photon emission in mammalian cells[14,15]. Using this approach, normal cells originating from young and adult donors were compared and in further experiments, spectral

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analysis in keratinocytes and fibroblasts were performed. This new biological development is an innovative and sophisticated analysis to shed new insights on the importance of light in cell biology and alternative medicine.

Material and Methods

Young skin cultures—Foreskin derived normal human fibroblasts 3229 derived from a one day old boy were a gift from Dr. Robert Zimmerman (Boston, USA). Skin samples from young donors (HF-Peal, 18 mo old female; NP/RA, 7 yr old female) were obtained in the Department of Dermatology in the University Hospital of Lausanne (Switzerland) from non-sun-exposed skin sites with written, informed consent and approval from the Medical School Ethics Committee. Skin samples were washed three times for 10 min each in PBS containing penicillin (100 U/ml) and streptomycin (100 µg/ml). Tissue was treated for ~15 min with trypsin/EDTA and the epidermal cell layer scraped gently away from the dermal tissue with the aid of a dissecting microscope.

Young keratinocyte culture preparation—Epidermal tissue was fragmented and centrifuged at 2000 *g* for 15 min. The pellet was then transferred to small tissue culture flasks which contained irradiated (2500 rads) Swiss mouse 3T3 cells at 70% confluence and keratinocyte complete medium as follows: Dulbecco's minimal essential medium:Hams diluted 3:1 (Flow); 10% FCS;1% glutamine; 0.4 µg/ml hydrocortisone;10 mM cholera toxin; 5.0 mg/ml insulin; 1.2 mg/ml adenine; 2.5 mg/ml transferrin; 0.14 mg/ml triiodothyronine; 10 µg/ml epidermal growth factor. Keratinocytes were grown at 37°C in a humidified atmosphere with 90% air/10% CO_2. Cells were used for experimentation at passages 1 and 2.

Young fibroblast culture preparation and fibroblast cell cultures—Dermal tissue was dissected into <0.5 mm^3 fragments and grown in DMEM supplemented with 10% FCS and 5% glutamine and the cells were used for experimentation at passages 1 and 2. They were grown to confluence before splitting and rinsed twice with PBS and counted. Skin fibroblasts GM 1717 (p 9) from a 40 year old male adult were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ, USA). Normal cells from a 8 year old boy (CRL 1221; p 16-22) were purchased from the American Type culture collection (Rockeville, USA). Normal cells were plated 1:2. For the determination of ultraweak photon emission, cells were cultured in tissue culture plastic flasks (surface, 75cm^2, Gibco Basel, Switzerland) in 15 ml Dulbecco's modified Eagle's medium (DMEM; Gibco, Basel Switzerland) supplemented with 10% fetal calf serum and 100 units (U) ml-1 penicillin-streptomycin as previously described[12,14] and grown for 3-7 days, depending on the cell type, until confluency. Cells were counted in a haemocytometer from Neubauer (Flow Laboratories, Baar, Switzerland) and cells were counted in triplicates (±10%).

Preparation of cells for ultra-weak photon measurements before and after transport to Catania—Cells were frozen in liquid nitrogen using the cryopreservation apparatus from Biotech Research Laboratories (Rockeville, MD, USA). Controlled gradual temperature reduction during cryopreserv-ation was critical for the maintenance of cell life and viability. This apparatus preserves cells at the rate of 1°C/min when placed in a -70°C freezer. After 5 hr or overnight, frozen samples were transferred to the liquid nitrogen transport storage system and transferred to Catania (Italy) for ultraweak photon measurements. For ultraweak photon measurements, cells were prepared as follows: All cells were gently thawed, centrifuged at 1200 rpm and the medium was discarded. The cells were washed in 5 ml phosphate buffered saline (PBS), centrifuged at 1200 rpm and resuspended in 250 µl. For ultraweak photon measurements 100 µl of cell suspensions were used.

Ultraweak photon detections—The delayed luminescence or photoinduced ultraweak photons was measured using the device ARETUSA[14], developed at the Southern National Laboratory in Catania. This measuring system consists of a cooled single photon count photomultiplier, a highly efficient optical system able to collect the emitted light from the sample and to transmit it to the photomultiplier, a high intensity pulsed nitrogen laser with pulses at 337 nm and an electronic set-up able to coordinate the measurement procedure and to collect, analyse and elaborate the signals from the photomultiplier (Fig. 1). The spectral analysis has been determined by a set of broadband interference filters (Thermo-Oriel) as published elsewhere[14]. One of the main problems in ultraweak photon measurements is the necessity to protect the photomultiplier (PMT) during the illumination time. This action has been performed until now by electromechanical shutters whose operational times are the order of 150 milliseconds[4-9]. In the system (Fig. 1), an electronic gate was developed which was controlled by a digital signal and able to establish on all dynodes. If digital signals have a lower level (the same tension as a normal voltage divider circuits) and if digital signals have his higher level it can invert the sign of the voltage between the cathode and the first dynode and between the third and the second dynode. Using this tool, it allows us to start the photon counting only a few microseconds after the laser pulses have reached the sample.

Results

UVA-laser induced ultraweak photon emission of young normal and adult normal fibroblastic cells—Figure 2 depicts ultraweak photon emission after induction with an ultraviolet A laser system (DL) in young normal and adult normal fibroblastic cells. In all cells DL is induced linearly as a function of cell density. A significant decrease ranging from young to adult cells was observed. It is evident that young donor cells can be induced more significantly by the UVA-laser system than cells originating from adult normal tissue as shown in this same Fig. 2.

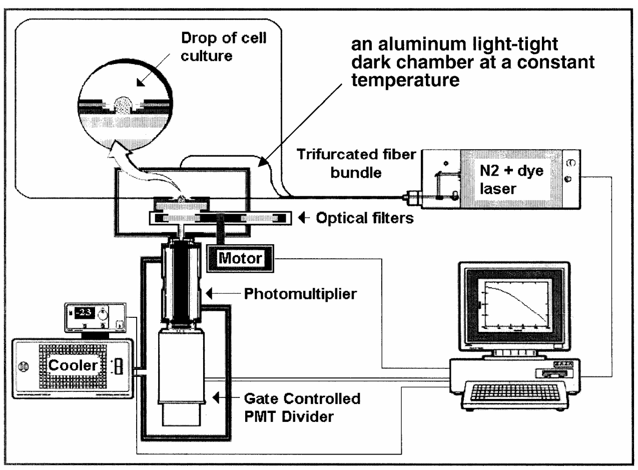

Fig. 1—Experimental design for determination of ultraviolet A laser induced ultraweak photon emission in cultured cells.

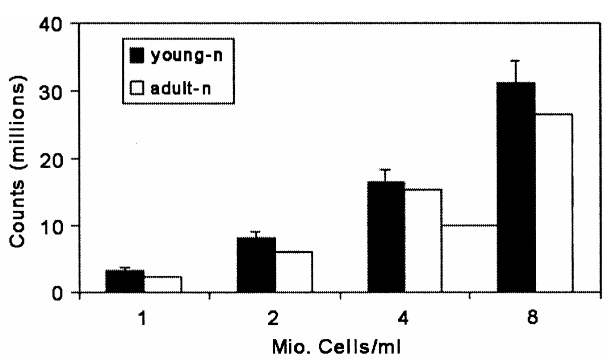

Fig. 2—Initial rates of DL of normal young skin fibroblasts (3229 p 13 and 117; 1221 p 16 and 22), adult fibroblasts (GM 1717; p 9) after pre-illumination with UVA-laser light in nanoseconds at suspension densities ranging from 0-8×10^6 cells. One experimental point included in the graphic represents the average of 100 determinations with standard deviation less than 5%.

Spectral distribution of ultravioletA-laser induced ultraweak photon emission in fibroblasts and keratinocytes—Figure 3 depicts ultraweak photon emission after ultraviolet-A laser induction in keratinocytes as well as fibroblasts stemming from the same young donors (for detail see Material and Methods). There is a small discernible difference found between fibroblasts and keratinocytes in the spectral range from 395–763 nm. The most significant differences are between 567 to 645 nm.

Ultraweak photon flux at 567nm for fibroblasts and keratinocytes—At highest level of measured photons in the spectrum, in the region of 567nm, the ultraweak photon flux from 15 to 5000 μs decreases significantly in both cell types (Fig. 4).

Ultraweak photon flux between 395-763nm in skin fibroblasts—In the spectrum region of 509 nm the ultraweak photon flux from 15 to 5000 μs decreases in fibroblastic cells as depicted in Fig. 5 (similar results were obtained for keratinocytes; data not shown). While at 460nm (Fig. 5) and 645 nm (Fig. 6) the photon flux was quite stable, in the spectrum region of 689-763 nm the ultraweak photon flux from 15 to 5000 μs increases in fibroblastic cells as shown in Fig. 6 (similar results were obtained for keratinocytes; data not shown).

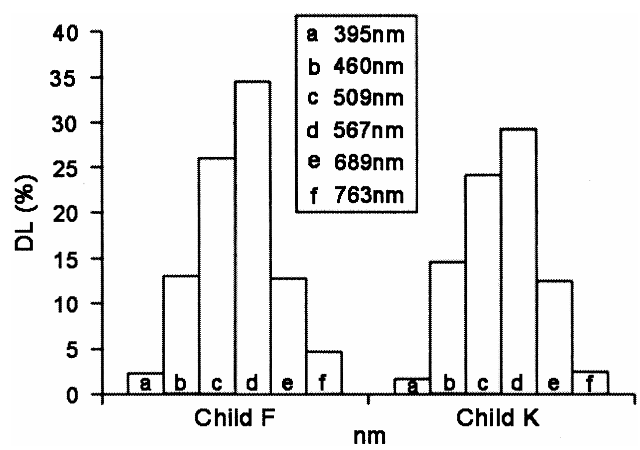

Fig. 3—Emission spectra of initial rates of DL in % (15 microseconds) of normal young skin cells (HF-Peal, NP/RA). Fibroblasts (F) and keratinocytes (K) were pre-illuminated with UVA-laser light in nanoseconds at a suspension density of 4×10^6 cells. One experimental point included in the graphic represents the average of 100 determinations with standard deviation less than 5%.

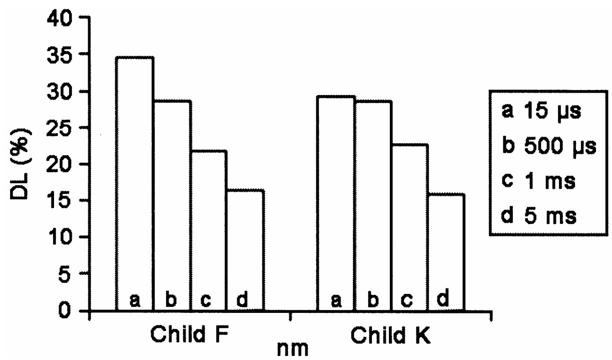

Fig. 4—Rates of DL in % at different time intervals (15-5000 microseconds) at 567 nm of normal young skin cells (HF-Peal, NP/RA). Fibroblasts (F) and keratinocytes (K) were pre-illuminated with UVA-laser light in nanoseconds at a suspension density of 4×10^6 cells. One experimental point included in the graphic represents the average of 100 determinations with standard deviation less than 5%.

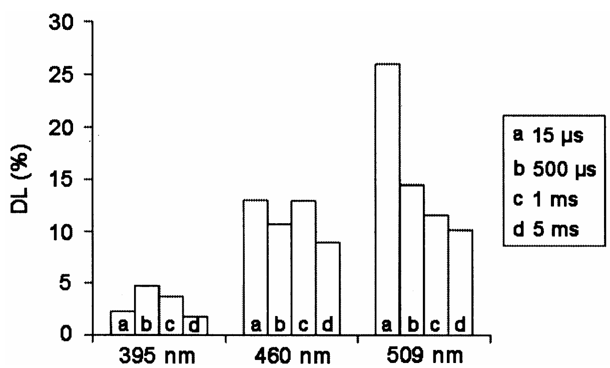

Fig 5—Emission spectra (395-509 nm) of DL in % at four different time intervals between 15-5000 microseconds of normal fibroblasts. The cells were pre-illuminated with UVA-laser light in nanoseconds at a suspension density of 4×10^6 cells. One experimental point included in the graphic represents the average of 100 determinations with standard deviation less than 5%.

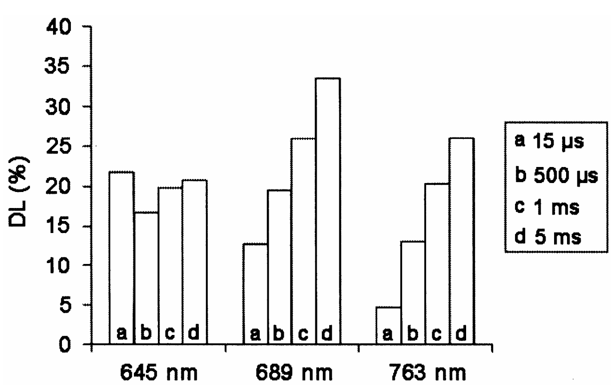

Fig 6—Emission spectra (645-763 nm) of DL in % at four different time intervals between 15-5000 microseconds of normal fibroblasts. The cells were pre-illuminated with UVA-laser light in nanoseconds at suspension density of 4×10^6 cells. One experimental point included in the graphic represents the average of 100 determinations with standard deviation less than 5%.

Discussion

A very sensitive method was used for ultraweak photon determination induced by a UVA nitrogen laser system in skin cells. Two important changes were made compared to classical determinations[6-8]: (i) the cell quantity used was reduced to a density as low as 100 µl before it was exposed to UVA laser light, and (ii) electronic closing of the photomultiplier system was used. As a consequence of these modifications the first time interval for measuring ultraweak photon is as low as several µs leading to light intensities in the range of more than 10^7 photons at higher cell densities (≈10 million cells/ml). The amelioration factor for the detection of ultraweak photons in cells relative to earlier procedures[1,4-9] is million-fold. Work is in progress to improve the instrumentation in order to reduce the time delay of acquisition up to 100 ns.

Figure 2 show differences in ultraweak photon emission after UVA-laser induction between fibroblasts derived from young and adult donors. There was no differences between low passages (13) and high passages (117) of foreskin derived human skin fibroblasts 3229 as well as normal fibroblasts 1221 as published elsewhere[15]. Normal cells tend to absorb significantly ultraweak photons after artificial sunlight-irradiation[6]. Ultraviolet light-induced ornithine decarboxylase response in human skin fibroblasts may serve as a marker of aging and differentiation[16,17]. Work on biophotonic measurements in differentiated fibroblasts pointed to ultraweak photons as sensitive markers in fibroblastic differentiation[8,18]. The report presented here, together with earlier data, indicate that this improved highly sophisticated analysis of ultraweak photons in skin cells may serve as an excellent tool for aging research, especially for specific questions concerning skin aging.

As seen in Figure 3 spectral analysis of UVA-laser induced ultraweak photon emission can discriminate between different cell types. Musumeci *et.al.*[19] illustrated remarkable differences between normal and cancer cells using time-resolved emission spectrum of UVA-laser induced ultraweak photons. In this respect this powerful and noninvasive technique can be applied in a variety of skin research, such as the investigation of skin abnormalities and to test the effect of products involved in regeneration and remedies for alternative medicine.

Albrecht-Buehler[13] showed that ultraviolet light induces light fluxes from the UV to the infrared of the spectrum in mitochondria using a sensitive CCDcamera. The main peaks he observed at wavelengths of 530 and 600 nm, while 4 additional minor peaks at 450, 560, 650 and 750 nm were detected. As depicted in figures 4–6, the present sensitive analysis with highly sensitive photomultiplier tubes confirms this finding, although the intensities of the peaks in present experiments were significantly lower.

As already pointed out above, Musumeci *et.al.*[19] discriminated between normal and cancer cells by using spectral analysis of delayed luminescence. They showed that the time trends of the blue-green region are significantly different compared to the orange-red components of the spectrum. Further, they observed that the emission spectrum depended on the time window of the measurements, a finding which was confirmed in the present experiments using skin cells as shown in Figs 4–6.

From the results presented in figures 2-6 it can be concluded that normal cells absorb ultraweak photons by a photochemical process and slowly release these light as delayed luminescence. In this respect it was recently demonstrated that the ultraweak delayed luminescence is closely connected to the differentiation stage of the biological systems[19]. Brizhik *et.al*[20] proposed a model which connects DL emission to the formation of soliton states inside polymeric chains that constitute the cytoskeleton. As shown here and some years ago[7], cultured cells are efficiently induced in biophotonic emission by light in the UVA range. Not only proteins but also nucleic acids, can act as chromophores for sunlight[18]. Therefore, photochemical products in cells may initiate biochemical processes and serve as molecular signals as described by Voeikov[21] for free radical reactions. Pyrimidine photodimers may serve as communication signal in human cells[22] which confirms a report of Eller *et.al*[23]. The biophysical data in the work presented here using human skin cells after irradiation with UVA-light indeed imply that there is a powerful light absorbing system in cells. Further research is necessary to prove that this light energy will be converted to biochemical signals in order to regulate life processes in cell biology.

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