Red light photodynamic therapy for actinic keratosis using 37 J/cm 2 : fractionated irradiation with 12.3 mW/cm 2 after 30 minutes incubation time compared to standard continuous irradiation with 75 mW/cm 2 after three hours incubation time using a mathematical modeling

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Abstract: Photodynamic therapy (PDT) is an emerging treatment modality for various diseases, especially for dermatological conditions. Although the standard PDT protocol for the treatment of actinic keratoses in Europe has shown to be effective, treatment-associated pain is often observed in patients. Different modifications to this protocol attempted to decrease pain have been investigated. The decrease in fluence rate seems to be a promising solution. Moreover, it has been suggested that light fractionation significantly increases the efficacy of PDT. Based on a flexible light-emitting textile, the FLEXITHERALIGHT device specifically provides a fractionated illumination at a fluence rate more than six times lower than that of the standard protocol. In a recently completed clinical trial of PDT for the treatment of actinic keratosis, the non-inferiority of a protocol involving illumination with the FLEXITHERALIGHT device after a short incubation time and referred to as the FLEXITHERALIGHT protocol has been assessed compared to the standard protocol. In this paper, we propose a comparison of the two above mentioned 635 nm red light protocols with 37 J/cm^2 in the PDT treatment of actinic keratosis: the standard protocol and the FLEXITHERALIGHT one-through a mathematical modeling, which slightly differs from the one we have already published. This comparison performed in terms of the local damage induced by the therapy demonstrates that the FLEXITHERALIGHT protocol with lower fluence rate, light fractionation and shorter incubation time is somewhat less efficient than the standard protocol. Nevertheless, from the clinical trial results, the FLEXITHERALIGHT protocol results in non-inferior response rates compared to the standard protocol. This finding raises the question of whether the PDT local damage achieved by the FLEXITHERALIGHT protocol (respectively, the standard protocol) is sufficient (respectively, excessive) to destroy

actinic keratosis cells…

I. Introduction

Photodynamic therapy (PDT) is a cancer treatment modality combining light of an appropriate wavelength, a nontoxic photosensitizer, and sufficient molecular oxygen to generate reactive oxygen species and destroy (pre-) malignant cells [1]. PDT using 5-aminolevulinic acid (ALA) (ALA-PDT) and PDT using 5-aminolevulinic acid methyl ester (MAL) (MAL-PDT) have been widely used for dermatological applications in recent decades [2-8]. Topical administration of ALA or MAL induces the selective accumulation of the endogenous photosensitizer protoporphyrin IX (PpIX) within the target cells and subsequent light irradiation leads to the target destruction. ALA-PDT and MAL-PDT have in particular proven to be an efficient treatment modality for actinic keratoses (AK) [9,10].

Actinic keratoses are scaly or crusty lesions that develop on sun-exposed areas, such as the face, scalp, neck, arms… in response to prolonged exposure to ultraviolet radiation. Confined to the epidermis (the basement membrane is intact), AKs are carcinomas in situ and, in approximately 10% of patients, will progress to invasive squamous cell carcinomas (SCCs) [11]. In order to reduce the subsequent risk of developing SCCs, most clinicians routinely treat AKs. Treatment options include lesion-directed destructive therapies, such as cryotherapy and surgical procedures, for individual lesions and field-directed therapies, such as topical medications and PDT, for areas with multiple or subclinical AKs. Compared to the other treatment options, the main advantage of PDT is the non-invasive nature and the excellent cosmetic results of this method [4,12].

A variety of PDT protocols with different photosensitizers, photosensitizer incubation times, light sources, light fluence rates… have been used for the treatment of AKs [12]. MAL-PDT using 635 nm red light with a total light dose of 37 J/cm^2, a fluence rate of 75 mW/cm^2 and three hours of incubation time is a standard protocol, widely used in Europe for the treatment of actinic keratosis. This protocol has been reported to be an effective PDT treatment option for AK and to result in similar response rates and improved cosmetic outcomes compared with standard therapies [9]. However, with these light dose parameters, high pain scores have been demonstrated and concurrent use of cold air analgesia may be required to prevent

discomfort [13,14]. Alternative red light protocols with lower fluence rates, as effective as the standard protocol while being much better tolerated by patients, have been studied for the treatment of AK [15-17]. Furthermore, fractionated irradiation with alternating light and dark periods, intended to allow tissue re-oxygenation and photosensitizer re-synthesis during the dark periods, has been demonstrated to increase the efficiency of the PDT for AK treatment [18,19].

Developed in the framework of the French National Research Agency (ANR) Project FLEXITHERALIGHT (http://www.flexitheralight.com/), the FLEXITHERALIGHT device is composed of three adjacent light emitting textiles [20], which sequentially emit red light (635 nm) at low fluence rate (12.3 mW/cm^2) for one minute allowing a fractionated illumination (1 minute light, 2 minutes dark). The illumination duration of 2.5 hours already programmed in the device enables a light dose of 37 J/cm^2 to be delivered in contact with the textiles. Combining illumination with the FLEXITHERALIGHT device with 30 minutes of incubation time, the FLEXITHERALIGHT protocol is being investigated for the treatment of actinic keratoses by the FLEXITHERALIGHT project. A phase II clinical trial approved by the French National Agency for Medicines and Health Products Safety (ANSM) on 27 November 2013 (registration number: 2013-A1096-39) and aiming to assess the non-inferiority of the FLEXITHERALIGHT protocol compared to the above mentioned standard 635 nm red light protocol for the treatment of actinic keratoses has just ended.

Based on this research project, we propose in this paper to compare the efficiency of the standard 635 nm red light protocol (incubation time: three hours, irradiation type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2, treatment duration: 493 s) to the one of the FLEXITHERALIGHT protocol involving lower fluence rate, light fractionation and lower incubation time (incubation time: 30 minutes, illumination type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2, treatment duration: 9024 s) through a mathematical modeling. This mathematical modeling greatly inspired by our previous works [21] and involving an improved model for both the biological clearance of PpIX and the conversion of MAL into PpIX enables the local damage induced by the therapy to be estimated.

II. Clinical materials

A. Presentation of the two red light protocols

Two different 635 nm red light protocols with 37 J/cm^2 were considered: the standard protocol using a three hours incubation period and a continuous irradiation with 75 mW/cm^2 fluence rate [16,22,23] and the FLEXITHERALIGHT protocol using a 30 minutes incubation period and a fractionated irradiation (1 minute light, 2 minutes dark) with 12.3 mW/cm^2 fluence rate (Table 1).

The FLEXITHERALIGHT protocol has been proposed in the French National Research Agency (ANR) Project FLEXITHERALIGHT (http://www.flexitheralight.com/) in which our research unit is involved. This project aims to develop a biophotonic device based on a flexible light emitting textile [20] and dedicated to the treatment of dermatologic diseases and carcinoma. The major advantage of the flexible light emitting textile is its optimal conformation to the area to be treated, thus leading to a more homogeneous irradiation than that delivered by the standard rigid light sources (Figure 1). Consisted of three adjacent textiles of size 21.5 cm × 5 cm sequentially emitting red light as illustrated in Figure 1, the FLEXITHERALIGHT device enables to obtain a fractionated irradiation (1 minute light, 2 minutes dark) with a fluence rate of 12.3 mW/cm^2 leading to a light dose of 37 J/cm^2 after 9024 seconds of treatment (12.3 mW/cm^2 × 9024 s × 1 minute light / (1 minute light + 2 minutes dark)). Moreover, based on the comparative study of Wiegell et al. [24], the FLEXITHERALIGHT protocol involves a 30 minutes incubation with MAL under occlusive dressing and no MAL removal before irradiation (Table 1).

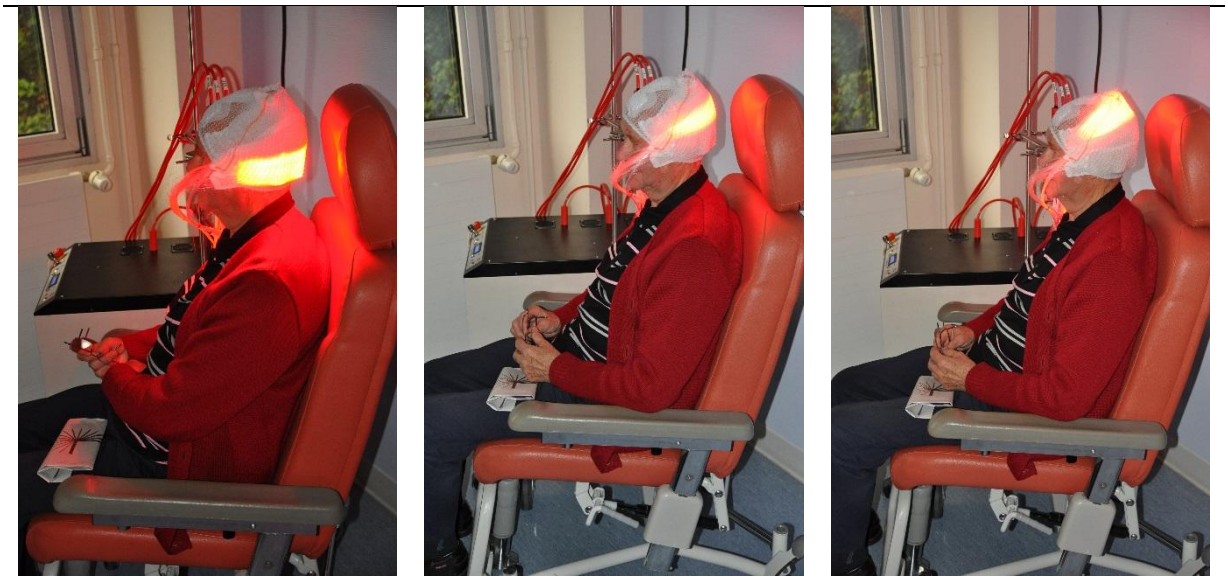


Figure 1: The three flexible light emitting textiles of the FLEXITHERALIGHT device are sequentially activated for one minute (http://www.flexitheralight.com/)

Protocol name	Incubation time	Irradiation type	Fluence rate	Treatment duration
Standard protocol	3 hours	Continuous	75 mW/cm^2	~493 s
FLEXITHERALIGHT protocol	30 minutes	Fractionated	12.3 mW/cm^2	~9024 s

Table 1: Parameters for the two different 635 nm red light protocols with 37 J/cm^2 investigated in this paper

B. Clinical trial for the comparison of the two protocols

A phase II clinical trial approved by the French National Agency for the Safety of Medicines and Health Products (ANSM) (authorization number: 2013-A01096-39) and the French Ethics Committee (CPP) (authorization number: CPP-03/051/2013) for the assessment of the non-inferiority of the FLEXITHERALIGHT device compared to the standard photodynamic therapy for the treatment of actinic keratoses was initiated at the end of 2013 and was recently completed.

This clinical trial was designed somewhat similarly to the study of Wiegell et al., which aims to compare standard MAL-PDT with daylight MAL-PDT [24]. First, the lesions of the

forehead and scalp were counted, photographed and divided into two symmetrical areas, which were then randomized to receive either PDT using the standard protocol or PDT using the FLEXITHERALIGHT protocol. After gentle surface preparation of the lesions and MAL application to the lesions, an occlusive dressing was placed for 30 minutes (respectively, 3 hours) over the area randomly assigned to receive PDT using the FLEXITHERALIGHT protocol (respectively, the standard protocol). After 30 minutes, PDT using the FLEXITHERALIGHT protocol was applied to the corresponding assigned area without dressing removal. Once this treatment was completed, the treated area was protected with an aluminum foil and the area randomized to receive PDT using the standard protocol, was treated after dressing removal and lesions cleaning.

At the end of the procedure, the patients indicated the level of pain experienced during PDT using the FLEXITHERALIGHT protocol and the one experienced during PDT using the standard protocol through a visual analogue scale (VAS) ranging 0 (no pain) to 10 (maximum pain). Pain was also assessed at 7 days after the treatment.

The treatment response was evaluated at 3 and 6 months after the treatment based on comparisons with baseline photographs.

III. Modeling method

Except for the change made regarding the biological clearance of PpIX and the conversion of MAL into PpIX in section III.C.2, the modeling method is the same as in our previously validated work [21] and therefore only outlines are referred to in this paper without further discussion.

A. Skin sample model

Our simplified skin sample model consists of an epidermis section represented by a 150 μm thick parallelepiped and of an AK designed as an ellipsoid as already published in [21].

As AKs are confined to the epidermis, the ellipsoid is included in the parallelepiped in such a way that it lies on, but does not cross, the lower boundary of this parallelepiped which

represents the boundary between the epidermis and the dermis. To account for the thickening of the epidermis generally observed with AK, the thickness of the ellipsoid is set to 200 μm which leads, according to the curettage usually performed prior to PDT, to the skin sample model displayed on Figure 2.

The epidermis and AK tissues are both assumed to be homogeneous and $\vec{z}$ is assumed to be the beam direction, which is also the depth direction of the skin sample model (Figure 2).

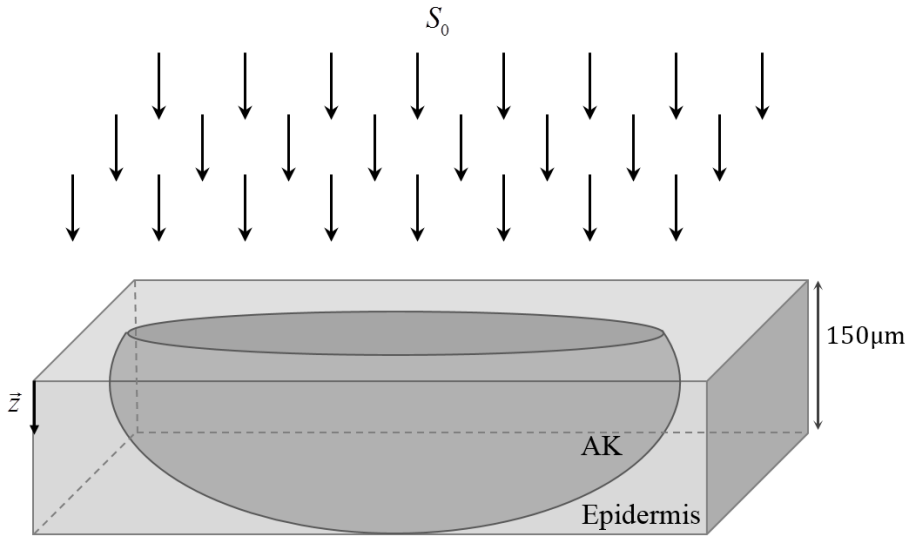


Figure 2: Skin sample model

B. Models for the two fluence rates

In this paper, the spectral fluence rate for the standard protocol (respectively, for the FLEXITHERALIGHT protocol) was modeled as a 75 mW/cm^2 (respectively, 12.3 mW/cm^2) - weighted Gaussian distribution with mean 635 nm and full width at half maximum (FWHM) of 19 nm as measured by Moseley et al. [25] from the Aktilite CL16 and CL128 (Galderma SA, Lausanne, Switzerland) (Figure 3). The total light dose of 37 J/cm^2 is achieved with a treatment duration of 493 s using the standard protocol and 9024 s using the FLEXITHERALIGHT protocol (Table 1).

For the standard protocol, after 3 h of incubation, a primary planar broad beam with a spectral fluence rate S_0 of 75 mW/cm^2 (blue curve in Figure 3) is assumed to continuously perpendicularly irradiate, for 493 s, the surface of the skin sample model as illustrated in

Figure 2. For the FLEXITHERALIGHT protocol, the irradiation for 9024 s is assumed to be performed, after 30 minutes of incubation, using a spectral fluence rate S_0 of 12.3 mW/cm^2 (red curve in Figure 3) during the light periods and a fluence rate S_0 of 0 mW/cm^2 during the dark periods.

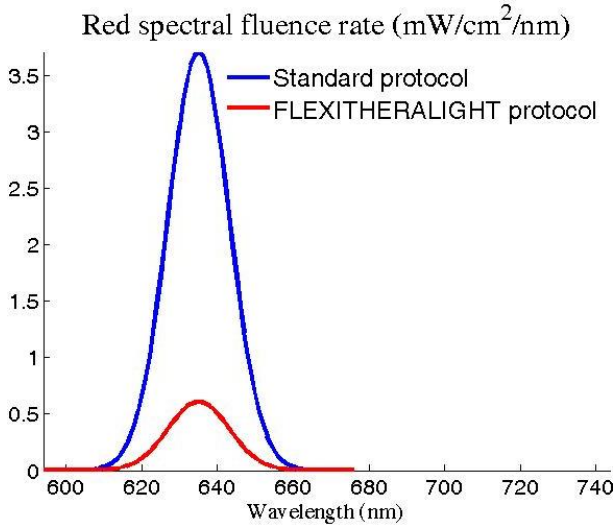


Figure 3: The 75 mW/cm^2 and 12.3 mW/cm^2 spectral fluence rates used for the standard and the FLEXITHERALIGHT protocols, respectively.

C. Modeling of the PDT process

Let the incubation start at time $t = 0\,s$ and let the light irradiation start at time $t = t_{start}$ ($t_{start} = 3$ hours and $t_{start} = 30$ minutes for the standard protocol and the FLEXITHERALIGHT one, respectively) and last until $t = t_{end}$ ($t_{end} \approx t_{start} + 493\,s$ and $t_{end} \approx t_{start} + 9024\,s$ for the standard protocol and the FLEXITHERALIGHT one, respectively).

The model we developed consists of two steps that are iteratively repeated until the end of treatment: determination of the local fluence rate and updating of the PpIX absorption coefficient.

1. Determination of the fluence rate

Similarly to Farrell et al. [26], based on a PpIX concentration varying only with depth, z,

below the irradiated surface (Figure 3), the local total fluence rate at time t, depth z and wavelength λ, denoted by $\varphi(t,z,\lambda)$, is given by equation 1 [21,26,27]:

$$\varphi(t,z,\lambda)=\begin{cases} 0 & \text{for } t\in[0;t_{start}[\text{ and } t>t_{end} \\ S_0\left\{\begin{array}{l}\dfrac{b}{\sqrt{\mu_{eff}(t,z,\lambda)}}\exp\left[-\displaystyle\int_0^z \mu_{eff}(t,w,\lambda)dw\right] \\ +P(t,z,\lambda)\exp\left[-\displaystyle\int_0^z \mu_t'(t,w,\lambda)dw\right]\end{array}\right\} & \text{for } t\in[t_{start};t_{end}] \end{cases} \quad (1)$$

Where:

- The above defined S_0 (Figure 3) is the spectral fluence rate of the primary planar broad beam,
- The total absorption coefficient, μ_a, is the sum of the PpIX absorption coefficient, $\mu_{a,PpIX}$, and the actinic keratosis absorption coefficient, $\mu_{a,AK}$,
- The total transport coefficient, μ_t', is the sum of the total absorption coefficient, μ_a, and the actinic keratosis reduced scattering coefficient [28], $\mu_{s,AK}'$,
- The effective attenuation coefficient, μ_{eff}, is defined as $\sqrt{3\mu_a\mu_t'}$,
- The two parameters, b and P, depending on both the optical properties of the actinic keratosis and the boundary conditions at the actinic keratosis surface, are computed as described in [26].

2. Updating of the PpIX absorption coefficient

During a PDT treatment, three processes affect the PpIX absorption coefficient: the biological clearance of PpIX, the conversion of ALA or MAL into PpIX and the PpIX photobleaching.

In our previous work [21], the conversion of MAL into PpIX was modeled using an exponential growth function resulting in an controversial unlimited increase in time of the number of new PpIX molecules. In this paper, a more realistic model for the conversion of MAL into PpIX also taking into account the biological clearance of PpIX is defined based on clinical data from several studies while the photobleaching model is the same as in our previous work [21].

In order to model the time evolution of the PpIX absorption coefficient when considering only the biological clearance of PpIX and the conversion of MAL into PpIX, we use the fluorescence data reported by Wiegell et al. [24]. These data that have been collected from 30 patients during three hours of MAL application without light irradiation suggest a logistic growth in time of the number of PpIX molecules. Based on this suggestion that is supported by the fluorescence data measured from 23 actinic keratoses during 28 hours of MAL incubation by Angell-Petersen et al. [29], equation 2 can be established. The limited growth in the number of PpIX molecules assumed by equation 2 is also observed for the PpIX concentration data computed by Star et al. during four hours of MAL application at 0 and 0.2 mm depth in the epidermis [30]. This latest data further demonstrates a depth-dependent shape of the logistic growth in time (the shape of the logistic growth depends on the depth in the epidermis). This dependence arising from the progressive skin penetration of MAL is taken into account in equation 2 through the limiting value of the logistic function as we deduced from the PpIX concentration data reported in [30]:

$$M_{PpIX}^{BC}(t,z)=\frac{L(z)}{1+\exp\left(-k\times(t-\tau)\right)} \qquad (2)$$

Where:

- $M_{PpIX}^{BC}(t,z)$ is the number of PpIX molecules present in an unit volume, V_U, at time t and depth z, when considering only the biological clearance of PpIX and the conversion of MAL into PpIX,
- $L(z)$, k and τ are the limiting value, the steepness and the midpoint position of the logistic function representing the time evolution of $M_{PpIX}^{BC}(t,z)$, respectively.

Assuming a standard exponential depth decay with constant, η, for $L(z)$, equation 2 becomes equation 3:

$$M_{PpIX}^{BC}(t,z)=\frac{L(0)\times\exp(-\eta z)}{1+\exp\left(-k\times(t-\tau)\right)} \qquad (3)$$

From equation 3, the variation in the number of PpIX molecules resulting from the biological clearance of PpIX and the conversion of MAL into PpIX during the time interval $]t;t+dt]$ can be expressed as follows (equation 4):

$$dM_{PpIX}^{BC}(t,z) = M_{PpIX}^{BC}(t+dt,z) - M_{PpIX}^{BC}(t,z)$$
$$= L(0)\times\exp(-\eta z)\times\left[\frac{1}{1+\exp(-k\times(t+dt-\tau))} - \frac{1}{1+\exp(-k\times(t-\tau))}\right] \quad (4)$$

Regarding the photobleaching process, based on our previous work [21], the number of PpIX molecules eliminated by photobleaching during the time interval $]t;t+dt]$, denoted by $dM_{PpIX}^{P}(t,z)$, can be estimated using equation 5:

$$dM_{PpIX}^{P}(t,z) = dt\times\frac{\kappa}{\aleph V_U}\times M_{PpIX}(t,z)$$
$$\times\int_{\tilde{\lambda}}\left\{dt\times\gamma_{\tilde{\lambda}}\times\tilde{\lambda}\times\frac{\varphi(t,z,\tilde{\lambda})}{\eta c}\times V_U\times\mu_{a,PpIX}(t,z,\tilde{\lambda})\right\}d\tilde{\lambda} \quad (5)$$

Where:

- $M_{PpIX}(t,z)$ is the number of PpIX molecules present in an unit volume, V_U, at time t and depth z,
- κ is the bimolecular rate constant for the reaction of singlet oxygen with PpIX,
- $\aleph$ is the Avogadro number (6.022×10^{23} /mol),
- $\gamma_{\tilde{\lambda}}$ is the singlet oxygen quantum yield (i.e. the number of singlet oxygen molecules generated for each photon of wavelength $\tilde{\lambda}$ absorbed by a PpIX molecule when the PDT process is not limited by the availability of oxygen),
- η is the Planck constant (6.626×10^{34} J×s),
- c is the speed of light (3×10^{8} m/s).

Combining equations 4 and 5 results in the following approximation equation for the number of PpIX molecules present at time $t+dt$ (equation 6):

$$M_{PpIX}(t+dt,z) = M_{PpIX}(t,z) + dM_{PpIX}^{BC}(t,z) - dM_{PpIX}^{P}(t,z)$$
$$= M_{PpIX}(t,z)$$
$$+ L(0)\times\exp(-\eta z)\times\left[\frac{1}{1+\exp(-k\times(t+dt-\tau))} - \frac{1}{1+\exp(-k\times(t-\tau))}\right] \quad (6)$$
$$- dt\times\frac{\kappa}{\aleph V_U}\times M_{PpIX}(t,z)\times\int_{\tilde{\lambda}}\left\{dt\times\gamma_{\tilde{\lambda}}\times\tilde{\lambda}\times\frac{\varphi(t,z,\tilde{\lambda})}{\eta c}\times V_U\times\mu_{a,PpIX}(t,z,\tilde{\lambda})\right\}d\tilde{\lambda}$$

Based on the relationship $\mu_{a,PpIX}(t,z,\lambda)=\frac{\varepsilon_{PpIX}(\lambda)}{\aleph V_U}\times M_{PpIX}(t,z),\forall t$ where $\varepsilon_{PpIX}(\lambda)$ is the PpIX molar extinction coefficient for wavelength λ, equation 6 can be rewritten in terms of the PpIX absorption coefficient giving the following updating formula for the PpIX absorption coefficient (equation 7):

$$\begin{aligned}\mu_{a,PpIX}(t+dt,z,\lambda) &= \mu_{a,PpIX}(t,z,\lambda)\\ &+\frac{\varepsilon_{PpIX}(\lambda)}{\aleph V_U}\times L(0)\times\exp(-\eta z)\times\left[\frac{1}{1+\exp(-k\times(t+dt-\tau))}-\frac{1}{1+\exp(-k\times(t-\tau))}\right]\\ &-dt\times\frac{\kappa}{\aleph V_U}\times\mu_{a,PpIX}(t,z,\lambda)\times\int_{\tilde{\lambda}}\left\{dt\times\gamma_{\tilde{\lambda}}\times\tilde{\lambda}\times\frac{\varphi(t,z,\tilde{\lambda})}{\eta c}\times V_U\times\mu_{a,PpIX}(t,z,\tilde{\lambda})\right\}d\tilde{\lambda}\end{aligned} \quad (7)$$

From equation 1, assuming a given distribution for the PpIX absorption coefficient at time 0, $\{\mu_{a,PpIX}(0,z,\lambda)\}_{z,\lambda}$, the local total fluence rate at time 0, $\varphi(0,z,\lambda)$, can be calculated for any point of the skin model (Figure 2) and any wavelength of the spectrum. Equation 7 then enables the distribution for the PpIX absorption coefficient at time dt, $\{\mu_{a,PpIX}(dt,z,\lambda)\}_{z,\lambda}$, to be computed. From this new distribution for the PpIX absorption coefficient, any local total fluence rate at time dt, $\varphi(dt,z,\lambda)$, can be calculated using equation 1... Iterating equations 1 and 7 therefore enables all the necessary PpIX absorption coefficients and local total fluence rates to be determined.

3. Initialization

We naturally assume that: $\forall z, M_{PpIX}(0,z)=\frac{L(0)\times\exp(-\eta z)}{1+\exp(k\times\tau)}$ and subsequently $\forall(z,\lambda),\mu_{a,PpIX}(0,z,\lambda)=\frac{\varepsilon_{PpIX}(\lambda)}{\aleph V_U}\times M_{PpIX}(0,z)=\frac{\varepsilon_{PpIX}(\lambda)}{\aleph V_U}\times\frac{L(0)\times\exp(-\eta z)}{1+\exp(k\times\tau)}$.

4. Parameters setting

The optical properties for actinic keratosis mentioned in equation 1 are derived from the data reported in Garcia-Uribe et al. [31].

The parameters related to the photobleaching process (equation 5 and last term in equation 7) were assigned to the values used in our previous work [21] (Table 2). These values reported in

the literature have been empirically determined as stated in [21].

Parameters	Value
dt	1×10^{-5} s
κ	5.3×10^{9} l/mol/s
$\gamma_{\tilde{\lambda}}$	0.56

Table 2: Specification of the model parameters from [21]

Regarding the biological clearance of PpIX and the conversion of MAL into PpIX (second term in equation 7), four parameters remain to be specified: $L(0)$, k and τ both introduced in equation 2 and η introduced in equation 3.

From the fitting of equation 2 to the PpIX concentration data computed at zero depth in normal human epidermis by Star et al. [30], $\alpha L(0)$ (α is the conversion factor between $M_{PpIX}^{BC}(t,z)$ and the corresponding PpIX concentration expressed in µg/g in [30]), k and τ were estimated to be 3.43 AU, 2.93×10^{-4} /s and 1.01×10^{4} s, respectively. Computed from the PpIX concentration data at zero depth and the ratio between the PpIX concentration at 0.2 mm and at 0 mm both reported in [30], the PpIX concentration data at 0.2 mm depth were fitted to equation 2 leading to the values of 2.82 AU, 3.25×10^{-4} /s and 1.01×10^{4} s for $\alpha L(0.2mm)$, k and τ, respectively. Regarding k and τ, these values are close to the ones obtained using the PpIX concentration data computed at zero depth and are therefore consistent with the use of a single value as assumed in equation 2.

Assuming $\alpha L(0)=3.43$ AU, $k=2.93\times10^{-4}$/s and $\tau=1.01\times10^{4}$ /s, the fitting of equation 3 to the PpIX concentration data at 0.2 mm depth [30], enables the depth decay constant, η, to be deduced (Table 3).

Finally, $L(0)$ was determined combining equation 3 with the concentration at the skin surface $(z=0)$ of 11.8 pmol/ml obtained by Smits et al. [32] from 11 patients with AK incubated with 20% ALA for 3 hours (equation 8):

$$\left.\begin{aligned} C_{PpIX}^{BC}(3\text{h},0) &= \frac{M_{PpIX}(3\text{h},0)}{\aleph V_U} \\ &\overset{Equation\ 3}{=} \frac{1}{\aleph V_U} \times \frac{L(0)}{1+\exp(-k\times(3\text{h}-\tau))} \\ C_{PpIX}^{BC}(3\text{h},0) &\overset{Smits\ et\ al.\ [32]}{=} 11.8\,\text{pmol/ml} \end{aligned}\right\} \Rightarrow L(0) = 11.8\,\text{pmol/ml}\times\aleph V_U \times\left[1+\exp(-k\times(3\text{h}-\tau))\right] \quad (8)$$

Parameters	Value
k	2.93×10^{-4} /s
τ	1.01×10^{4} s
η	0.89 /mm
$L(0)$	1.29×10^{4} using $V_U = (10\mu\text{m})^3$

Table 3: Specification of the parameters for the biological clearance of PpIX and the conversion of MAL into PpIX

D. Quantification of the PDT local damage

The integral in the photobleaching term of equation 7 (last part of the right hand side) represents the number of singlet oxygen molecules generated during the time interval $]t;t+dt]$ in an unit volume, V_U, located at depth z in the skin sample model when the PpIX molecules, excited by absorption of photons, return to the ground state [21]. Therefore, the sum of this integral over the time intervals $]0;dt],]dt;2dt],]2dt;3dt],\ldots,]t_i-dt;t_i]$ with $t_i = t_{start} + i\times dt$ provides the total cumulative singlet oxygen molecules produced during the time interval $]0;t_i]$. Following several studies on PDT [26,33,34], this cumulative parameter enables the quantification of the PDT local damage, below-denoted as D, over time (equation 9).

$$D(t_i = t_{start} + i\times dt, z) = \sum_{\substack{j=0 \\ t_j = t_{start}+j\times dt}}^{i-1} \left[\int_{\tilde{\lambda}} \left\{ dt\times\gamma_{\tilde{\lambda}}\times\tilde{\lambda}\times\frac{\varphi(t_j,z,\tilde{\lambda})}{\eta c}\times V_U\times\mu_{a,PpIX}(t_j,z,\tilde{\lambda}) \right\} d\tilde{\lambda} \right] \quad (9)$$

IV. Results

A. From the clinical trial

The preliminary results obtained for the first 14 patients of the clinical trial [35] are summarized in Tables 4 and 5. From these results, PDT using the FLEXITHERALIGHT protocol is not inferior in terms of complete response rate when compared to PDT using the standard protocol (Table 4) while being more comfortable for patients (Table 5).

Complete response rate	At 3 months	At 6 months
Standard protocol	54.2%	64%
FLEXITHERALIGHT protocol	65.3%	71.7%

Table 4: Estimation of the complete response rate for PDT using the standard protocol (second row) and for PDT using the FLEXITHERALIGHT protocol (third row) at 3 (second column) and 6 months (third column) from the results of the first 14 patients of the clinical trial [35].

Pain (0: no pain and 10: unbearable pain)	At day 0 (treatment day)	At day 7
Standard protocol	Mean: 5.2 (standard deviation: 2.8)	Mean: 0.1 (standard deviation: 0.2)
FLEXITHERALIGHT protocol	Mean: 0.4 (standard deviation: 0.6)	Mean: 0.1 (standard deviation: 0.2)

Table 5: Estimation of the pain for PDT using the standard protocol (second row) and for PDT using the FLEXITHERALIGHT protocol (third row) at day 0 (second column) and day 7 (third column) from the results of the first 14 patients of the clinical trial [35].

B. From the mathematical modeling study

All the computations were performed using a Matlab™ program on a standard personal computer (Intel Xeon CPU E3-1240 V2 3.40 GHz–8Go of RAM–Windows 7 64 bits).

Figure 4 shows the evolution of the number of PpIX molecules as a function of time when using the standard and the FLEXITHERALIGHT protocols (equation 6).

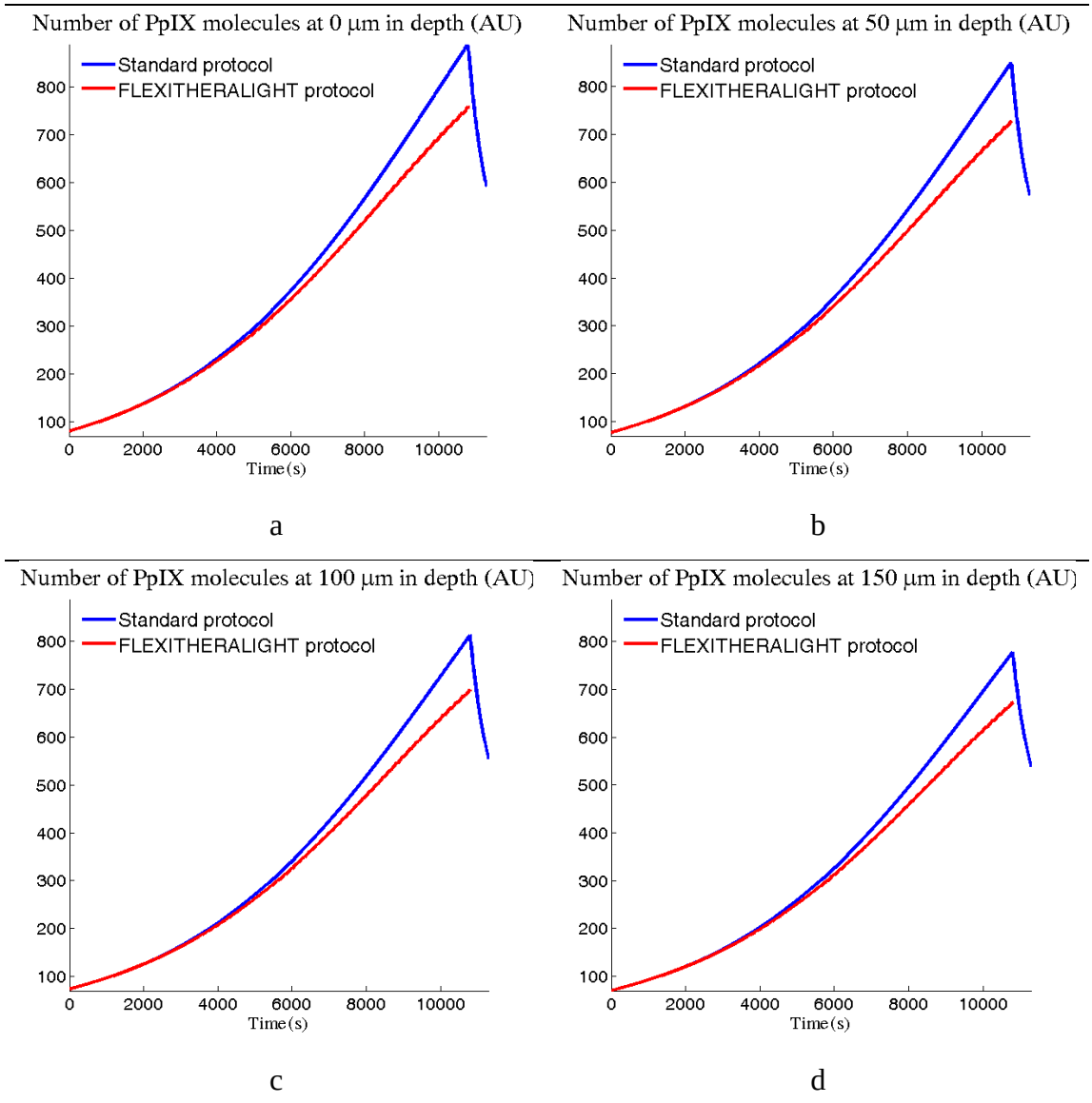


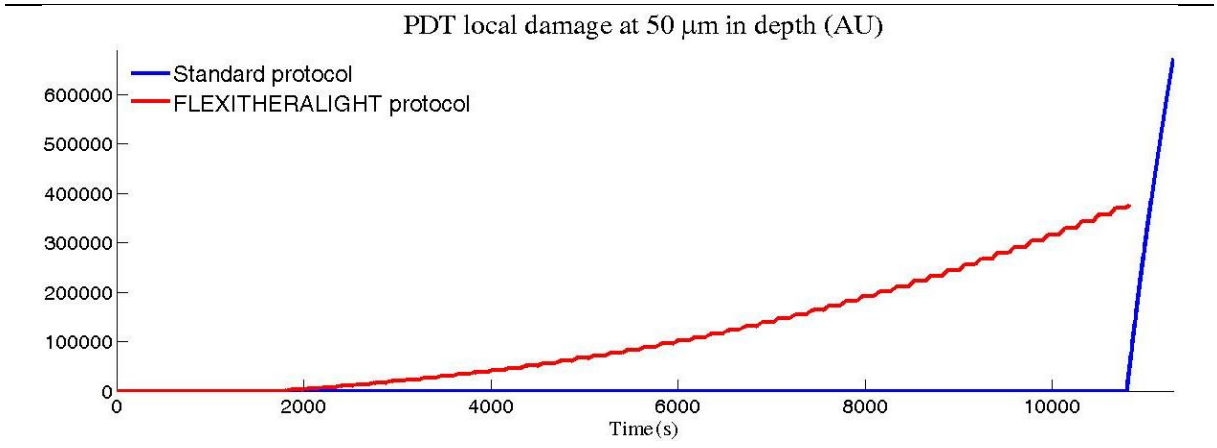
Figure 4: Evolution in time of the number of PpIX molecules when using the standard protocol (incubation time: three hours, irradiation type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2, treatment duration: 493 s) (blue curves) and the FLEXITHERALIGHT one (incubation time: 30 minutes, irradiation type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2, treatment duration: 9024 s) (red curves) at 0 (a), 50 (b), 100 (c) and 150 (d) µm in depth in AK.

From Figure 4, whatever the depth in AK, the number of PpIX molecules corresponding to the FLEXITHERALIGHT protocol continues to increase even after the beginning of

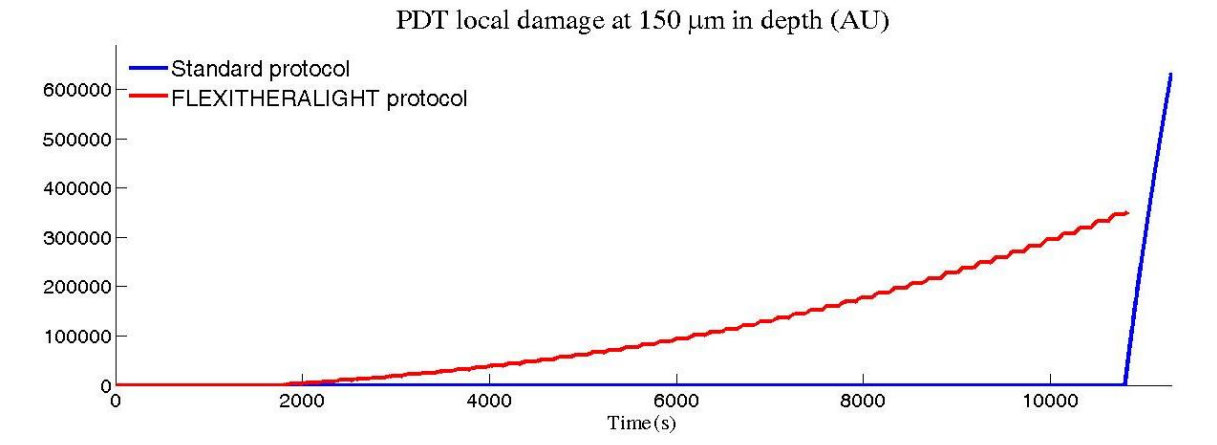
irradiation ($t_{start} = 30$ minutes). This means that the number of PpIX molecules generated from the conversion of MAL is always higher than the number of PpIX molecules removed by either the biological clearance or the photobleaching of PpIX. Regarding the standard protocol, the irradiation leads to a mean percent drop of 32.04% in the number of PpIX molecules, that is close to the 27 percent drop computed from data reported in Wiegell et al. [24].

Whatever the protocol, an important number of PpIX molecules is still present at the end of the irradiation (Figure 4): for the standard protocol (respectively, for the FLEXITHERALIGHT protocol), this number is more than 7.59 (respectively, 9.59) times higher than the number of PpIX molecules present at the beginning of the incubation (i.e., at time $t = 0$ s).

The time evolution of the PDT local damage achieved when using the standard and the FLEXITHERALIGHT protocols is illustrated for different depths in AK in Figures 5 and 6.

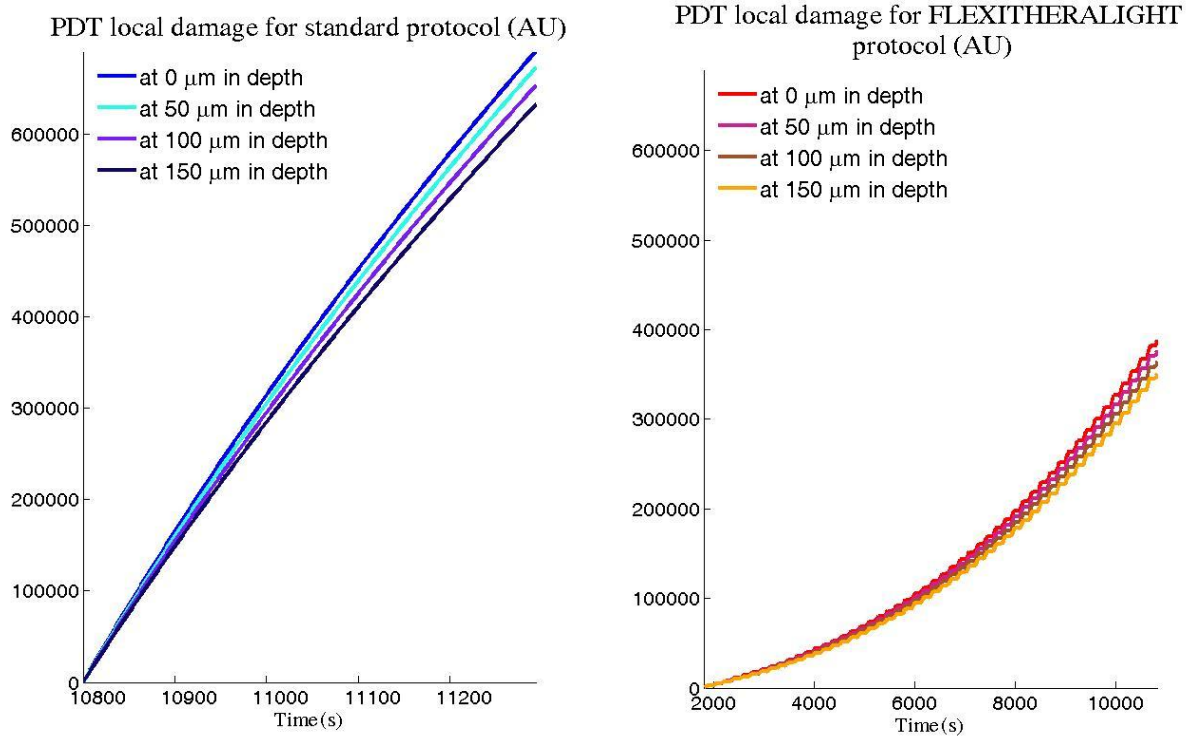


a



b

Figure 5: Evolution in time of the PDT local damage achieved when using the standard protocol (incubation time: three hours, irradiation type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2, treatment duration: 493 s) (blue curves) and the FLEXITHERALIGHT one (incubation time: 30 minutes, irradiation type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2, treatment duration: 9024 s) (red curves) at 50 (a) and 150 (b) µm in depth in AK.



a b

Figure 6: Time evolution of the PDT local damage as a function of the depth position in AK. Results are displayed for the standard protocol (incubation time: three hours, irradiation type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2, treatment duration: 493 s) (a) and for the FLEXITHERALIGHT one (incubation time: 30 minutes, irradiation type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2, treatment duration: 9024 s) (b).

As expected looking at the involved fluence rates and irradiation types, the PDT local damage produced using the standard protocol increases much faster than that produced using the FLEXITHERALIGHT protocol (Figure 5). From a linear regression of the PDT local damage

versus time performed for all depths in AK, the PDT local damage obtained using the standard protocol increases, on average, about 33.30 times faster than the one obtained using the FLEXITHERALIGHT protocol.

Furthermore, as can be seen in Figures 5 and 6, whatever the depth position in AK, the PDT local damage achieved at the end of the treatment using the standard protocol is higher than the one achieved at the end of the treatment using the FLEXITHERALIGHT protocol. Ranging from 1.78 at 0 µm depth to 1.80 at 150 µm depth, the ratio of the PDT local damage achieved at the end of the treatment between using the standard protocol and using the FLEXITHERALIGHT protocol is a slightly increasing function of the depth position in AK.

In addition, for both the protocols, an increasing impact of the depth in AK on the PDT local damage over time is observed in Figure 6. Moreover, the shape of the time course of the PDT local damage for the standard protocol tends to demonstrate a very slight logarithmic trend (Figure 6.a) while that for the FLEXITHERALIGHT protocol suggests an exponential trend (Figure 6.b).

The local damages obtained at the end of the treatment using the standard protocol and the FLEXITHERALIGHT protocol are displayed according to depth in Figure 7.

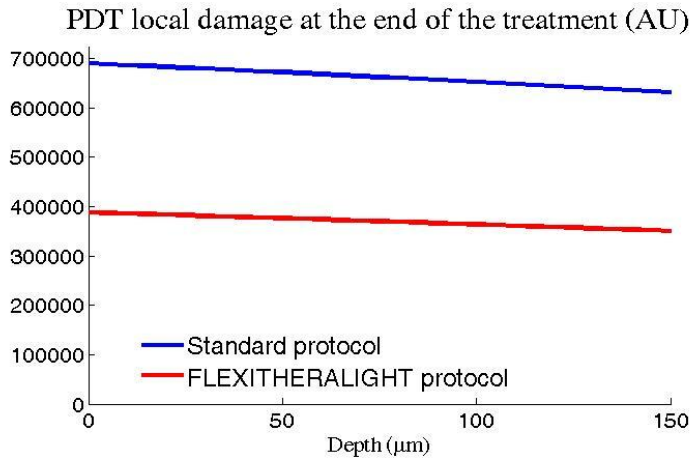


Figure 7: Depth evolution of the PDT local damage for the standard protocol (incubation time: three hours, illumination type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2, treatment duration: 493 s) (blue curves), and the FLEXITHERALIGHT protocol (incubation time: 30 minutes, illumination type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2, treatment duration:

9024 s) (red curves).

From Figure 7, the depth-related decrease rate in the PDT local damage obtained at the end of the treatment using the FLEXITHERALIGHT protocol seems to be similar to those obtained using the standard protocol. Nonetheless from a linear regression, the depth-related decrease rate for the FLEXITHERALIGHT protocol is around one-third smaller than that obtained using the standard protocol.

V. Discussion

In this paper, a comparison between the two following 635 nm red light protocols is performed for the PDT treatment of actinic keratosis using a mathematical modeling of the PDT process:

- Protocol 1 with incubation time: three hours, irradiation type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2
- Protocol 2 with incubation time: 30 minutes, irradiation type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2.

The continuous 75 mW/cm^2 red light protocol was considered due to its standardized use across Europe [16,22,23] while the choice of the fractionated 12.3 mW/cm^2 red light protocol was motivated by the FLEXITHERALIGHT Project (http://www.flexitheralight.com/). This French National Research Agency Project focuses on the development of a biophotonic device based on a flexible light emitting textile enabling a fractionated irradiation with a 12.3 mW/cm^2 fluence rate for the PDT treatment of actinic keratosis. Based, on one hand, on the published results of some alternative red light protocols with lower fluence rates than the standard 75 mW/cm^2 red light protocol [15-17], and on the other hand, on the tissue re-oxygenation and photosensitizer re-synthesis during the dark periods of the fractionated irradiation, the FLEXITHERALIGHT protocol is expected to be at least as effective as the standard protocol while being much better tolerated by patients. The preliminary results from the phase II clinical trial (ANSM authorization number: 2013-A01096-39) live up to these expectations since they demonstrate that the FLEXITHERALIGHT protocol is not inferior in terms of complete response rate to the standard protocol and is much more comfortable for

patients.

A 200-µm thick partial ellipsoid included into a 150-µm thick parallelepiped was used to model a post-curettage AK in epidermis and an iterative procedure alternating determination of the fluence rate and updating of the optical properties was derived from our previous work [21] to model the PDT process. The determination of the fluence rate involves solving the one-dimensional diffusion equation (equation 1) while the updating of the optical properties takes the biological clearance of PpIX, the conversion of MAL into PpIX and the PpIX photobleaching into account (equation 7). In this paper, the biological clearance of PpIX and the conversion of MAL into PpIX were described using a single logistic growth model (equations 2 and 3), that is, according to [24,29,30], a more realistic model compared to the two exponential models used in our previous work [21]. Regarding the photobleaching, we used the original model that we proposed in [21] (equation 5). This original photobleaching model involves the calculation of the number of singlet oxygen molecules generated over time assuming unlimited oxygen availability. This assumption that is made through the singlet oxygen quantum yield in equation 5, is deemed reasonable according to the thickness of the AK [36]. In fact, as mentioned in [21,36], the epidermis layer is almost exclusively supplied by diffused oxygen from the atmosphere, and the unlimited source of atmospheric oxygen allows unlimited oxygen availability in the skin sample model to be reasonably assumed. Finally, estimation of the cumulative number of singlet oxygen molecules produced during the treatment enables the quantification of the PDT local damage (equation 9).

From the above-mentioned suitable assumption of unlimited oxygen availability, the fractionated irradiation aimed at allowing tissue re-oxygenation and photosensitizer re-synthesis during the dark periods, is, in the proposed model, only taken into account for photosensitizer re-synthesis purposes.

All the parameters involved in this model are set to published values obtained using PpIX and either normal human epidermis or AK [21].

Applying to the standard protocol and to the FLEXITHERALIGHT one, the model allows evaluation and comparison of their performance in terms of the PDT local damage.
From the results, the higher fluence rate of the standard protocol compared to that of the FLEXITHERALIGHT protocol combined with the continuous irradiation type of the standard

protocol opposed to the fractionated irradiation of the FLEXITHERALIGHT protocol logically leads to a higher increase rate for the PDT local damage of the standard protocol — deduced to be more than 30 times higher than that for the PDT local damage of the FLEXITHERALIGHT protocol — (Figure 5).

Furthermore, in spite of the identical light dose of 37 J/cm^2 for the two protocols, using the well-known efficient standard protocol results in a PDT local damage at the end of the treatment of, on average, 1.79 times as high as that obtained using the FLEXITHERALIGHT protocol (Figures 5 and 6).

However, the above-mentioned clinically-demonstrated non-inferiority in terms of complete response rate of the FLEXITHERALIGHT protocol versus the standard protocol, seems to highlight that the PDT local damage achieved using the FLEXITHERALIGHT protocol is sufficient to destroy any cancer cells and therefore that the parameters of the standard protocol should be revised accordingly. Thus, among the possible changes in parameters, reducing by half the treatment duration of the standard protocol (and therefore the light dose) may lead to a PDT local damage equivalent to the sufficient one obtained using the FLEXITHERALIGHT protocol (Figure 5).

Regarding the time evolution of the PpIX molecules number in Figure 4, the beginning of the irradiation is clearly identifiable for the standard protocol with a more than 30 percent drop which is in close agreement with results from [24]. On the contrary, the steady growth curves observed for the FLEXITHERALIGHT protocol makes the identification of the beginning of the irradiation impossible: the 12.3 mW/cm^2 fluence rate and the fractionated irradiation do not allow a photobleaching of the PpIX molecules important enough to outweigh the conversion of MAL into PpIX. Consideration also needs to be given to the important number of PpIX molecules still present at the end of the treatment for both the protocols (Figure 4). This important number tends to demonstrate that the incubation time of the two protocols — or the cream concentration in MAL —could be reduced [37].

Moreover, based on various studies on the impact of fluence rates on pain [15,16], a better tolerability in terms of pain is expected using the FLEXITHERALIGHT protocol. This expectation has been verified from the above-mentioned phase II clinical trial.

Finally, these results confirmed by a first analysis of the clinical trial data emphasize the need to redefine standard protocols and better determine treatment parameters for a similar

efficiency but an improved tolerability and a more manageable clinical practice.

VI. Conclusions

In this paper, we have proposed to evaluate and compare two protocols: the standard protocol (wavelength: 635 nm, incubation time: three hours, illumination type: continuous, light dose: 37 J/cm^2, fluence rate: 75 mW/cm^2) and an alternative one, the FLEXITHERALIGHT protocol (http://www.flexitheralight.com/) (wavelength: 635 nm, incubation time: 30 minutes, illumination type: fractionated with two minutes dark intervals every three minutes, light dose: 37 J/cm^2, fluence rate: 12.3 mW/cm^2). The evaluation tends to demonstrate that an optimization of the two protocols parameters and especially of the incubation times could lead to a similarly efficient and more suitable treatment while the comparison tends to prove a slightly better efficiency of the standard protocol in term of the PDT local damage.

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References

[1] Plaetzer K., Krammer B., Berlanda J., Berr F., and Kiesslich T., "Photophysics and photochemistry of photodynamic therapy: fundamental aspects.", Lasers Med Sci, 24, 2, 259-268, 2009.

[2] Bissonette R., Bergeron A., and Liu Y., "Large surface photodynamic therapy with aminolevulinic acid: treatment of actinic keratoses and beyond.", J Drugs Dermatol, 3, 1 Suppl, S26-S31, 2004.

[3] Santos MA., Belo VG., and Santos G., "Effectiveness of photodynamic therapy with topical 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone in the treatment of acne vulgaris: comparative study.", Dermatol Surg, 31, 8 Pt 1, 910-915, 2005.

[4] Braathen LR., Szeimies RM., Basset-Seguin N., Bissonette R., Foley P., Pariser D., Roelandts R., Wennberg AM., and Morton CA., "Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus.", J Am Acad Dermatol, 56, 1, 125-143, 2007.

[5] Morton CA., McKenna KE., and Rhodes LE., "Guidelines for topical photodynamic therapy: update.", Br J Dermatol., 159, 6, 1245-1266, 2008.

[6] Lu YG., Wu JJ., He Y., Yang HZ., and Yang YD., "Efficacy of topical aminolevulinic acid photodynamic therapy for the treatment of verruca planae.", Photomed Laser Surg, 28, 4, 561-563, 2010.

[7] Sotiriou E., Apalla Z., Chovarda E., Panagiotidou D., and Ioannides D., "Photodynamic therapy with 5-aminolevulinic acid in actinic cheilitis: an 18-month clinical and histological follow-up.", J Eur Acad Dermatol Venereol, 24, 8, 916-920, 2010.

[8] Cai H., Wang YX., Zheng JC., Sun P., Yang ZY., Li YL., Li XY., Li Q., and Liu W., "Photodynamic therapy in combination with CO2 laser for the treatment of Bowen's disease.", Lasers Med Sci., 30, 5, 1505-1510, 2015.

[9] Morton CA., Campbell S., Gupta G., Keohane S., Lear J., Zaki I., Walton S., Kerrouche N., Thomas G., and Soto P., "Intraindividual, right-left comparison of topical methyl aminolaevulinate-photodynamic therapy and cryotherapy in subjects with actinic keratoses: a multicentre, randomized controlled study.", Br J Dermatol., 155, 5, 1029-1036, 2006.

[10] Wiegell SR., "Update on photodynamic treatment for actinic keratosis.", Curr Probl Dermatol., 46, 122-128, 2015.

[11] Glogau RG., "The risk of progression to invasive disease.", J Am Acad Dermatol, 42, 1 Pt 2, 23-24, 2000.

[12] Ericson MB., Wennberg AM., and Larko O, "Review of photodynamic therapy in actinic keratosis and basal cell carcinoma.", Ther Clin Risk Manag., 4, 1, 1-9, 2008.

[13] Tyrrell J., Campbell SM., and Curnow A., "The effect of air cooling pain relief on protoporphyrin IX photobleaching and clinical efficacy during dermatological photodynamic therapy.", J Photochem Photobiol B., 103, 1, 1-7, 2011.

[14] Stangeland KZ. and Kroon S., "Cold air analgesia as pain reduction during photodynamic therapy of actinic keratoses.", J Eur Acad Dermatol Venereol., 26, 7, 849-854, 2012.

[15] Ericson MB., Sandberg C, Stenquist B., Gudmundson F., Karlsson M., Ros AM., Rosen A., Larko O, Wennberg AM., and Rosdahl I., "Photodynamic therapy of actinic keratosis at varying fluence rates: assessment of photobleaching, pain and primary clinical outcome.", Br J Dermatol., 151, 6, 1204-1212, 2004.

[16] Apalla Z., Sotiriou E., Panagiotidou D., Lefaki I., Goussi C., and Ioannides D., "The impact of different fluence rates on pain and clinical outcome in patients with actinic keratoses treated with photodynamic therapy.", Photodermatol Photoimmunol Photomed., 27, 4, 181-185, 2011.

[17] Enk CD. and Levi A., "Low-irradiance red LED traffic lamps as light source in PDT for actinic keratoses.", Photodermatol Photoimmunol Photomed., 28, 6, 332-334, 2012.

[18] De Haas ERM., De Vijlder HC., Sterenborg HJ., Neumann HA., and Robinson DJ., "Fractionated aminolevulinic acid–photodynamic therapy provides additional evidence for the use of PDT for non-melanoma skin cancer.", J Eur Acad Dermatol Venereol., 22, 4, 426-430, 2008.

[19] Sotiriou E., Apalla Z., Chovarda E., Goussi C., Trigoni A., and Ioannides D., "Single vs. fractionated photodynamic therapy for face and scalp actinic keratoses: a randomized, intraindividual comparison trial with 12-month follow-up.", J Eur Acad Dermatol Venereol., 26, 1, 36-40, 2012.

[20] Cochrane C., Mordon SR, Lesage JC., and Koncar V., "New design of textile light diffusers for photodynamic therapy.", Mater Sci Eng C Mater Biol Appl., 33, 3, 1170-1175, 2013.

[21] Vignion-Dewalle AS., Betrouni N., Tylcz JB., Vermandel M., Mortier L., and Mordon S., "Comparison of three light doses in the photodynamic treatment of actinic keratosis using mathematical modeling.", J Biomed Opt, 20, 5, 58001- 2015.

[22] Tyrrell J., Campbell S., and Curnow A., "Protoporphyrin IX photobleaching during the light irradiation phase of standard dermatological methyl-aminolevulinate photodynamic therapy.", Photodiagnosis Photodyn Ther., 7, 4, 232-238, 2010.

[23] Sotiriou E., Apalla Z., Vrani F., Lallas A., Chovarda E., and Ioannides D., "Photodynamic therapy vs. imiquimod 5% cream as skin cancer preventive strategies in patients with field changes: a randomized intraindividual comparison study.", J Eur Acad Dermatol Venereol, 29, 2, 325-329, 2015.

[24] Wiegell SR., Haedersdal M., Philipsen PA., Eriksen P., Enk CD., and Wulf HC., "Continuous activation of PpIX by daylight is as effective as and less painful than conventional photodynamic therapy for actinic keratoses; a randomized, controlled, single-blinded study.", Br J Dermatol., 158, 4, 740-746, 2008.

[25] Moseley H., "Light distribution and calibration of commercial PDT LED arrays.", Photochem Photobiol Sci., 4, 11, 911-914, 2005.

[26] Farrell TJ., Hawkes RP., Patterson MS., and Wilson BC., "Modeling of photosensitizer fluorescence emission and photobleaching for photodynamic therapy dosimetry.", Appl Opt, 37, 31, 7168-7183, 1998.

[27] Carp SA., Prahl SA., and Venugopalan V., "Radiative transport in the delta-P1 approximation: accuracy of fluence rate and optical penetration depth predictions in turbid semi-infinite media.", J Biomed Opt, 9, 3, 632-647, 2004.

[28] Garcia-Uribe A., Zou J., Duvic M., Cho-Vega JH., Prieti VG., and Wang LV., "In vivo diagnosis of melanoma and nonmelanoma skin cancer using oblique incidence diffuse reflectance spectrometry.", Cancer Res., 72, 11, 2738-2745, 2012.

[29] Angell-Petersen E., Sorensen R., Warloe T., Soler A.M., Moan J., Peng Q., and Gierchsky K.E., "Porphyrin formation in actinic keratosis and basal cell carcinoma after topical application of methyl 5-aminolevulinate.", J Invest Dermatol., 126, 2, 265-271, 2006.

[30] Star WM., Aalders MC., Sac A., and Sterenborg HJ., "Quantitative model calculation of the time-dependent protoporphyrin IX concentration in normal human epidermis after delivery of ALA by passive topical application or lontophoresis.", Photochem Photobiol., 75, 4, 404-432, 2002.

[31] Garcia-Uribe A, Zou J, Duvic M, Cho-Vega JH, Prieto VG, and Wang LV, "In Vivo diagnosis of melanoma and nonmelanoma skin cancer using oblique incidence diffuse reflectance spectrometry", Cancer Research, 72, 11, 2738-2745, 2012.

[32] Smits T and Moor ACE, "New aspects in photodynamic therapy of actinic keratoses", Journal of Photochemistry and Photobiology, 96, 3, 159-169, 2005.

[33] Valentine RM., Brown CT., Moseley H., Ibbotson S., and Wood K., "Monte Carlo modeling of in vivo protoporphyrin IX fluorescence and singlet oxygen production during photodynamic therapy for patients presenting with superficial basal cell carcinomas.", J Biomed Opt, 16, 4, 048002- 2011.

[34] Zhu TC., Liu B., Kim MM., McMillan D., Liang X., Finlay JC., and Busch TM., "Comparison of singlet oxygen threshold dose for PDT.", Proc SPIE Int Soc Opt Eng, 8931-89310I, 2014.

[35] Vicentini C., Tylcz JB., Maire C., Betrouni N., Mortier L., and Mordon S., "Phase II study evaluating the non-inferiority of the device FLEXITHERALIGHT compared to conventional PDT.", American Society for Laser Medicine and Surgery Abstracts, Lasers in Surgery and Medicine, 48, S27, 1-92, 2016.

[36] Stücker M., Struk A., Altmeyer P., Herde M., Baumgärtl H., and Lübbers DW., "The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis.", J Physiol., 538, Pt 3, 985-994, 2002.

[37] Christiansen K., Bjerring P., and Troilius A., "5-ALA for photodynamic photorejuvenation--optimization of treatment regime based on normal-skin fluorescence measurements", Lasers in Surgery and Medicine, 39, 4, 302-310, 2007.