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The application of ultra-weak photon emission in dermatology

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ABSTRACT

Ultra-weak photo emission (UPE) is a phenomenon closely associated with life and provides us a rare window to look into oxidative reactions in life directly without the aid of other agents. Dozens of independent studies have investigated UPE in skin in the last 2 decades. Skin serves as a convenient target for the application of UPE. As the outmost layer of our body, skin is also subjected to the influences from environmental factors such as ultraviolet light. Therefore UPE measurement can help us better understand the interaction between skin and the outside world.

A variety of dermatological interventions may benefit from UPE studies. In particular, those treatments aiming to manage the oxidative status of the skin can be monitored directly by UPE measurements. In recent years, UPE has already been used as a valuable in vivo tool to assist the selection of better skin care ingredients and products. The knowledge gained by UPE studies of skin may also help generate new insights and new targets for future treatments.

This review emphasizes in vivo and clinical measurement of UPE in skin. The applications of UPE in skin research related to antioxidants and sunscreens are discussed.

1. Introduction

Ultra-weak photon emission (UPE) is sometimes also called biophoton or low-level chemiluminescence. It refers to weak photon emission from a very broad range of biological systems with intensity of 10–1000 photons/s $*cm^2$ at 37 °C and in the spectrum of 300–800 nm. The detection of these photons usually does not require the aid of any light-enhancing agents such as luciferin or its analogs. This feature of universally observed weak light emission distinguishes UPE from the traditional chemiluminescence or bioluminescence where the light emission is more abundant but the observations are linked to the specific probe systems involved. UPE is also very different from fluorescence or phosphorescence because it does not depend on photon excitation. The spontaneous weak photon emission can be measured at any given time for almost any given biological processes. When biological systems are triggered by either physical or chemical stimuli, the induced light emission following the excitation albeit weak can last for more than a few seconds and often minutes, longer than the relaxation time of fluorescence.

The nature of this ultra-weak photon emission has been reviewed by Mei, Sies and several other authors previously [2–4,16,30,33,56,60,68]. Even though the universal origin and the physiology associated with this weak photon emission is difficult to know since they are probably different in different situations, experimental evidences support that UPE in general is probably a by-product of oxidation reactions in biological systems. It is related to the formation of reactive oxygen species (ROS) including singlet oxygen and/or the formation of triplet excited carbonyl which is probably originated from reactions initiated by ROS. UPE signals are mostly observed in visible light range and spectral analysis of the signal often shows a broad peak in the region of 500–700 nm, which overlap with the emission wavelength of triplet carbonyl and dimol reaction of singlet oxygen [2,4]. The presence of molecular oxygen is often founded to be critical to the UPE signal. Anaerobic conditions have been shown to be able to significantly reduce the UPE signal [20,38,48]. On the other hand, the presence of pro-oxidants such as hydrogen peroxide or UV can significantly enhance the UPE signal (induced ultra-weak photon emission). Studies on the effect of free radical scavengers confirmed the involvement of free radicals such as hydroxyl radicals in the generation of UPE signals [48]. In addition, the correlation of UPE measurement with other detection methods such as electron paramagnetic resonance measurements corroborates the involvement of free radicals in UPE [11,13,14,24,45].

UPE is closely associated with life as the living systems are continuously emitting light and the properties of the light reflect the status of the living systems. This is not surprising since oxidation is at the center stage of biological process and life itself. UPE measurements for cell cycle, seeding process, metabolism and even tissues or cells in pathological states have been carried out to explore novel insight about life [23,28–31,34–37]. At the same time, simple model systems have been used to study the key elements or

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mechanisms of UPE to address the question how it is unique for biological systems [19,41,58]. While the UPE related to oxidation of carbohydrates has not been reported, protein, DNA and lipid can all be the targets of ROS and have all been linked to the generation of UPE signals when oxidized. Several reports emphasized that protein oxidation probably plays a very significant role in overall UPE [1,19,40,55]. The UPE signal related to protein oxidation may also not be the summation of the UPE signals of all the individual amino acid components, indicating UPE is very much related to the structure and function of the protein [19]. Since protein is the molecular instrument of biological function, UPE studies associated with protein and ROS could have many potential applications.

UPE measurements have been made to skin tissues both in vivo and in vitro in the last two decades. Keratinocytes, fibroblast, skin homogenate, ex vivo skin tissues as well as malignant skin cells have been measured in vitro [12,19,28,34,36,54,56,58,65,69]. Spontaneous UPE and UPE induced by external factors such as UV, smoke and chemicals have also been documented for human skin as well as for animals [8,9,39]. An early review on in vivo UPE measurement of skin was published by Sauermann and coworkers in 1999 [56]. The current article reviews some recent UPE studies relevant to dermatology with an emphasis on in vivo clinical UPE measurements and how UPE can help better understand and manage oxidative stress in skin.

2. Pros and cons of UPE for studying skin

Skin as the outmost layer of body provides a convenient target for in vivo UPE research because the photon emission does not need to pass other tissues before being captured. The non-invasive feature of optical measurement presents a clear advantage over some other clinical methods such as histology studies where invasive skin biopsies need to be obtained before histological staining can be made for the biopsy samples, or tape stripping studies where stratum corneum needs to be removed by adhesive tapes before chemical analysis can be made to the tapes [54,61,62]. In comparison, direct UPE measurement of skin by itself at physiological relevant conditions can be conducted relatively easily in vivo.

The very nature of UPE requires no involvement of any enhancing agents, which makes the measurements and the experiments more straightforward because it avoids any potential interference caused by the addition of other agents to the system. UPE can also be measured at a time frame relevant to skin physiology since spontaneous UPE does not change rapidly and induced UPE in skin can often last for minutes. UPE measurement is typically made in a continuous mode for every second or faster, allowing detailed profiling of skin variations under different circumstances and following different treatments or stimuli. The ability to continuously monitoring skin to gain a full dynamic picture is a feature not many other techniques can offer.

UPE measurement utilizes detectors with very broad spectral sensitivities in 300–800 nm to capture signals from all the relevant ROS reactions. We cannot seem to decouple the ultra-weak photon emission from any biological process we studied therefore UPE may provide us a tool to monitor the overall oxidative stress in skin. This is a feature different from some other analytical methods such as EPR where spin traps specific to certain reactive species need to be used for detection [18], or fluorescence or resonance Raman spectroscopy where measurement are only conducted at specific wavelengths related to specific chemicals or processes [24,63]. Since UPE measurement provides information directly related to ROS in skin, it may also help simplify those complex clinical endpoints such as erythema or pigmentation, which involve but are not limited to the changes in skin oxidative status. Overall, UPE has the potentials to be used as a diagnostic tool in clinical settings to assess the oxidative status of skin.

There are also challenges associated with the application of UPE to skin research. The extremely weak signal of spontaneous UPE in skin is one of the major limitations. Extensive studies of spontaneous UPE could illustrate the long-term effects of temperature, humidity, various cosmetic treatments and help shed light to skin aging and photoaging. However, because the signal is ultra-weak (often at the level of 10 photons/s $*cm^2$) there have not been many published reports in which spontaneous UPE in skin in vivo was systematically investigated. On the bright side, skin is particularly prone to acute insult and is serving as the protective layer. UPE signal often increases by multiple folds following external insults. It is therefore suitable by studying induced ultra-weak photon emission to better understand the interactions between skin and the outside world and how we can potentially influence these interactions.

Another limitation for the application of UPE in dermatology lies on the lack of specificity in UPE signal despite the signal is related free radicals. Detailed biochemical information about complex oxidation process in skin cannot be obtained by UPE measurement alone. UPE may be more powerful when it is used in combination with other physicochemical techniques including EPR and fluorescence.

One challenge related to UPE and other in vivo optical measurements of skin lies in the complexity of skin optics in the visible wavelength range. Skin has a layered structure and the optical properties are different in different layers. The presence of skin pigments such as melanin and hemoglobin that can extinct the visible photons may potentially interfere with the UPE measurements. Therefore, extra attention may need to be paid to take into account the signals that can be affected by optical features of the skin. This is particularly relevant when we investigate the spatial variations of the UPE signals by imaging techniques since the pigment distribution in skin is not uniform among different sites.

3. The set-up of in vivo UPE measurement

The experimental set-up for in vivo UPE measurement has been described extensively by Inaba, Boveris, Sauermann, Van Wijk, Kobayashi and Pospisil et al. [2,3,16,22,48,49,56,71]. Due to the ultra-weak signal, the measurement must rely on highly sensitive detectors with sufficient spectral responses in the red and the in vivo measurement must be conducted in a light-tight dark room to reduce the background noise (Fig. 1). Since the spontaneous photon emission in skin can be affected by many factors, especially the lighting and the heat. It is always recommended to conduct the measurement after sufficient acclimation of the skin to the dark room.

A photomultipler (PMT) made by either Hamamatsu or Electron Tubes is often used for a spot measurement. The PMT needs to be cooled thermoelectrically to −20 to 30 °C to reduce the dark current and an optimal high voltage needs to be applied to enhance the performance of the PMT. PMT with the cooling housing should generally be put very close to the skin surface (1–2 cm) to avoid any photon loss and PMTs with large cathode surface area (1–2 in. in diameter) are often selected to maximize the collection efficiency because the measurement counts the total photons emitted from all the areas covered. The amplified signal from PMT may be recorded in a photon-counting device. The opening of the PMT is often controlled by shutters with light-tight enclosure to avoid any photon leak. Spectral filters may be placed in front of the PMT to collect spectral distribution of the signal and attention should be paid to mitigate any light emission caused by the filter addition.