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Photodynamic therapy for skin cancer : How to enhance drug penetration ?

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Abstract

Photodynamic therapy (PDT) induced by protoporphyrin IX (PpIX) has been widely used in dermatological practices such as treatment of skin cancers. Clearance rate depends on different factors such as light irradiation, skin oxygenation and drug penetration. The poor penetration of 5-aminolevulinic acid (5-ALA) with topical application is limited and restrains the production of PpIX which could restrict PDT outcomes. This review will focus on techniques already used to enhance drug penetration in human skin, and will present their results, advantages, and drawbacks. Chemical and physical pretreatments will be discussed. Chemical pre-treatments comprise of drug formulation modification, use of agents that modify the heme cycle, enhance PpIX formation, and the combination of differentiation-promoting agent prior to PDT. On the other hand, physical pretreatments affect the skin barrier by creating holes in the skin or by removing stratum corneum. To promote drug penetration, iontophoresis and temperature modulation are interesting alternative methods. Cellular mechanisms enrolled during chemical or physical pretreatments have been investigated in order to understand how 5-ALA penetrates the skin, why it is preferentially

metabolized in PpIX in tumour cells, and how it could be accumulated in deeper skin layers. The objective of this review is to compare clinical trials that use innovative technology to conventional PDT treatment. Most of these pretreatments present good or even better clinical outcomes than usual PDT.

1. Introduction

Protoporphyrin IX-induced PDT has been widely used in dermatological practice such as treatment of skin cancers [1].

Historically, treatments using light appeared during antiquity and were used by the Egyptians, Indians, Greeks and Chinese to treat skin diseases. Therapy using an exogenous substance reacting with sun's photons was observed during the 15th century BC in India. At the end of the 19th century, interaction between light and molecules was considered for medical treatment. The concept of PDT was first introduced by Van Tappeiner who mentioned the necessity of oxygen to obtain a chemical reaction over a century ago [2]. When PDT depends on the combination of three components to induce tumour destruction : a photosensitive drug, light, and oxygen (**Fig.1**). None of these elements is individually toxic, but together they produce a photochemical reaction that generates singlet oxygen which is a highly reactive product causing cell death [3]. (**Fig.2**) displays the different energy states and the different pathways leading to fluorescence and other photochemical processes. First the photosensitizer (PS) at its ground and singlet state absorbs a photon and moves to its excited energy state. Because of the internal conversion the PS energy decreases and reaches the lower allowed transition of its excited state. Inter-system conversion causes this singlet state to become a triplet state. The singlet state relaxation produces fluorescence, and the triplet produces phosphorescence. A molecule with high triplet energy quantum yield can initiate chemical reactions (type I) that produce free radicals, and then could react with oxygen to form singlet oxygen. Singlet oxygen is a reactive oxygen species that is responsible for degradation of several cell components, and induce cell death by type II chemical reactions [4].

PDT treatment in dermatology consists of the administration of a non-toxic drug known as a photosensitizer (PS) to a patient presenting a lesion, which is frequently, but not always cancerous [5].

Photosensitizers should have characteristics such like being nontoxic, water soluble, having a chemical and physical stability, being selective for neoplastic tissues with the quickest time interval between administration and maximal accumulation, and able to be eliminated by the body in a short time in order to avoid photoreactions [5]. Historically, hematoporphyrin and hematoporphyrin derivative (HpD) were the first PS to be studied in detail [6]. However these compounds present photosensitivity after a long time (8weeks), so patients had to avoid sunlight which was very constraining. Moreover, tumour-localizing properties of these PSs were not as pronounced as first thought [7].

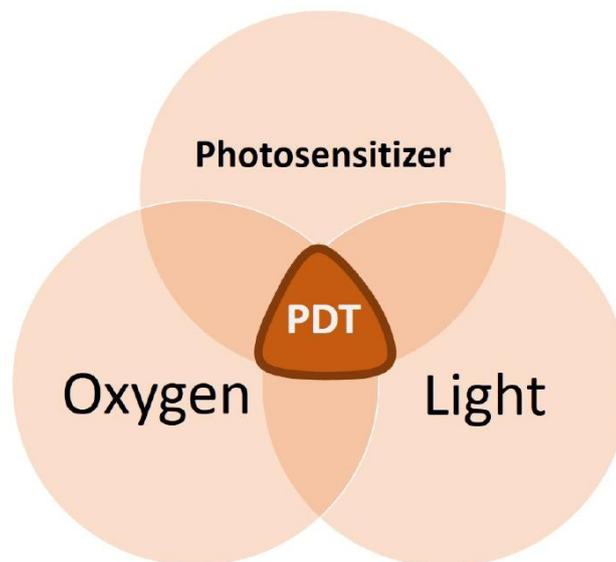


Figure 1 : Synergy between three parameters produces PDT.

These deficiencies and the fact that these products were orally administered made their use difficult for dermatology. So a new kind of PS has been developed : endogenous photosensitizers. Endogenous PS are synthesized in the body from non photosensitizing precursors (also called prodrugs). A precursor's chemical conversion is established by metabolic pathways and particularly by the heme cycle.

Nowadays in the field of dermatology, topical application of 5-aminolevulinic acid (5-ALA) (a non-photosensitizing precursor) is used to induce *in situ* synthesis of endogenous PS according to the heme cycle [8]. (Fig.3) displays the heme cycle where 5-ALA is the first metabolite in

the heme biosynthesis pathway in humans. 5-ALA-when not voluntarily added-is originally produced by glycine and succinyl coenzyme A, and then migrates to the cytosol. From there, porphobilinogen is formed due to the condensation of two molecules of 5-ALA. Four molecules of porphobilinogen are connected together, and after porphobilinogen deaminase they form the cyclic uroporphyrinogen III. Decarboxylation of uroporphyrinogen leads to coproporphyrinogen III which then generates within mitochondria protoporphyrin IX after coproporphyrinogen oxidase (CPO) acts upon coproporphyrinogen III. This last molecule is further oxidized to form PpIX. PpIX is subsequently chelated with iron to form heme [9]. 5-ALA is also valuable for PDT because it has the possibility to be preferentially accumulated in tumour cells. This fact could be explained by a change in mitochondrial function (lack of energy), alteration in porphyrin transporters, or a perturbation in heme biosynthetic enzyme in tumour cells. Indeed individual steps of the heme cycle are delayed by an high concentration of porphobilinogen deaminase and low concentration of ferrochelatase (FC). More over the enzyme responsible for converting protoporphyrin IX (PpIX) in heme has been found in reduced levels in a variety of tumour tissues including liver, bladder, colorectal, esophageal, gastric, and rectal [9]. This deregulation causes the accumulation of various intermediate products leading to PpIX accumulation [8]. This accumulation has been also show in different skin lesions (psoriatic plaques, actinic keratosis, and basal cell carcinoma) where PpIX fluorescence was at higher levels as compared to healthy skin. [10,11]

For most clinical trials [12–15], 5-ALA was administrated at a high dose ($\approx 80\text{mg}$) as a cream on the lesion to promote PpIX accumulation [16]. After an incubation period¹, lesions were illuminated with a special wavelength (usually 635 nm) appropriate for tissue transmission and able to stimulate the PS [1,3]. Stimulated PS (PS*) follow the process presented in Fig.2 and PDT occurs.

PDT efficiency depends on several parameters such as light dosimetry, fluence rate, time between PS administration and irradiation (also called *incubation period*), exposure time, rate of oxygen, kinetic distribution of PS in tissues, PS selectivity, and concentration [3,18,19]

Table 1 : Prodrugs used for enhancing photodynamic therapy.

Type of Lesions	Prodrugs	Method	Efficacy	Ref.
Superficial BCC (sBCC) and Bowen disease (BD) on 40 human patients	MAL	Application of 5-ALA (20%) or MAL (16%) cream on lesions after surface preparation.	No significant difference was found between 5-ALA or MAL. PpIX fluorescence induced in lesions of sBCC and BD was similar and secondly, patients pain was not different with one or another prodrug.	[33]
Extensive AK on 16 human patients	MAL	Visible layer of 5-ALA cream (20%) or MAL cream (20%) was applied on lesions	Both 5-ALA-PDT and MALPDT result in a significant reduction in scalp AK. 5-ALAPDT was more painful than MAL-PDT in the treatment of extensive scalp AK.	[34]
Healthy excised human stratum corneum	ButylALA	Ex vivo Franz cell. ButylALA (10%) in 4 different cream formulation, 5-ALA (10%) in 5 different cream formulation.	Permeation coefficient butylALA in SC was higher than that of 5-ALA, no matter the cream formulation.	[35 , 36]
Actinic keratosis (AK) Grade I, II, III on human patients (200 lesions)	HexylALA	Daylight PDT study compared 0.2% hexyl-ALA and 16% MAL in the treatment of AK.	Hexyl-ALA was less effective for grade II-III AKs compared with MAL. For grade I AKs the clearance was equal.	[37]

PDT optimization is difficult because all parameters are correlated. Production and distribution of PS remains the most important parameters since there after photodynamic therapy (PDT) result on it. Photosensitizer formation and epidermal penetration depth represent basic predictors of drug efficacy in dermatological PDT [20]. Concentration, distribution, aggregation, and accumulation of PS modifies the photobleaching² kinetic [20].

Despite progress concerning few parameters, PDT is currently limited by poor tissue penetration of the porphyrin precursor 5-aminolevulinic acid. This review will focus on one of the three important parameters needed for PDT. Lack of 5-ALA penetration through the skin

is due to molecule hydrophilicity that prevents it to cross the *stratum corneum* (SC) [21]. The aim of this review is to present the chemical and physical methods to increase drug penetration before PDT. To increase penetration of 5-ALA in to deep epidermal regions different adaptations to standard PDT treatment have been considered. Proposals have been made concerning chemical modification of the drug precursor that could lead to a better penetration. Others strategies have been developed to control the heme cycle by adding special agents in order to promote PpIX accumulation. Modification of the skin surface has been researched by physical pretreatment such as creating holes, changing the temperature, or a voltage shocks have been proposed [22].

2. Chemical Methods

Although 5-ALA has been widely use for PDT treatment, the preparation is quite difficult because of its properties. 5-ALA-stability with in solution or topical formulation is low because of 5-ALA dimerization to 2,5-pyrazinedipropionic acid (PY) which makes its formulation quite challenging.

2.1 Extrinsic Parameters

To overcome 5-ALA-degradation, pH range, concentration and appropriate temperature should be well controlled [23]. According to [24], stability of 5-ALA was maintained at low pH. Solutions of 5-ALA in buffer (0.060 molL^{-1}) were investigated at 50°C . At $\text{pH} = 2.53$ no degradation was observed for 37 days where as at $\text{pH} = 4.81$ and $\text{pH} = 7.42$ after respectively 257 h and 3 h half of the 5-ALA compound was degraded [24]. Similarly, [25] showed a decrease of 5-ALA-degradation when pH values were lower. A solution of 5-ALA at 0.1% was investigated at 21°C . These solutions were stable for 128 days at $\text{pH} \leq 6$ where as at $\text{pH} \geq 7$, 5-ALA content declined below 90% within a few days (≈ 10 days) [25]. Nevertheless, for some applications it is sometimes necessary to work with a physiological pH ($\text{pH} \approx 7$). So it was recommended to decrease the 5-ALA concentration to increase its stability according to a kinetic equation proposed by Elfsson et al. [24]. De Blois et al. [25] had proven that a solution of 5-ALA at $\text{pH} = 5$ at 0.5% could be stable for 178 days where as solutions with initial concentrations of 2, 5 and 10% had an 5-ALA content which dropped below 90% respectively

after 150, 94, and 29 days [25]. Finally to overcome 5-ALA degradation temperature should be controlled too, because an increase of temperature enhanced 5-ALA degradation which followed Arrhenius law [25].

2.2 Intrinsic Parameters

Once a stable formulation is developed, there is still the problem of poor penetration of 5-ALA through the skin. This fact is accounted for by the physicochemical properties of 5-ALA. The drug is highly hydrophilic with an octanol : water partition coefficient at -1.5 (logP) which does not match with the lipophilic character of the skin surface.

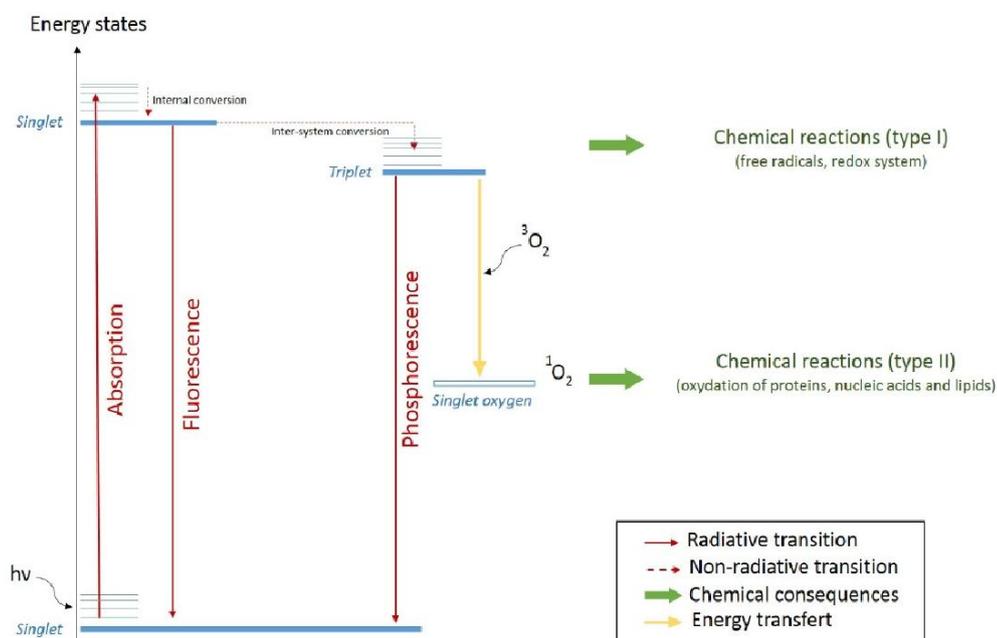


Figure 2 : Jablonski diagram representing the diferent processes leading to singlet oxygen production responsible for cell death.

Therefore, no interaction between the skin and the prodrug that could initiate 5-ALA penetration occurs. Moreover, this drug has a zwitterionic nature which means that at any pH the molecule is charged and it is wellknown that charged molecules do not cross the *stratum corneum* (SC) easily because they could interact with components from skin [26,27]. Although 5-ALA has a low molecular weight (131 gmol^{-1}) penetration through the *stratum corneum* is

limited to a maximum depth of 1 mm and is nonhomogenous [28]. To overcome these limitations different strategies have been developed such as using others prodrugs Instead of 5-ALA, or making new drug delivery system to ensure 5-ALA penetration in to the skin.

2.3. Use of Different Prodrugs

Because of 5-ALA properties and in order to enhance drug penetration through the stratum corneum, other prodrugs with longer carbon chains, more lipophilic, have been developed [29,30]. Chemical structures of these prodrugs are presented in Fig. 4 and their use in clinical trials on human patients is presented in Table 1.

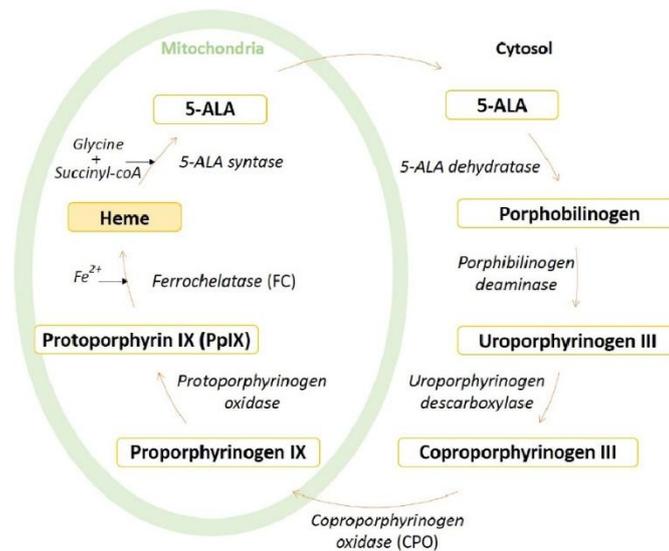
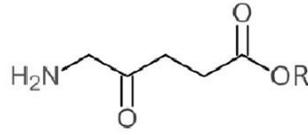


Figure 3 : Heme cycle



Carbonyl group -R	Name
-H	5-ALA
	MAL
	Ethyl-ALA
	Propyl-ALA
	Butyl-ALA
	Hexyl-ALA
	Octyl-ALA

Less lipophilic

 Carbon number
 More lipophilic

Figure4 : Chemical structure of the different prodrugs used for enhancing drug penetration and PDT outcomes.

Methyl-5-aminolevulinic acid (MAL) is currently used in clinic similarly to 5-ALA [36]. Due to its methyl group, methyl-5-aminolevulinic acid (MAL) is more lipophilic and has better selectivity for tissue. MAL-PDT could be more effective than 5-ALA-PDT in the case of deep lesions (squamous cell carcinoma (SCC)) [37]. Overall 5-ALA-PDT and MAL-PDT are both successful dermatologic therapies with high clearance rates 3 from 75% to 80% [32,36]. In order to increase the penetration of drug through the stratum corneum, ethyl-ALA, propyl-ALA, butyl-ALA have been produced. These prodrugs improved PpIX penetration after topical application to skin *in vivo* [30], but their formulation (esterification) required numerous chemical steps [33,38,39]. Similarly, hexyl-ALA and octyl-ALA have been developed and tested *in vitro* [29] and *in vivo* [35]. These two prodrugs have great penetration and could be utilized in smaller concentrations than 5-ALA or MAL [35]. Nevertheless, these prodrugs are too lipophilic, and can be accumulated in the skin which compromises their biological action [40]. So 5-ALA and MAL remain the most used drugs. To enhance their penetration through the SC new vehicles or drug delivery systems have been developed.

2.4. Skin Penetration of 5-ALA with Different Vehicles

Due to the difficulties encountered with long carbon chain prodrugs 5-ALA and MAL remain the two most used PSs for PDT on skin cancers, despite their limited penetration through the skin. Therefore, new vehicles for drug administration have been developed in order to enhance drug permeation and promote improved PpIX formation. Topical delivery is considered to be the delivery route of choice, and is the most adequate for skin cancer since it localizes the photosensitizer to the site of application and avoids skin photosensitivity everywhere in the body [36]. So different vehicles such as creams⁴, gels⁵, patch systems, lotions, liposomes, nanoparticles, powders and microemulsions have been developed to be applied on skin lesions to overcome the penetration issue [43,44]. Each formulation was developed in an environment favorizing 5-ALA stability.

2.4.1. Cream Formulations

5-ALA has been clinically used as a cream formulation. Lipophilic cream can enhance drug affinity with skin and increase the penetration of 5-ALA. The most employed formulation consists of 5-ALA at 20% wv in acid solution with 48% ethanol, water, laureth-4, isopropylalcohol, and polyethylene glycol [36,44]. This composition is FDA-approved and commercialized as Levulan[®] KerastickTM in the U.S. This product has been principally used in the U.S [45]. In Europe, a similar cream composed of 16% ww MAL in a solution containing different excipients such as cetostearyl alcohol, peanut oil, glycerol, methylparaben, propylparaben, and water is mainly used. This formulation is employed as frequently as the one composed of 5-ALA, and is commercialized by Galderma under the trademark Metvix[®]. Although crossing the SC is not fully overcome, Levulan[®] and Metvix[®] have been widely used and has acceptable clearance rates and cosmetic outcomes. However, side effects such as burning, stinging, itching, erythema, or even oedema have been detected during light exposure and could become more intense after few minutes of light exposure [46,47].

2.4.2. Gel Formulations

In order to avoid side effects during 5-ALA-PDT and MAL-PDT, some gel formulations containing the drug have also been developed. To enhance the physical stability of a cream for the treatment of AKs, Risaliti et al. [43] developed three galenic gel formulations (named Natrosol, Sepigel and Carbopol) each containing 10% ww of 5-ALA. In terms of viscosity, microbiological stability and storage stability, the Sepigel formulation composed of 10% of 5-ALA, 87% of water and 3% of polyacrylamide, isoparaffin and laureth-7 displayed the best results for skin cancer treatment. Comparison between this gel and aliphilic cream used in the study on 15 patients proved that Sepigel caused less unpleasant effects than a cream during coating and during irradiation. This formulation also has the advantage to be better absorbed by the skin [43]. This improved penetration was also observed in the other gel formulations. One was prepared by mixing surfactant, water, cetylstearyl-2-ethylhexanoate, and 1.5% (w/w) of 5-ALA and displayed excellent skin permeation [48]. Another gel composed of isopropyl alcohol, dimethylisobutylate, medium chain triglycerides, water, Pluronic F127, and 10% w/w of 5-ALA presented better permeation coefficient through human *stratum corneum* than German Pharmacopoeia creams [49]. On the other hand, a 5-ALA nanoemulsion gel was compared to a MAL cream on pig skin. A nanoemulsion of 5-ALA penetrated deeper skin layers and produced higher fluorescence signals (5fold) than MAL cream [50]. Since 5-ALA stability was increased due to the presence of the nanoemulsion, this formulation was used for AK treatment on 122 patients to test the efficacy and safety of this product. At the end of the study (12 weeks), lesions complete clearance rate was 81% with the nano emulsion gel, where as it was 22% with the placebo treatment. This study proved that the nanoemulsion gel was well suited for PDT of AK with high efficacy despite the low 5-ALA concentration (10%) [51]. Another study with 630 enrolled patients proved the efficacy of the 5-ALA nanoemulsion by comparing it with the MAL cream. At a long follow-up (12 months), the proportion of patients fully cleared was, respectively, 47% for the 5-ALA nanoemulsion and 36% for the MAL treatment [52]. Since these results, the 5-ALA nanoemulsion formulation has been FDA – approved and put on the market as Ameluz® in 2016. The efficacy was established on AKs, and is under clinical investigation on 186 patients suffering from superficial basal cell carcinoma (BCC) (NCT03573401). To facilitate gel use, they can also be integrated into patches.

2.4.3. Patches

Patches are small, soft and adhesive devices that contain a drug which could be delivered when applied onto the surface of the skin. Patches containing 5-ALA were developed to facilitate classical PDT that consisted of applying a cream on neoplastic lesions and keeping them under occlusion. The patch is able to adhere to the skin, allow to deliver a precise 5-ALA dose, and blocking the lesion due to a backing layer. A schematic representation is presented in Fig.5 [53].

Different compositions were developed. For instance, a solution was first prepared by mixing cetylsteryl-2-ethylhexanoate, water, surfactant, and 1.5%w/w of 5-ALA. Then it was mixed with a carrageenan hydrogel and freeze-dried to form a homogenous aerogel (film). Permeation of 5-ALA was investigated by using porcine abdominal skin in Franz diffusion cells. After 48 h, diffusion was improved with the patch than with a hydroxyethylcellulose gel containing 1.5% w/w of 5-ALA. Nevertheless, a degradation of 5-ALA was detected in the patch after 14 days which make its use impossible after a long term storage [48].

A second example is a square, self-adhesive patch (PD P 506 A[®]) containing 8 mg of 5-ALA for 4 cm² tested on 25 patients during a clinical phase I study. The aim of the study was to evaluate PpIX fluorescence corresponding to patch application duration and the 5-ALA pharmacokinetics. Maximum fluorescence was observed after 4h of patch application, and was more intense in AK lesions than in normal skin. Pharmacokinetics study revealed a small increase of 5-ALA in the blood, but normal values returned rapidly after patch removal. These experiments had proven that use of patches for skin cancer was safe and tolerable [54]. This same patch was clinically used on 218 patients suffering from AKs. Efficacy rate on a lesion was superior to 82% when 3 to 8 patches were applied per patient. The use of this patch was favorable because it was easy to handle, apply (no skin preparation was necessary), and induced very good efficacy and cosmetic results compared to placebo-PDT or cryosurgery [55]. Because of these satisfactory clinical outcomes, this patch was then commercialized as Alacare[®]. Alacare[®]-PDT has the potential to become the first-line treatment in the therapy of AK. However, in case of thick lesions and without a skin surface pretreatment, this therapy might be less efficient. Also the patch area is quite small (32 cm²) which limits its use for large skin lesions [56].

2.4.4. Drug Delivery Systems

Micro or nanocarriers are commonly used as drug delivery systems since they can penetrate through small capillaries and are taken up by cells, which allows efficient drug accumulation in certain locations. Their use in topical formulation for 5-ALA delivery is very attractive since they might easily cross the SC. Some of these different vehicles (liposomes, nanoparticles, micelles, nanofibers, patches) are presented in **Fig.6**.

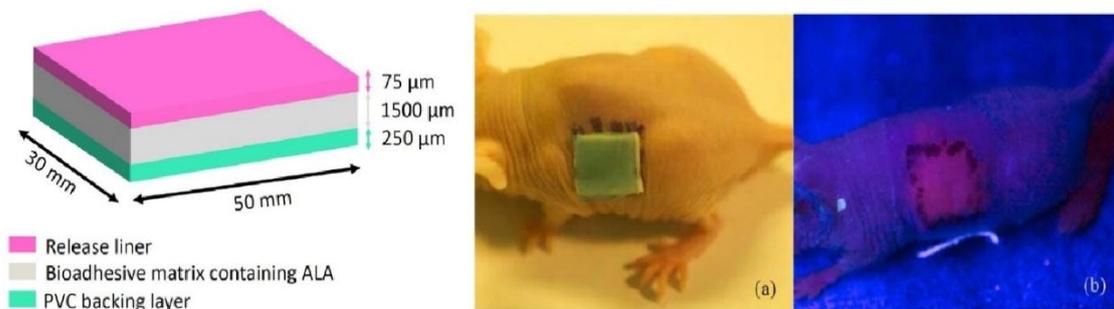


Fig.5. Patch formulation and its *in vivo* use on nude mouse. PpIX fluorescence was observed 4h after patch removal [53]

2.4.4.1. Liposomes.

Liposomes are spherical vesicles with a diameter from 70 nm to few micrometers. Liposomes are formed by one or more lipid concentric bimolecular layers often made of phospholipids and present an *aqueous core* (**Fig. 6a**) [41]. Liposomes have been used *in vitro* and *ex vivo* in topical photodynamic therapy [60]. This drug delivery system has the advantage to encapsulate lipophilic molecules within the lipid bilayers or hydrophilic drugs such as 5-ALA within the *aqueous score*. [61] reviewed the use of different types of liposomes for encapsulating 5-ALA for topical PDT [61]. In most presented studies, Liposomes promote higher 5-ALA penetration in to skin than free 5-ALA. Phospholipids present on the particule surface could interact with the SC and act as penetration enhancers. This accumulation of 5-ALA in the skin enhanced PpIX formation which should lead to better

PDT efficacy and enable topical PDT of deep skin lesions [61,62]. Liposomes are extensively used as controlled and targeted drug delivery systems which allow to use a low concentration of 5-ALA [63]. Compared to free 5-ALA, liposomes can reduce drug absorption into systemic circulation, limit its cytotoxicity, the risk of photosensitivity after the treatment, and could possibly avoid pain and side effects (itching, burning) induced by the use of an high 5-ALA concentration [61]. To our knowledge, except for a study on tumour mouse models [60] and a study on patients suffering from acne [64,65], no in vivo studies on patients suffering from skin cancer have been conducted.

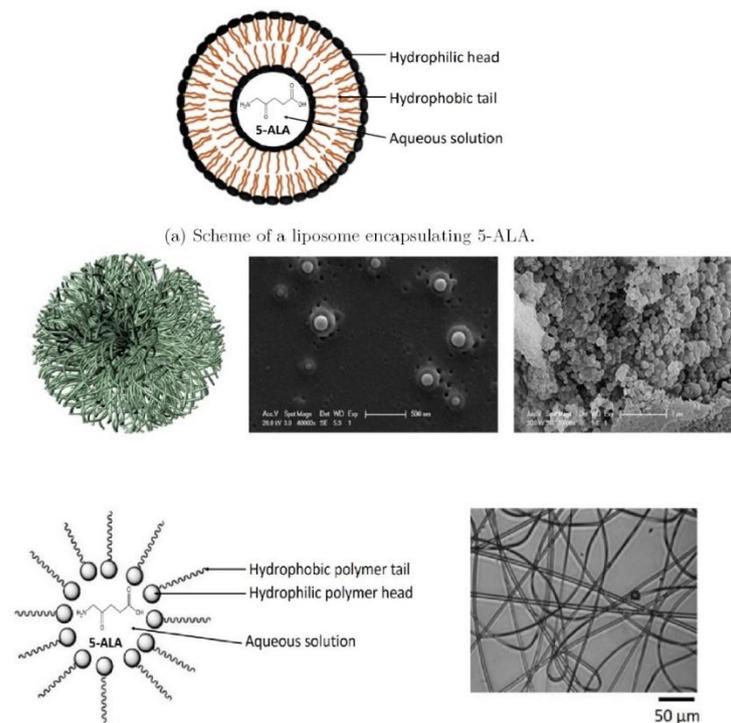


Fig.6. Representation of the different vehicles used for topical delivery of 5-ALA. (a) Scheme of a liposome encapsulating 5-ALA. (b) Scheme of polymeric PLGA nanoparticles loaded with 5-ALA [57] and scanning electron microscope (SEM) observations of nanoparticles [58]. (c) Scheme of a 5-ALA loaded micelle. (d) SEM observations of electrospun nanofibers from 25% w/w PMVEMA-ES solutions in ethanol combined with 5-ALA (16.7%w/w) [59].

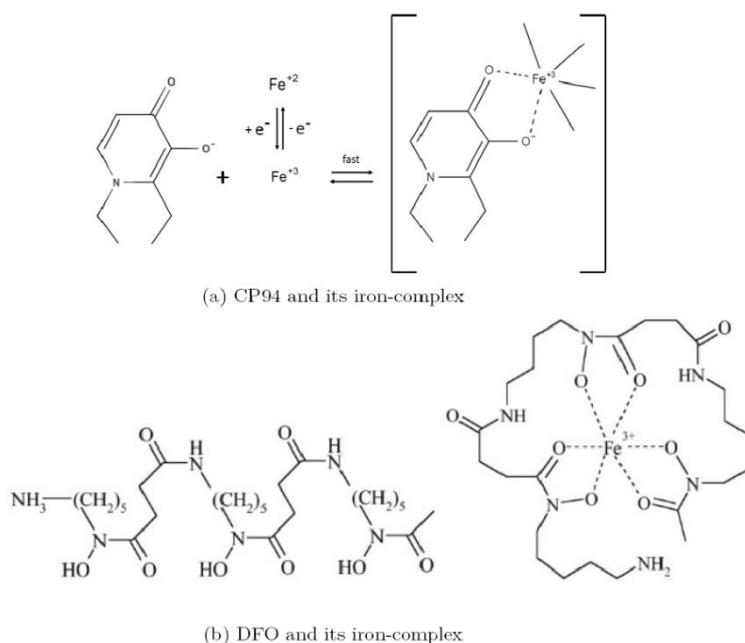


Fig.7. Representation of iron-complex involved to promote PpIX accumulation.
 (a) CP94 and its iron-complex. (b) DFO and its iron-complex.

2.4.4.2. Polymer Nanoparticles.

Nanoparticles are colloidal systems with a diameter between 10 nm and 1000 nm. They are generally composed of biodegradable polymers or lipids and are able to entrap drugs by adsorption or encapsulation [41]. A new 5-ALA delivery approach using poly (lactic- co-glycolic acid) (PLGA) nanoparticles has been investigated (Fig.6b). Due to their size the drug have the ability to go through the skin pores and penetrate more easily the epidermis [62]. An *in vitro* experiment on human epidermoid squamous cells carcinoma revealed that 5-ALA loaded in nanoparticles induced more PpIX fluorescence and a higher photocytotoxicity than free 5-ALA at the same concentration (0.1 mM) [58]. PLGA nanoparticles provided a promising 5-ALA delivery strategy for topical PDT on SCC but *in vivo* investigations should be pursued.

2.4.4.3. Polymeric Micelles.

Polymeric micelles are composed of a core and a shell formed by the self-assembly of block copolymers. They have a spherical shape with a particle diameter from 10 nm to 100 nm. Their composition and size enhance their permeation through the skin. Polymeric micelles are starting to be used to encapsulate 5-ALA (Fig. 6c). Tong et al. [66] chose to study the interaction between α -cyclodextrin and a poly (ethylene glycol) (PEG) conjugated to form a

pseudopolyrotaxane for entrapping 5-ALA. The produced micelles were stable in a physiological environment, were able to release 5-ALA, and enhanced cellular uptake. Therefore, a PpIX accumulation was observed and a higher photodynamic cytotoxicity than free 5-ALA [66]. Such prodrug micelles may provide new opportunities for the development of 5-ALA-based photodynamic cancer therapy and further investigation on *in vivo* systems might be interesting.

2.4.4.4. Nanofibers.

Nanofibers can be generated from different polymers, and hence have different physical properties. Nanofibers possess extremely high specific surface area that can be use full for drug delivery due to their nanoscale diameter. The method most commonly used to produced nanofibers is electrospinning. This technique consists of applying a high electric force to a polymer solution to form charged threads. Electrospun nanofibers with 5-ALA (Fig. 6d) were first synthetized with poly (methyl vinyl ether-alt-maleic acid) (PMVEMA) and poly (methyl vinyl ether-alt-maleic ethyl monoester) (PMVEMAES), and 5-ALA was incorporated within these nanofibers. The prodrug was stable in this conformation. Franz cells were used to evaluate the kinetic release of 5-ALA, and showed that nanofibers loaded with 5-ALA were able to deliver the drug and served as a substrate for the production of PpIX [59].

A combination of 5-ALA and micro or nanocarriers might provide an interesting strategy to enhance clinical out comes with PDT, but in some drug delivery systems *in vivo* investigations are necessary to conclude their safety and therapeutic efficacy [61].

2.5. Agents that Control Heme Cycle and Promote PpIX Accumulation

Some prodrugs pass through the stratum corneum, but sometimes the amount is not sufficient to produce enough PpIX for PDT treatment. Other strategies have been developed to control the heme cycle in order to ensure enough PpIX production. To make sure that PpIX will accumulate in cells it is important that PpIX does not convert in to heme (Fig.3). An iron-chelating agent can be used to inhibit the conversion of PpIX to heme by removing Fe^{2+} from the cellular system which will result in a temporary increased accumulation of PpIX (3-5 fold) [67] (Fig.3).

The adding of iron chelators like desferrioxamine (DFO) or hydroxypyridinone (HPO) can act on heme cycle and increase amount on PpIX in cells. DFO and HPO are ligands able to bind with iron as central metal atom to form a coordination complex. The binding involves donation of electrons. DFO and CP94 complex are presented in **Fig.7 [68]**. Other strategies for iron chelating are reported in **Table2 [69]**.

The hydroxypyridinone iron chelator CP94 (1,2-diethyl-3-hydroxypyridin-4-one hydrochloride) has been used *in vivo* with human cells [67] or on patients [73]. Comparison studies between CP94+MAL-PDT and MAL-PDT highlighted that use of an iron chelator was useful to obtain PpIX accumulation and caused a better clearance rate as presented in **Table2 [71]**. DFO was also used, but Curnow et al. Have recently reported that CP94 was a more efficient enhancer of PpIX-induced PDT *in vitro* than DFO [1].

It is also important to note that iron has other functions than converting PpIX in heme. Iron is necessary for a number of important cellular functions such as DNA synthesis. Decreasing iron concentration in the cell due to iron-chelators results in antiproliferative effects via inhibition of DNA synthesis. 1,2-diethyl-3-hydroxypyridin-4-one (CP94) can particularly induce these effects due to faster access into intracellular iron pools [71]. Even if CP94 has a proven good efficacy in treatment of skin cancer with PDT, to our knowledge there has been no more clinical trial on patients since 2008.

Table 2 : Iron chelators used for enhancing photodynamic therapy.

Type of Lesions	Prodrugs	Method	Efficacy	Ref.
Human epidermoid squamous carcinoma cells	CP94	Comparison of MAL PDT (500 μmolL^{-1}) and MAL + CP94 PDT (500 μmolL^{-1} + 150 μmolL^{-1})	Addition of CP94 increased PpIX accumulation. Necrosis increased with MAL+CP94.	[66]
Nodular BCC (nBCC) on 36 human patients	CP94	Topical formulation of 5ALA (20%) was mixed with an increasing concentration of CP94 (from 0% to 40%) before PDT treatment	40% CP94 resulted in significantly greater clearance rates in nBCC than with 5-ALA (20%) alone. There was also no apparent increase in pain noticed during irradiation.	[69]
nBCC on 12 human patients	CP94	Topical formulation of MAL (Metvix® 16%) was mixed with an increasing concentration of CP94 (from 0% to 40%) before PDT treatment.	Combining CP94 with MAL is a safe and effective way of increasing the efficacy of MAL PDT.	[70]

49 patients with superficial or nBCC, BD or AK.	DFO	Topical formulation of 20% 5-ALA and 3% DFO	DFO increased the selectivity of the 5-ALA-induced porphyrin synthesis in neoplastic. Clearance rates were interesting: sBCC (88%), nBCC (32%), AK (81%) and BD (30%).	[71]
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2.6. Chemical Enhancers for PpIX Accumulation

Instead of influencing the heme cycle, some chemical products such as methotrexate (MTX), 5-fluorouracil (5-FU), and tazarotene (TZ) act directly on the cell process as will be presented in the further paragraphs. They are called differentiation-promoting agents⁶. They can improve PDT due to their selectivity by preferentially enhancing PpIX accumulation in a variety of preneoplastic and neoplastic epithelial cancer cells while causing very little damage to non-cancerous cells. [74].

MTX is an antifolate which inhibits cell proliferation and triggers cellular differentiation. Presence of MTX activates the first apoptosis signal (FAS) which is a receptor on the surface of the cells that leads to programmed cell death [75]. MTX can be used as an oral pretreatment before ALA-PDT as presented in Table 3, and it results in the improvement of cell death [76]. Moreover, pretreatment with MTX is selective since the presence of MTX stimulates mitochondrial CPO activity. CPO is particularly present in malignant cells, and its stimulation leads to a hyperproduction of PpIX (**Fig.3**) [77]. Even if a low dose of methotrexate renders PDT more effective, MTX is a toxic chemical compound with a lot of side effects and contraindications [78]. PDT has to be easy, patient-friendly treatment and use of this chemical enhancer is not fully in agreement with this.

Vitamin D is a differentiation-promoting hormone with six different forms (including cholecalciferol which is present in food, and calcitriol which plays a role in PDT. The goal of vitamin D in the body is to maintain a normal range of calcium and phosphorus in fluids. Calcitriol is the most potent and active hormonal form of vitamin D that maintains calcium homeostasis by its actions in bones, kidneys, intestines, and parathyroid glands. Calcitriol and its receptor are also present in a wide variety of cells and tissues, including cancer cells. Its goal is to regulate cellular proliferation and differentiation. Concentration of calcitriol in the body is very important since at a concentration smaller than nanomolar, it promotes cell

proliferation whereas at higher concentrations it inhibits proliferation. Also, whatever the concentration, cell differentiation still occurs [83]. Anand et al. [84] showed that coproporphyrinogen oxidase and ferrochelatase were increased and decreased respectively after vitamin D pretreatment (oral administration) which promoted formation of PpIX and inhibited its transformation into heme (Fig. 3). Calcitriol pretreatment caused a marked increase in PpIX accumulation in the tumour site. Yet, pretreatment with vitamin D induced a small increase (2-fold) in tumour necrotic factor (TNF) and after PDT-irradiation TNF levels were highly elevated (70 fold above baseline). High TNF levels promoted an activation of the extrinsic apoptotic pathway, and enhanced 5-ALA-PDT-mediated cell death. In addition, markers of cellular differentiation and proliferation were increased (4–6 folds) in tumours compared to normal skin. Tumours cell proliferation might be more susceptible to PDT in the presence of vitamin D [85], but it could be a problem if some cells are not destroyed by the 5-ALA-PDT-treatment and continue to proliferate after [83]. Nevertheless, some studies have been conducted on cell cultures [86,87] and principally on small animals. They are presented in Table 3. Despite some disadvantages such as hyper proliferation or hypercalcemia, some clinical trials are under investigation in order to establish whether oral Vitamin D/5-ALA-PDT is an interested innovative combination therapy for 50 patients with BCC skin cancer. (NCT03483441orNCT03467789)

Table 3 : Combination of a differentiation-promoting agent with PDT.

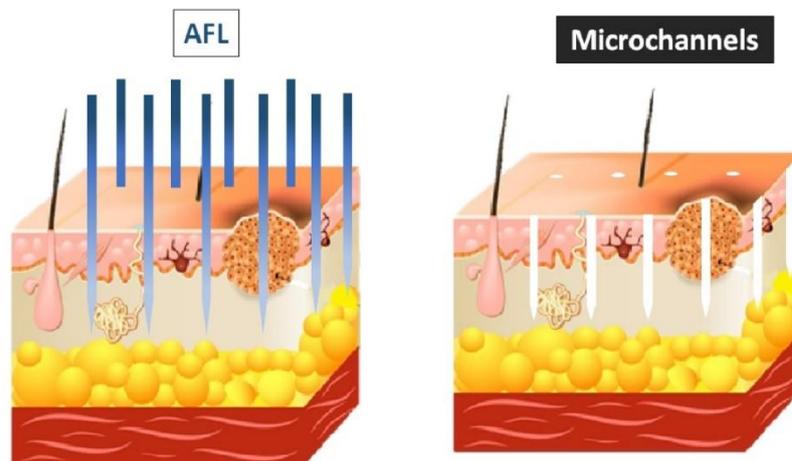
Type of Lesions	Prodrugs	Method	Efficacy	Ref.
Skin carcinoma cells in vitro and in vivo. Cultured human cells, normal keratinocytes, and in vivo skin tumor models.	MTX	Préincubation with methotrexate for 72h then incubation with ALA for 4h.	Intracellular PpIX was increased (2 to 4 fold) in carcinoma cells versus normal keratinocytes. Photodynamic killing was enhanced by the combined therapy compared with PDT alone. In vivo, methotrexate preconditioning enhanced PpIX accumulation in cultures of keratinocytes, skin tumors in mice and human squamous cell tumors implanted in mice	[75]
Basal cell carcinoma (BCC) and squamous cell carcinoma on mice.	Natural vitamin D (cholecalciferol)	Mice were fed for 10 days with vitamin D or vitamin was applied topically on mice.	Oral Vitamin D improved PpIX accumulation in SCC. Topical application of active vitamin D enhanced PpIX accumulation in BCC in mice.	[78]

Human SCC cell line implanted in a subcutaneous tumor model in nude mice.	Vitamin D (calcitriol)	Systemic calcitriol (0.5µg/kg to 10µg/kg daily) was given for 3 days.	Systemically administered vitamin D enhanced PpIX levels in subcutaneous tumors in mice by decreasing FC levels and increasing cell differentiation.	[79]
Actinic keratosis.	5-FU	Patients applied 5-FU cream once daily for 6 days, prior to the PDT treatment visit (Application of MAL for 3 hours then illumination).	PpIX accumulation was higher when the lesions were pretreated with 5-FU. Clinical response measured at 3 months after PDT was better after 5-FU pretreatment.	[80]
24 patients with AKs on the hands.	5-FU	Lesions were pretreated for seven days twice daily with 5-FU before daylight PDT.	Response rate was higher for the combination of 5-FU and daylight-PDT (63%) than for daylight-PDT alone (52%)	[81]

Contrary to MTX, 5-fluorouracil (5-FU) is FDA-approved and can be used topically. 5-FU is a drug developed in the 1960s and has been widely used alone especially for dermatology and skin cancer as a neoadjuvant⁷ cream [74,88]. Logically, 5-FU can be used in PDT. 5-FU is able to rapidly enter the cell and is converted to several active metabolites. One metabolite is a thymidylate synthase inhibitor that interrupts the synthesis of pyrimidine thymidine, which is a nucleoside required for DNA replication [89]. Lack of pyrimidine thymidine causes growth arrest and apoptosis. In addition, upregulation of coproporphyrinogen oxidase and downregulation of ferrochelatase were observed and lead to PpIX accumulation [88]. Combination of 5-FU with PDT has been extensively studied and is an effective method to improve clearance rate as presented with some examples in **Table 3**. 5-FU seems to be a good candidate and its combination with PDT improve treatment outcome. Contrary to MTX and calcitriol, 5-FU does not present huge disadvantages except that sometimes severe inflammatory and erosive reactions can appear [90].

Retinoid prodrugs are also used prior to PDT. Tazarotene (TZ) is part of the acetylenic class of retinoids and is known to be converted by deesterification in cognate carboxylic acid that is its active form. Then it is able to disrupt gene expression, modify cell differentiation, and modulate cell proliferation or hyperplasia [91]. TZ on human BCC cells has shown an

activation of caspases⁸ due to reactive oxygen species. In parallel, a decrease of anti-apoptosis proteins and cell cycle arrest have also been detected. These observations contributed to apoptosis of human BCC cells [92]. Use of TZ alone on 109 patients suffering of sBCC or nBCC was effective with regression of tumour size (upto50%) which was possibly due to antiproliferative and proapptotic actions [93]. So TZ has a metabolic effect and a dermabrasion effect too. These previous clinical studies show favorable clinical outcomes and the production of reactive oxygen species lead researchers to believe that TZ administration prior to PDT might enhance lesion clearance rate. [94] conducted a clinical study on 10 patients with at least 4 AK lesions. The pretreatment consisted of applying TZ gel 0.1% twice daily on lesions 1week before 5-ALA-PDT. Eight weeks after 5-ALA-PDT treatment, more subjects (8 vs. 3) achieved higher clearance rate (lesion count reduction $\geq 50\%$) in the TZ pretreatment than in the control group. These results suggest a slight tendency toward enhancement of 5-ALA-PDT outcome when lesions were pretreated with TZ [94]. Oral retinoid delivery prior to PDT has been investigated as well. A daily dose of 0.5mg/kg of etretinate during 2 months led to thinner and less keratotic tumours that allowed a better 5-ALA penetration and a potential increase of PpIX [95]. As the other alternatives proposed for pretreatments, tazarotene has also side-effects and may increase erythema, dry skin, burning, craking of skin, or itchiness [91].



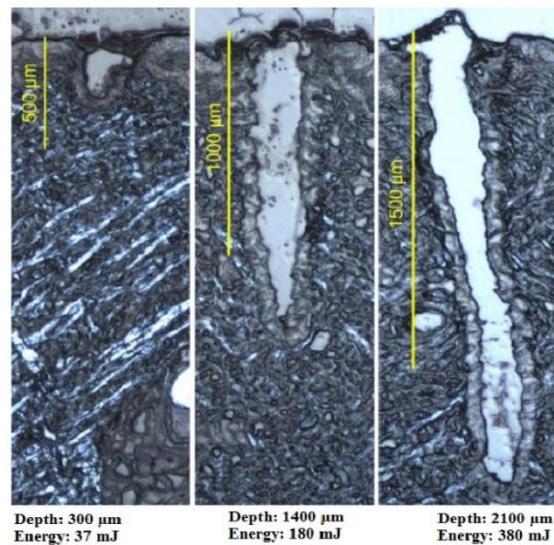


Fig.8. Principle and results of AFL on skin tissues. (a) Skin was pulverized and microchannels were created where AFL was applied. (b) The depth of microchannels created with ablative fractional laser (AFL) depends on laser energy. Vertical section of frozen biopsies [97].

3. Physical Methods

Physical pretreatments of the skin help the penetration of the photosensitizing agent and then promote better outcomes for treatment of skin cancers with PDT. Several techniques will be presented and each affects the skin barrier differently. Holes can be created with a laser or microneedles, or SC removal can take place with curettage or microdermabrasion. Temperature modification or application of a voltage gradient on the skin can also increase skin permeability and enhance drug penetration [22].

3.1. Skin Puncture

3.1.1. Ablative Fractional Laser (AFL)

This technique affects the skin barrier as presented in **Fig.8 a**. The laser beam strikes the skin surface and creates vertical, ablated channels by vaporizing a small column of tissue. [96]. The depth of the microchannels created can be controlled by changing the laser energy. When the

energy was between 32 mJ and 380 mJ, microchannels depth was between 300 μm and 2100 μm (Fig.8 b) [97].

Lasers with wavelength in the far infrared spectrum are particularly used since they are strongly absorbed by tissue water (human skin is composed of 70% by water) [98–100]. Their use started around 2007 [101] and three groups of AFL are currently used : CO₂-laser (10600 Nm), Er : YAG-laser (Erbium yttrium aluminium garnet, 2940 nm), and Er:YSSG-laser (Yttrium scandium gallium garnet, 2790nm).

The creation of vertical micropores facilitates the photosensitizing agent uptake and may improve PDT efficacy [102]. Moreover, modification of the skin barrier increases drug delivery and then could decrease the incubation time and gives similar treatment efficacy as compared to conventional PDT [103]. Because drug penetration is enhanced, PpIX fluorescence is more intense and is more homogeneous in deeper skin regions [103]. The fluorescence intensities could be controlled by choosing an adequate laser density [104]. The combination of skin pretreatment with AFL followed by PDT has been studied in clinical trial and some results are presented in **Table 4**.

Although AFL seems to be a reliable and effective method, it remains an ablative method and is aggressive. Therefore it presents a high risk of potential skin damage with scarring, discoloration and skin infection [101]. In addition, the device is large and costly which make its use quite restrictive and not allowed in all medical facilities.

3.1.2. Microneedles(MNs)

Needles and especially microneedles represent a unique technological approach to enhance drug permeation through the *stratum corneum*. Infact, it is not necessary to use needles with large dimensions because drugs to deliver have microsize [105]. Microneedle (MN) have been developed with lengths inferior to 900 μm which could limit pain because they do not penetrate in deep vascular and nervous regions [106]. Due to their micro size MN cannot be seen by patients and reduce needle phobia [107]. The small length improves patient compliance and safety because length is adequate enough to successfully reach the dermal layers without inducing bleeding and touching active immune cells. Design of MNs with special materials can have strong mechanical properties to enable disruption and penetration through the *stratum corneum*. More over they can exist in a few shapes : tetrahedron [108],

pyramidal, conical [109], beveled tip, or tapered cone [110] which promote different insertion pathways and permeation in the skin [111]. So hydrophilic or high molecular weight drugs can be diffused in skin tissues [112]. There are four types of MNs : solid, coated, hollow and dissolving MNs [113,114] and they can be assembled as a roller (Fig.9a) [115] or as low cost patches (Schema9b). These noninvasive transdermal patches can be used easily by anyone [111] and make skin pretreatment rapid.

Solid MNs have been commonly used for skin pretreatment before conventional PDT. They improve the cutaneous delivery of 5-ALA or MAL [106], and could reduce incubation time about 20 min [117]. Some clinical studies are presented in Table 4. 5-ALA-coated MNs have also been studied on mouse skin tumour model. An higher formation of PpIX ($\times 3.2$) and PpIX in deeper skin regions (480 μm vs 150 μm) were observed with coated MNs compared to topical application [16]. To our knowledge hollow MNs and dissolvable MNs have not been part of clinical or preclinical studies yet.

MNs promote good PDT-out come for actinic keratosis and have also good cosmetic outcome since they create small holes that stimulate collagen and elastin production [118,119]. They also up regulate the expression of genes which promote extracellular matrix production and remodeling [28,120]. Although MN have displayed promising results (Table4), their use for the treatment of basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) have not been investigated yet.

3.2. Stratum Corneum Removal

Removal of stratum corneum (SC) can be done by abrasion, curettage, debulking, or tape stripping. In each technique the objective is to disrupt or reduce thickness of the SC by removing hyperkeratosis layers. [69,125]

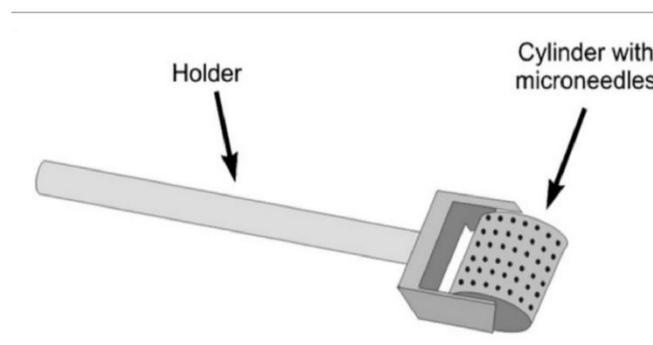
3.2.1. Curettage

It consists of using a sharp curette ring to remove hard keratotic tissue. To minimize the possibility of recurrences, the practitioner needs to remove 5 mm of the surrounding tissue with normal appearance [125]. This technique has been recommended for skin preparation since it delineates well the tumour [126], it is effective, acceptable, and quite inexpensive

[127]. This gentle removal is often used for superficial basal cell carcinoma (sBCC) treatment and consists of scraping crust and scale [128]. Curettage increased PpIX fluorescence [129] and according to [130] MAL-PDT with prior curettage was a promising method for the treatment of BCC with a clearance rate of 91% for sBCC (131 lesions treated), 93% for thin nBCC (82 lesions) and 86% for thick nBCC (86 lesions treated). Cosmetic out comes were excellent (i.e lesion area not visible) and recurrence rate after ALA-PDT with prior curettage was low which make it a good skin pretreatment method [126,130]. Nevertheless some authors report that curettage may damage surrounding healthy tissue and cause pain for patients [126,129].

3.2.2. Abrasion

This technique also called microabrasion uses a pad made of aluminium oxide crystals [127,131]. When a pressure is exerted, it will remove hyperkeratosis layers, crusts and the stratum corneum (SC) in order to improve transdermal skin delivery of 5-ALA or MAL cream [125]. This technique has proved to enhance absorption of 5-ALA. The method could avoid the use of high drug concentration, and reduce incubation times from hours to minutes. Those evolutions may enhance the efficacy of PDT [132].



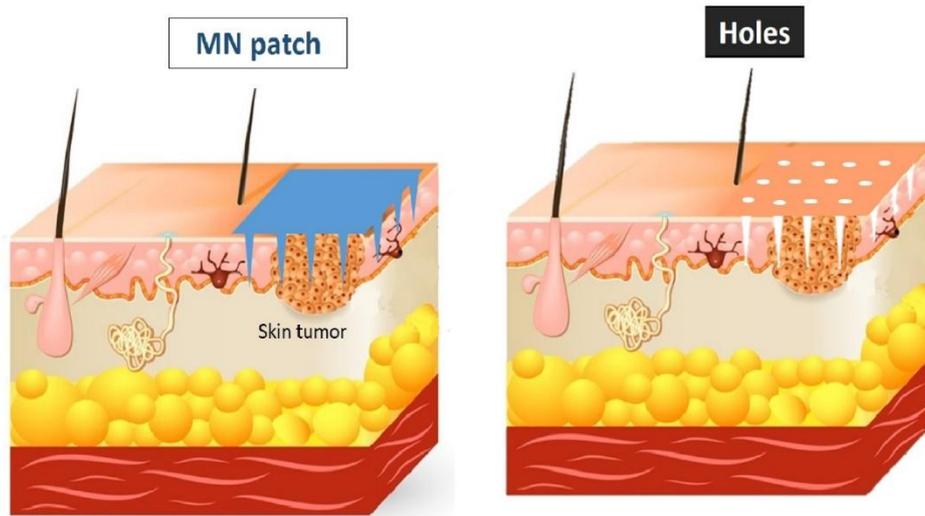


Fig.9. Microneedles systems to create holes in the skin. (a) Schematic presentation of a Dermaroller® [116]. (b) Microneedles patch is applied on skin thanks to finger pressure then is removed and creates holes in the skin.

3.2.3. Debulking

It is a surgical act used when the lesions are large. It consists of reducing as much as possible the bulk of the tumour. This technique is often used for thicker lesions such as nBCC [125]. After this partial removal it is necessary to treat the edge with PDT. Three weeks before 5-ALA-PDT, [133] had chosen to debulk nBCCs (24) on 23 patients. 92% of the nBCC showed a complete response on clinical and histopathological examination with excellent cosmetic outcomes and no serious side effects. nBCC are difficult to treat with only PDT since either photosensitizer or light can not penetrate deep enough. Debulking could be used prior to PDT to enhance clinical response [133].

3.2.4. Tape Stripping

It can be used for the treatment of AK on the lips and in the preparation of large skin areas. Tape is applied on to the skin lesions and is then pulled off sharply which can reduce uniformly SC by 30% [125,134]. This technique does not require effort, is inexpensive, safe, and minimally invasive.

3.3. Other Methods

3.3.1. Temperature

Hyperthermia consists of elevating temperature in a body region. Contrary to the previous physical techniques presented in this review, this method is known to be slightly invasive and without large side effects [135]. Historically (5000 years ago) this technique was used to treat diseases or to cauterize tumours by using a metal heated stick. With the evolution of medicine and emergence of precise surgery, radiotherapy, chemotherapy, etc. this technique was put aside [136]. Nowadays hyperthermia is experiencing a resurgence and is used as a complement to standard cancer treatments, and is particularly combined with PDT for skin cancer treatment.

Increase of a temperature to a body area can lead to cell alteration. Protein and DNA synthesis can be disrupted or inactivated. The cell membrane can be destabilized which causes a burst release of chemicals in the intracellular structures. Inhibition of cell proliferation could occur which inactivates the development of cancer cells [135,136].

Elevating temperature may also enhance PDT. First, an increase of temperature led to an improved penetration of 5-ALA in mice skin [137]. Secondly, conversion of 5-ALA to PpIX was higher when the skin was heated during 5-ALA incubation. Increase of PpIX level was detected when temperature was elevated. After 4h, the fluorescence ratio (between the mean of PpIX fluorescence and autofluorescence) was equal to 1, 2, 7 when the temperature was respectively 26°C, 28.6°C, and 40.8°C. Like many metabolic pathways in the body and according to the Arrhenius law, the conversion of ALA is modulated by temperature [137,138]. This large amount of PpIX led to an elevation of the photobleaching rate in human cells during light exposure [139]. Thirdly, 5-ALA uptake in cells was better when the skin was warmed due to a disruption in the cell membrane [140,141]. Finally, increasing temperature led to the vasodilation of skin vessels which increased the amount of oxygen in the skin. Photodynamic therapy (PDT) generates singlet oxygen to kill cancerous cells. The more oxygen, the higher chances of inducing cell death. An increase of apoptotic cell death was observed due to an increase of superoxide singlet oxygen during a thermal PDT on *in vitro* human skin fibroblasts [142].

All the above reasons cause an increase in PDT efficiency when combined with thermal treatment as long as the temperature is well controlled to prevent damage to surrounding tissues [140]. Willey et al. tried thermal-PDT on 18 patients suffering of AK on the extremities (head, fingers, toes). Warming the skin during 5-ALA incubation was well tolerated, did not create side effects, improved PDT efficacy even at 1 year follow-up with a medium clearance rate of 90% [138,143].

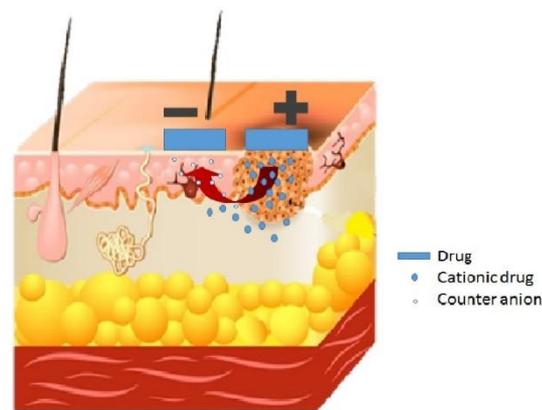


Fig.10. Transport of ionic drug through the skin thanks to iontophoresis

While highly increased temperature treatments have been effective other studies have indicated that temperatures greater than 10°C can be used effectively in PDT [144,145]. This necessity has been showed during daylight PDT treatment which consists in not using a laser with a special wavelength that could be costly or painful but rather using the sunlight which allows to excitate PpIX continuously [146]. Daylight MAL-PDT (Metvix® cream) is starting to be used for actinic keratosis and displays good clearance rate after three months, 77% when sunexposed 244 min (30 patients) [147] or 77% when sun-exposed 150 min (120 patients) [148]. Daylight MAL-PDT is a convenient and effective treatment but presents some disadvantages since its arrangement is dependant on weather and especially on temperature. This treatment could be difficult to perform in northern countries [149] contrary to southern countries [150].

3.3.2. Iontophoresis

Iontophoresis is a method to improve transdermal drug delivery and is based on the use of a voltage gradient applied to an electrolytic formulation, using a positive (anode) and a negative (cathode) electrode on the skin (**Fig.10**) [151]. This technique is known to facilitate the transport of ionic species. In addition, drugs transport should be well controlled since drugs should cross the SC, and reach the viable epidermis in the desired amounts but not enter into the circulatory system. [152]

Two mechanisms are involved for 5-ALA penetration and are dependent on pH. At physiological pH (pH \approx 7) 5-ALA is a zwitterion and its transport into skin layers is due to electroosmosis. At acidic pH=4, the acidic groups of 5-ALA are ionized and the transport is linked to electromigration because 5-ALA has greater cationic character [152,153].

Iontophoresis on skin excised from porcine ears showed that 5-ALA was delivered in higher concentrations (6 fold) than with a conventional topical application [153,154]. This technique should also reduce the incubation time before PDT treatment. Therapeutic levels of PpIX in *ex vivo human stratum corneum* were achieved faster with the use of iontophoresis than without [152]. After 10 min iontophoresis with a 2% 5-ALA solution, an amount of 0.065 $\mu\text{mol}/\text{cm}^2$ was transported across the SC whereas the same amount was obtained by passive topical diffusion after 6.5 h with a 20% 5-ALA solution [155].

Clinical trials on human patients with the comparison of iontophoresis and no pretreatment prior to 5-ALA-PDT were not extensively investigated on skin cancers. One study was conducted by [156] on 5 patients suffering from solar keratosis lesions and Bowen disease showed a PpIX production after delivery of 5-ALA by iontophoresis. One or 2 weeks after PDT treatment, histological examinations confirmed the complete disappearance of tumour cells [156]. Although iontophoresis-PDT is not usually compared to conventional PDT, this is a technique that could be combined with PDT. For example, iontophoresis-assisted AFL prior to PDT on 41 patients suffering of AK has been compared to AFL-PDT. Group A received iontophoresis-assisted AFL-PDT with a 2h incubation time. Group B and group C received AFL-PDT 2 or 3h incubation time, respectively. After 12 months, complete response rate was evaluated to be 83%, 66%, and 86% for group A, group B and group C, respectively. These results proved that the adding of iontophoresis in the clinical protocol is effective and reduces the incubation time by 1h [157].

Iontophoresis's advantages are numerous. This is a non-invasive technique that keeps the skin intact, controls and targets drug delivery. These points and the previous investigations should motivate researchers to use this technique in clinical trials more frequently. However, special attention should be taken to ensure drug accumulation in the tumour without entering the systemic circulation which can cause side effects [152].

4. Conclusion

Skin cancers are the most common malignancy of humans and particularly for caucasians with an increasing incidence rate. According to the World Health Organization, between 2 and 3 million cases of non melanoma skin cancers are diagnosed each year in the world. PDT appears to be a safe treatment, simple to set up, efficient, and favorable cosmetic outcomes which could make PDT a routine technique widely used in the world. Unfortunately, a lack of 5-ALA penetration through the skin has been observed during the treatment and reduces PDT benefits. Throughout the last decade, it has been proven with the above mentioned studies that drug chemical modification or skin pretreatment may optimize the efficacy of PDT by allowing better drug penetration and better PpIX metabolisation. Despite of the presented studies not having been conducted in large randomized clinical trials, the results up till now are promising. These new perspectives may allow better patient compliance by reducing drug incubation period and better clearance rates.

Among all these techniques some of them seem to be very promising since they present good efficiency with encouraging risk-benefit balance and they can be easily implemented. For instance, the application of TZ cream prior to PDT could be performed by the patient itself. Adjusting the temperature may also be easily performed during PDT treatment and does not present adverse side effects. The use of microneedles to create holes in the SC could be done rapidly prior to PDT, it is painless and allow sa more rapid and efficient treatment. In the future, it would be also interesting to combine different pretreatments (like pairing iontophoresis-assisted AFL prior to PDT as presented in the above results) to evaluate if there is synergy between them. It would be also possible to create heated microneedles that could deliver 5-ALA or a dermoabrasive and dissolvable patch containing an iron chelator and the prodrug. A lot of pretreatment combinations may be imagined, but to be routinely used in

hospitals clinician standardized PDT protocols are needed. These new and future protocols should be largely and randomly tested to evaluate which is the most safe, effective, easily to perform, and less costly. Bibliometric analysis and clinical trials still under investigation show an increasing interest of researchers to improve conventional PDT for skin cancers and in the next years the previous difficulties encountered may disappear and be replaced by a better, complete, and simple PDT protocol that could be standardized and used all over the world.

Acknowledgements

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References

- [1] A. Curnow, M. Aj, Enhancing protoporphyrin IX induced photodynamic therapy with a topical iron chelating agent in a normal skin model, *J. Heavy Metal Toxicity Dis.* 1 (1) (2016), <https://doi.org/10.21767/2473-6457.100002> ISSN24736457.
- [2] M.D. Daniell, J.S. Hill, A history of photodynamic therapy, *Aust. N. Z. J. Surg.* 61 (5) (1991) 340–348.

[3] P. Agostinis, K. Berg, K. A. Cengel, T. H. Foster, A. W. Girotti, S. O. Gollnick, S. M. Hahn, M. R. Hamblin, A. Juzeniene, D. Kessel, M. Korbelik, J. Moan, P. Mroz, D. Nowis, J. Piette, B. C. Wilson, J. Golab, Photodynamic therapy of cancer : an update, *CA Cancer J. Clin.* 61 (4) (2011) 250–281 ISSN00079235 [https://doi.org/ 10.3322/caac.20114](https://doi.org/10.3322/caac.20114).

[4] N. Basset-Seguin, La photothérapie dynamique en dermatologie : place actuelle et perspectives, *Annales de Dermatologie et de Vénérologie*, vol.133, Elsevier Masson, 2006, pp.421–423.

[5] C. Robertson, D. H. Evans, H. Abrahamse, Photodynamic therapy (PDT) : a short review on cellular mechanisms and cancer research applications for PDT, *J. Photochem. Photobiol. BBiol.* 96 (1) (2009) 1–8 ISSN10111344 [https://doi.org/ 10.1016/j.jphotobiol.2009.04.001](https://doi.org/10.1016/j.jphotobiol.2009.04.001).

[6] T. J. Dougherty, Photosensitizers : therapy and detection of malignant tumors, *Photochem. Photobiol.* 45 (S1) (1987) 879–889.

[7] A. P. Castano, T. N. Demidova, M. R. Hamblin, Mechanisms in photodynamic therapy : part one — photosensitizers, photochemistry and cellular localization, *Photodiagnosis Photodyn. Ther.* 1 (4) (2004) 279–293 ISSN15721000 [https://doi.org/10.1016/S1572-1000\(05\)00007-4](https://doi.org/10.1016/S1572-1000(05)00007-4).

[8] J. C. Kennedy, S. L. Marcus, R. H. Pottier, Photodynamic therapy (PDT) and photodiagnosis (PD) using endogenous photosensitization induced by 5-aminolevulinic acid (ALA) : mechanisms and clinical results, *J. Clin. Laser Med. Surg.* 14 (5) (1996) 289–304.

[9] X. Yang, P. Palasuberniam, D. Kraus, B. Chen, Aminolevulinic acid-based tumor detection and therapy : molecular mechanisms and strategies for enhancement, *Int. J. Mol. Sci.* 16 (10) (2015) 25865–25880 ISSN1422-0067 <https://doi.org/10.3390/ijms161025865>.

[10] A. L. Golub, E. G. Dickson, J. C. Kennedy, S. L. Marcus, Y. Park, R. H. Pottier, The monitoring of ALA-Induced protoporphyrin IX accumulation and clearance in patients with skin lesions by in vivo surface-detected fluorescence spectroscopy, *Lasers Med. Sci.* 14 (2) (1999) 112–122.

[11] T. Smits, C. A. Robles, P. E. van Erp, P. C. van de Kerkhof, M. -J. P. Gerritsen, Correlation between macroscopic fluorescence and protoporphyrin IX content in psoriasis and actinic keratosis following application of aminolevulinic acid, *J. Investig. Dermatol.* 125 (4) (2005) 833–839.

[12] A. Westers-Attema, B. G. Lohman, F. van den Heijkant, P. J. Nelemans, V. J. Winnepenninckx, N. W. Kelleners-Smeets, K. Mosterd, Photodynamic therapy in bowen's

disease : influence of histological features and clinical characteristics on its success, *Dermatology* 230 (1) (2015) 55–61 ISSN1018-8665, 1421-9832 <https://doi.org/10.1159/000366500>.

[13] M. Ericson, C. Sandberg, B. Stenquist, F. Gudmundson, M. Karlsson, A. -M. Ros, A. Rosen, O. Larko, A.-M. Wennberg, I. Rosdahl, Photodynamic therapy of actinic keratosis at varying fluence rates : assessment of photobleaching, pain and primary clinical outcome, *Br. J. Dermatol.* 151 (6) (2004) 1204–1212 ISSN0007-0963, 1365-2133 <https://doi.org/10.1111/j.1365-2133.2004.06211.x>.

[14] C. Sandberg, B. Stenquist, I. Rosdahl, A.-M. Ros, I. Synnerstad, M. Karlsson, F. Gudmundson, M. B. Ericson, O. Larkö, A.-M. Wennberg, Important factors for pain during photodynamic therapy for actinic keratosis, *Acta Derm. Venereol.* 86 (5) (2006) 404–408.

[15] R.-M. Szeimies, P. Calzavara-Pinton, S. Karrer, B. Ortel, M. Landthaler, Topical photodynamic therapy in dermatology, *J. Photochem. Photobiol. BBiol.* 36 (2) (1996) 213–219.

[16] A. K. Jain, C. H. Lee, H. S. Gill, 5-aminolevulinic acid coated microneedles for photodynamic therapy of skin tumors, *J. Control. Release* 239 (2016) 72–81 ISSN 01683659 <https://doi.org/10.1016/j.jconrel.2016.08.015>.

[17] A. Juzeniene, P. Juzenas, L.-W. Ma, V. Iani, J. Moan, Topical Application of 5 Aminolaevulinic Acid, Methyl 5-Aminolaevulinate and Hexyl 5-Aminolaevulinate on Normal Human Skin, *Br. J. Dermatol.* 155 (4) (2006) 791–799 ISSN0007-0963, 1365-2133 <https://doi.org/10.1111/j.1365-2133.2006.07484.x>.

[18] S. Berrahmoune, N. Fotinos, L. Bezdetsnaya, N. Lange, J. C. Guedenet, F. Guillemin, M. A. D’Hallewin, Analysis of differential PDT effect in rat bladder tumor models according to concentrations of intravesical hexyl-aminolevulinate, *Photochem. Photobiol. Sci.* 7 (9) (2008) 1018 ISSN1474-905X, 1474-9092 <https://doi.org/10.1039/b804921a>.

[19] L. Brancalion, S. W. Magennis, I. D. Samuel, E. Namdas, A. Lesar, H. Moseley, Characterization of the photoproducts of protoporphyrin IX bound to human serum albumin and immunoglobulin G, *Biophys. Chem.* 109 (3) (2004) 351–360 ISSN03014622 <https://doi.org/10.1016/j.bpc.2003.12.008>.

[20] L. Schmitz, B. Novak, A.-K. Hoeh, H. Luebbert, T. Dirschka, Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin, *Photodiagn. Photodyn. Ther.* 14 (2016) 40–46 ISSN15721000 <https://doi.org/10.1016/j.pdpdt.2015.11.004>.

- [21] R. F. Donnelly, D. I. Morrow, P. A. Mc Carron, A. D. Woolfson, A. Morrissey, P. Juzenas, A. Juzeniene, V. Iani, H. O. Mc Carthy, J. Moan, Microneedle-mediated intradermal delivery of 5-aminolevulinic acid : potential for enhanced topical photodynamic therapy, *J. Control. Release* 129 (3) (2008) 154–162 ISSN 01683659 <https://doi.org/10.1016/j.jconrel.2008.05.002>.
- [22] C. Bay, C. M. Lerche, B. Ferrick, P. A. Philipsen, K. Togsverd-Bo, M. Haedersdal, Comparison of physical pretreatment regimens to enhance protoporphyrin IX Up take in photodynamic therapy : a randomized clinical trial, *JAMA Dermatol.* 153 (4) (2017) 270 ISSN 2168-6068 <https://doi.org/10.1001/jamadermatol.2016.5268>.
- [23] A. Bunke, O. Zerbe, H. Schmid, G. Burmeister, H. P. Merkle, B. Gander, Degradation mechanism and stability of 5-aminolevulinic acid, *J. Pharm. Sci.* 89 (10) (2000) 1335–1341.
- [24] B. Elfsson, I. Wallin, S. Eksborg, K. Rudaeus, A. M. Ros, H. Ehrsson, Stability of 5 aminolevulinic acid in aqueous solution, *Eur. J. Pharm. Sci.* 7 (2) (1999) 87–91.
- [25] A. W. De Blois, R. J. E. Grouls, E. W. Ackerman, W. J. A. Wijdeven, Development of a stable solution of 5-aminolaevulinic acid for intracutaneous injection in photodynamic therapy, *Lasers Med. Sci.* 17 (3) (2002) 208–215.
- [26] M. R. Prausnitz, V. G. Bose, R. Langer, J. C. Weaver, Electroporation of mammalian skin : a mechanism to enhance transdermal drug delivery, *Proc. Natl. Acad. Sci.* 90 (22) (1993) 10504–10508.
- [27] B. W. Barry, Novel mechanisms and devices to enable successful transdermal drug delivery, *Eur. J. Pharm. Sci.* 14 (2) (2001) 101–114.
- [28] M.-C. Kearney, S. Brown, M.T. Mc Crudden, A. J. Brady, R. F. Donnelly, Potential of microneedles in enhancing delivery of photosensitising agents for photodynamic therapy, *Photodiagn. Photodyn. Ther.* 11 (4) (2014) 459–466 ISSN 15721000 <https://doi.org/10.1016/j.pdpdt.2014.09.003>.
- [29] F. S. De Rosa, A. C. Tedesco, R. F. V. Lopez, M. B. R. Pierre, N. Lange, J. M. Marchetti, J. C. G. Rotta, M. V. L. B. Bentley, In vitro skin permeation and retention of 5-aminolevulinic acid ester derivatives for photodynamic therapy, *J. Control. Release* 89 (2) (2003) 261–269.
- [30] R. Lopez, Photodynamic therapy of skin cancer : controlled drug delivery of 5-ALA and its esters, *Adv. Drug Deliv. Rev.* 56 (1) (2004) 77–94 ISSN 0169409X <https://doi.org/10.1016/j.addr.2003.09.002>.

[31] R. M. Valentine, S. H. Ibbotson, C. T. A. Brown, K. Wood, H. Moseley, A quantitative comparison of 5-aminolaevulinic acid- and methylaminolevulinate-induced fluorescence, photobleaching and pain during photodynamic therapy, *Photochem. Photobiol.* 87 (1) (2011) 242–249 ISSN00318655 <https://doi.org/10.1111/j.1751-1097.2010.00829.x>.

[32] F. Moloney, P. Collins, Randomized, double-blind, prospective study to compare topical 5-aminolaevulinic acid methyl ester with topical 5-aminolaevulinic acid photodynamic therapy for extensive scalp actinic keratosis, *Br. J. Dermatol.* 157 (1) (2007) 87–91 ISSN0007-0963,1365-2133 <https://doi.org/10.1111/j.13652133.2007.07946.x>.

[33] A. Winkler, C. Mullergoymann, The influence of topical formulations on the permeation of 5-aminolevulinic acid and its-butyl ester through excised human stratum corneum, *Eur. J. Pharm. Biopharm.* 60 (3) (2005) 427–437 ISSN 09396411 <https://doi.org/10.1016/j.ejpb.2005.01.014>.

[34] A. Winkler, C. C. Müller-Goymann, Comparative permeation studies for 5-aminolevulinic acid and its n-butyl ester through stratum corneum and artificial skin constructs, *Eur. J. Pharm. Biopharm.* 53 (3) (2002) 281–287.

[35] N. Neittaanmaki-Perttu, M. Gronroos, T. Karppinen, T. Tani, E. Snellman, Hexyl-5 aminolaevulinate 0.2% vs. methyl-5-aminolaevulinate 16% daylight photodynamic therapy for treatment of actinic keratoses : results of a randomized double-blinded Pilottrial, *Br. J. Dermatol.* 174 (2) (2016) 427–429 ISSN 00070963 <https://doi.org/10.1111/bjd.13924>.

[36] M. H. Gold, Therapeutic and a esthetic uses of photodynamic therapy part five of a five-part series : ALA–PDT and MAL–PDT what makes them different, *J. Clin. Aesthet. Dermatol.* 2 (2) (2009) 44.

[37] J. Lin, M. T. Wan, Current evidence and applications of photodynamic therapy in dermatology, *Clin. Cosmet. Investig. Dermatol.* (2014), <https://doi.org/10.2147/CCID.S35334145> ISSN1178-7015.

[38] G. F. Gola, G. M. DiVenosa, D. A. Sáenz, G. H. Calvo, G. M. Cabrera, A. G. Casas, J. A. Ramírez, Synthesis of chemically diverse esters of 5-aminolevulinic acid for photodynamic therapy via the multicomponent passerini reaction, *RSC Adv.* 6 (92) (2016) 89492–89498 ISSN2046-2069 <https://doi.org/10.1039/C6RA15832C>.

[39] W. Zhu, Y.-H. Gao, C.-H. Song, Z.-B. Lu, T. Namulinda, Y.-P. Han, Y.-J. Yan, L.X. Wang, Z.-L. Chen, Synthesis and evaluation of new 5-aminolevulinic acid derivatives as prodrugs of protoporphyrin for photodynamic therapy, *Photochem. Photobiol. Sci.* 16 (11) (2017) 1623–1630 ISSN1474-905X,1474-9092 <https://doi.org/10.1039/C7PP00203C>.

[40] J. Kloek, G. M. Beijersbergen van Henegouwen, Prodrugs of 5-aminolevulinic acid for photodynamic therapy, *Photochem. Photobiol.* 64 (6) (1996) 994–1000.

[41] M.-A. Bolzinger, S. Briancon, Y. Chevalier, P. Franois, *Formulation Des Systemes Pteux Ou Prparations Semi-Solides*, (2015).

[42] L. Buhse, R. Kolinski, B. Westenberger, A. Wokovich, J. Spencer, C. W. Chen, S. Turujman, M. Gautam-Basak, G. J. Kang, A. Kibbe, B. Heintzelman, E. Wolfgang, Topical drug classification, *Int. J. Pharm.* 295 (1–2) (2005) 101–112 ISSN 03785173 <https://doi.org/10.1016/j.ijpharm.2005.01.032>.

[43] L. Risaliti, V. Piazzini, M. G. DiMarzo, L. Brunetti, R. Cecchi, P. Lencioni, A. R. Bilia, M. C. Bergonzi, Topical formulations of delta-aminolevulinic acid for the treatment of actinic keratosis : characterization and efficacy evaluation, *Eur. J. Pharm. Sci.* 115 (2018) 345–351 ISSN 09280987 <https://doi.org/10.1016/j.ejps.2018.01.045>.

[44] R. F. Donnelly, P. A. McCarron, A. D. Woolfson, Drug delivery of aminolevulinic acid from topical formulations intended for photodynamic therapy, *Photochem. Photobiol.* (2005) 750–767.

[45] E. Jeffes, Levulan : the first approved topical photosensitizer for the treatment of actinic keratosis, *J. Dermatol. Treat.* 13 (sup1) (2002) s19–s23 ISSN 0954-6634, 1471-1753 <https://doi.org/10.1080/095466302317414663>.

[46] C. A. Morton, Methyl aminolevulinate (Metvix) photodynamic therapy-practical pearls, *J. Dermatol. Treat.* 14 (sup3) (2003) 23–26.

[47] K. Lang, K. W. Schulte, T. Ruzicka, Aminolevulinic acid (Levulan) in photodynamic Therapy of actinic keratoses, *Clin. Trials* 432 (2001) 18.

[48] C. Valenta, B. Auner, I. Loibl, Skin permeation and stability studies of 5-aminolevulinic acid in a new gel and patch preparation, *J. Control. Release* 107 (3) (2005) 495–501 ISSN 01683659 <https://doi.org/10.1016/j.jconrel.2005.07.006>.

[49] N. Grning, C. C. Mller-Goymann, Physico chemical characterisation of a novel thermogelling formulation for percutaneous penetration of 5-aminolevulinic acid, *J. Pharm. Sci.* 97 (6) (2008) 2311–2323 ISSN 00223549 <https://doi.org/10.1002/jps.21157>.

[50] T. Maisch, F. Santarelli, S. Schreml, P. Babilas, R.-M. Szeimies, Fluorescence induction of protoporphyrin IX by a new 5-aminolevulinic acid nanoemulsion used for photodynamic

therapy in a full-thickness ex vivo skin model : letter to the editor, *Exp. Dermatol.* 19 (8) (2009) e302–e305 ISSN09066705,16000625 <https://doi.org/10.1111/j.1600-0625.2009.01001.x>.

[51] R.-M. Szeimies, P. Radny, M. Sebastian, F. Borrosch, T. Dirschka, G. Krähn Senftleben, K. Reich, G. Pabst, D. Voss, M. Foguet, R. Gahlmann, H. Lübbert, U. Reinhold, Photodynamic therapy with BF-200 ALA for the treatment of actinic keratosis : results of a prospective, randomized, double-blind, placebo-controlled phase III study : PDT with BF-200 ALA for actinic keratosis, *Br. J. Dermatol.* 163 (2) (2010)386–394 ISSN00070963,13652133 <https://doi.org/10.1111/j.13652133.2010.09873.x>.

[52] T. Dirschka, P. Radny, R. Dominicus, H. Mensing, H. Brüning, L. Jenne, L. Karl, M. Sebastian, C. Oster-Schmidt, W. Klövekorn, Long-Term (6 and 12 months) follow-up of two prospective, randomized, controlled phase III trials of photodynamic therapy with BF-200 ALA and methyl aminolaevulinate for the treatment of actinic keratosis, *Br. J. Dermatol.* 168 (4) (2013) 825–836.

[53] P. A. Mc Carron, R. F. Donnelly, A. Zawislak, A. D. Woolfson, Design and evaluation of a water-soluble bio adhesive patch formulation for cutaneous delivery of 5 aminolevulinic acid to superficial neoplastic lesions, *Eur. J. Pharm. Sci.* 27 (2–3) (2006) 268–279 ISSN09280987 <https://doi.org/10.1016/j.ejps.2005.10.009>.

[54] J.-D. Fauteck, G. Ackermann, M. Birkel, M. Breuer, A. C. E. Moor, A. Ebeling, C. Ortland, Fluorescence characteristics and pharmacokinetic properties of a novel self-adhesive 5-ALA patch for photodynamic therapy of actinic keratoses, *Arch. Dermatol. Res.* 300 (2) (2008)53–60 ISSN0340-3696,1432-069X <https://doi.org/10.1007/s00403-007-0787-0>.

[55] A. Hauschild, E. Stockfleth, G. Popp, F. Borrosch, H. Brüning, R. Dominicus, H. Mensing, U. Reinhold, K. Reich, A. Moor, M. Stocker, C. Ortland, M. Brunnert, R.-M. Szeimies, Optimization of photodynamic therapy with a novel self-adhesive 5-aminolaevulinic acid patch : results of two randomized controlled phase III studies, *Br. J. Dermatol.* 160 (5) (2009) 1066 – 1074 ISSN00070963,13652133 <https://doi.org/10.1111/j.1365-2133.2009.09040.x>.

[56] F. Kuonen, O. Gaide, Nouvelle Lumière Sur La Thérapie Photodynamique Cutanée, *Rev. Méd. Suisse* 10 (2014) 754–759.

[57] M. Sechi, V. Sanna, N. Pala, Targeted therapy using nanotechnology : focus on cancer, *Int. J. Nano medicine* 467 (2014), <https://doi.org/10.2147/IJN.S36654> ISSN1178-2013.

[58] L. Shi, X. Wang, F. Zhao, H. Luan, Q. Tu, Z. Huang, H. Wang, H. Wang, In vitro evaluation of 5-aminolevulinic acid (ALA) loaded PLGA nano particles, *Int. J. Nano medicine* 2669 (2013), <https://doi.org/10.2147/IJN.S45821ISSN11782013>.

[59] A. Mira, C. R. Mateo, R. Mallavia, A. Falco, Poly (methyl vinyl ether-ALT-maleic acid) and ethyl monoester as building polymers for drug-loadable electrospun nanofibers, *Sci. Rep.* 7 (1) (2017), <https://doi.org/10.1038/s41598-017-17542-4> ISSN2045-2322.

[60] M.-W. Lin, Y.-B. Huang, C.-L. Chen, P.-C. Wu, C.-Y. Chou, P.-C. Wu, S.-Y. Hung, A formulation study of 5-aminolevulinic acid encapsulated in DPPC liposomes in melanoma treatment, *Int. J. Med. Sci.* 13 (7) (2016) 483–489 ISSN1449-1907 <https://doi.org/10.7150/ijms.15411>.

[61] N. Dragicevic-Curic, A. Fahr, Liposomes in topical photodynamic therapy, *Expert Opin. Drug Deliv.* 9 (8) (2012) 1015–1032 ISSN1742-5247,1744-7593 <https://doi.org/10.1517/17425247.2012.697894>.

[62] M. Schneider, F. Stracke, S. Hansen, U. F. Schaefer, Nanoparticles and their interactions with the dermal barrier, *Dermato-Endocrinology* 1 (4) (2009) 197–206.

[63] A. J. Plaunt, K. M. Harmatys, K. A. Hendrie, A. J. Musso, B. D. Smith, Chemically triggered release of 5-aminolevulinic acid from liposomes, *RSC Adv.* 4 (101) (2014) 57983–57990 ISSN2046-2069 <https://doi.org/10.1039/C4RA10340H>.

[64] J. An, J.-E. Kim, D.-H. Lee, B.Y. Kim, 0.5% liposome-encapsulated 5-aminolevulinic acid (ALA) photodynamic therapy for acne treatment, *J. Cosmet. Laser Ther.* 13 (1) (2011) 28–32, <https://doi.org/10.3109/14764172.2011.552613>.

[65] J. deLeeuw, N. van der Beek, P. Bjerring, H. Martino Neumann, Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid 0.5% liposomal spray and intense pulsed light in combination with topical keratolytic agents, *J. Eur. Acad. Dermatol. Venereol.* 24 (4) (2010) 460–469 ISSN09269959,14683083 <https://doi.org/10.1111/j.1468-3083.2009.03447.x>.

[66] H. Tong, Y. Wang, H. Li, Q. Jin, J. Ji, Dual pH-responsive 5-aminolevulinic acid pseudo polyrotaxane prodrug micelles for enhanced photodynamic therapy, *Chem. Commun.* 52 (20) (2016) 3966–3969 ISSN1359-7345,1364-548X <https://doi.org/10.1039/C6CC00450D>.

[67] Y. Dogra, D. C. Ferguson, N. J. Dodd, G. R. Smerdon, A. Curnow, P. G. Winyard, The hydroxypyridinone iron chelator CP94 increases methyl-aminolevulinate-based photodynamic cell killing by increasing the generation of reactive oxygen species, *Redox Biol.* 9 (2016) 90–99 ISSN22132317 <https://doi.org/10.1016/j.redox.2016.07.002>.

[68] D. S. Kalinowski, The evolution of iron chelators for the treatment of iron over load disease and cancer, *Pharmacol. Rev.* 57 (4) (2005) 547–583ISSN0031-6997 <https://doi.org/10.1124/pr.57.4.2>.

[69] M. Gerritsen, T. Smits, M. Kleinpenning, P. van de Ker khof, P. van Erp, Pretreatment to enhance protoporphyrin IX accumulation in photodynamic therapy, *Dermatology* 218 (3) (2009) 193–202ISSN1018-8665,1421-9832 <https://doi.org/10.1159/000183753>.

[70] S. Campbell, C. Morton, R. Alyahya, S. Horton, A. Pye, A. Curnow, Clinical investigation of the novel iron-chelating agent, CP94, to enhance topical photodynamic therapy of nodular basal cell carcinoma, *Br. J. Dermatol.* 159 (2) (2008) 387–393ISSN00070963,13652133 <https://doi.org/10.1111/j.1365-2133.2008.08668.x>.

[71] A. Pye, S. Campbell, A. Curnow, Enhancement of methyl-aminolevulinate photodynamic therapy by iron chelation with CP94 : an in vitro investigation and clinical dose-escalating safety study for the treatment of nodular basal cell carcinoma, *J. Cancer Res. Clin. Oncol.* 134 (8) (2008) 841–849ISSN0171-5216,1432-1335 <https://doi.org/10.1007/s00432-008-0358-6>.

[72] S. Fijan, H. Honigsmann, B. Ortel, Photodynamic therapy of epithelial skin tumours using delta-aminolaevulinic acid and desferrioxamine, *Br. J. Dermatol.* 133 (2) (1995) 282–288.

[73] C. Morton, S. Campbell, D. Gould, A. Curnow, Topical photodynamic therapy with the iron chelator, CP94, for nodular basal cell carcinoma, *J. Am. Acad. Dermatol.* 50 (3) (2004) P120.

[74] E. V. Maytin, S. Anand, C. Wilson, K. Iyer, 5-Fluorouracil as an Enhancer of Aminolevulinate-Based Photodynamic Therapy for Skin Cancer : New Use for a Venerable Agent ? 78860K (2011), <https://doi.org/10.1117/12.874188>.

[75] M. Nihal, J. Wu, G. S. Wood, Methotrexate inhibits the viability of human melanoma cell lines and enhances Fas/Fas-ligand expression, apoptosis and response to interferon-alpha : rationale for its use in combination therapy, *Arch. Biochem. Biophys.* 563 (2014) 101–107ISSN00039861 <https://doi.org/10.1016/j.abb.2014.04.019>.

[76] S. Anand, G. Honari, T. Hasan, P. Elson, E. V. Maytin, Low-dose methotrexate enhances aminolevulinate-based photodynamic therapy in skin carcinoma cells in vitro and in vivo, *Clin. Cancer Res.* 15 (10) (2009) 3333–3343ISSN1078-0432, 1557-3265 <https://doi.org/10.1158/1078-0432.CCR-08-3054>.

- [77] I. Postiglione, A. Chiaviello, G. Palumbo, Enhancing photodynamic therapy efficacy by combination therapy : dated, current and on coming strategies, *Cancers* 3 (2) (2011) 2597–2629 ISSN 2072-6694 <https://doi.org/10.3390/cancers3022597>.
- [78] S. Shen, T. O'Brien, L. M. Yap, H. M. Prince, C. J. Mc Cormack, The use of methotrexate in dermatology : are view : methotrexate in dermatology, *Australas. J. Dermatol.* 53 (1) (2012) 1–18 ISSN 00048380 <https://doi.org/10.1111/j.14400960.2011.00839.x>.
- [79] E. V. Maytin, S. Anand, K. Rollakanti, Clinical Potential for Vitamin D as a Neo adjuvant for Photodynamic Therapy of Non melanoma Skin Cancer, 93080H, (2015), <https://doi.org/10.1117/12.2077234>.
- [80] E. V. Maytin, S. Anand, N. Atanaskova, C. Wilson, Vitamin D as a potential enhancer of aminolevulinate-based photodynamic therapy for non melanoma skin cancer, *Optical Methods for Tumor Treatment and Detection : Mechanisms and Techniques in Photodynamic Therapy XIX*, 7551 International Society for Optics and Photonics, 2010755105.
- [81] E. V. Maytin, S. Anand, Combination Photodynamic Therapy Using 5-Fluorouracil and Aminolevulinate Enhances Tumor-Selective Production of Protoporphyrin IX and Improves Treatment Efficacy of Squamous Skin Cancers and Precancers, 96940^E (2016), <https://doi.org/10.1117/12.2218138>.
- [82] C. Nissen, I. Heerfordt, S. Wiegell, C. Mikkelsen, H. Wulf, Pretreatment with 5 fluorouracil cream enhances the efficacy of daylight-mediated photodynamic therapy for actinic keratosis, *Acta Derm. Venereol.* 97 (5) (2017) 617–621 ISSN 0001-5555 <https://doi.org/10.2340/00015555-2612>.
- [83] S. Anand, C. Wilson, T. Hasan, E. V. Maytin, Vitamin D 3 enhances the apoptotic response of epithelial tumors to aminolevulinate-based photodynamic therapy, *Cancer Res.* 71 (18) (2011) 6040–6050 ISSN 0008-5472, 1538-7445 <https://doi.org/10.1158/0008-5472.CAN-11-0805>.
- [84] S. Anand, K. R. Rollakanti, R. L. Horst, T. Hasan, E. V. Maytin, Combination of oral vitamin D 3 with photodynamic therapy enhances tumor cell death in a murine model of cutaneous squamous cell carcinoma, *Photochem. Photobiol.* (2014), <https://doi.org/10.1111/php.12286> ISSN 00318655.
- [85] L. Wyld, O. Smith, J. Lawry, M. W. R. Reed, N. J. Brown, Cell cycle phase influences tumour cell sensitivity to aminolaevulinic acid-induced photodynamic therapy in vitro, *Br. J. Cancer* 78 (1) (1998) 50.

- [86] N. Sato, B. W. Moore, S. Keevey, J. A. Drazba, T. Hasan, E. V. Maytin, Vitamin D enhances ALA-induced protoporphyrin IX production and photodynamic cell death in 3-D organotypic cultures of keratinocytes, *J. Investig. Dermatol.* 127 (4) (2007) 925–934.
- [87] E. Cicarma, M. Tuorkey, A. Juzeniene, L.-W. Ma, J. Moan, Calcitriol treatment improves methyl aminolaevulinate-based photodynamic therapy in human squamous cell carcinoma A431 cells, *Br. J. Dermatol.* 161 (2) (2009) 413–418 ISSN 00070963,13652133 <https://doi.org/10.1111/j.1365-2133.2009.09180.x>.
- [88] S. Anand, K. R. Rollakanti, N. Brankov, D. E. Brash, T. Hasan, E. V. Maytin, Fluorouracil enhances photodynamic therapy of squamous cell carcinoma via a P53-independent mechanism that increases protoporphyrin IX levels and tumor cell death, *Mol. Cancer Ther.* 16 (6) (2017) 1092–1101 ISSN 1535-7163,15388514,1538-8514 <https://doi.org/10.1158/1535-7163.MCT-16-0608>.
- [89] D. B. Longley, D. P. Harkin, P. G. Johnston, 5-fluorouracil : mechanisms of action and clinical strategies, *Nat. Rev. Cancer* 3 (5) (2003) 330–338 ISSN 1474175X, 14741768 <https://doi.org/10.1038/nrc1074>.
- [90] C. Berking, A. Hauschild, O. Kölbl, G. Mast, R. Gutzmer, Basal Cell Carcinoma Treatments for the Commonest Skin Cancer, *Deutsches Ärzteblatt Online* ISSN 1866-0452(2014), <https://doi.org/10.3238/arztebl.2014.0389>.
- [91] J. Lanoue, G. Goldenberg, Basal cell carcinoma : a comprehensive review of existing and emerging non surgical therapies, *J. Clin. Aesthet. Dermatol.* 9 (5) (2016) 26.
- [92] C.-S. Wu, G.-S. Chen, P.-Y. Lin, I.-H. Pan, S.-T. Wang, S.H. Lin, H.-S. Yu, C.-C. Lin, Tazarotene induces apoptosis in human basal cell carcinoma via activation of caspase-8/t-Bid and the reactive oxygen species-dependent mitochondrial pathway, *DNA Cell Biol.* 33 (10) (2014) 652–666 ISSN 1044-5498,1557-7430 <https://doi.org/10.1089/dna.2014.2366>.
- [93] L. Bianchi, A. Orlandi, E. Campione, C. Angeloni, A. Costanzo, L. Spagnoli, S. Chimenti, Topical treatment of basal cell carcinoma with tazarotene : a clinicopathological study on a large series of cases, *Br. J. Dermatol.* 151 (1) (2004) 148–156 ISSN 0007-0963,1365-2133 <https://doi.org/10.1111/j.1365-2133.2004.06044.x>.
- [94] B. I. Galitzer, Effect of retinoid pretreatment on outcomes of patients treated by photodynamic therapy for actinic keratosis of the hand and forearm, *J. Drugs Dermatol.* 10 (10) (2011) 1124–1132.

[95] M.-H. Lin, J.Y.-Y. Lee, C.-Y. Ou, T.-W. Wong, Sequential systemic retinoid and photodynamic therapy for multiple keratotic pigmented nodular basal cell carcinomas on the scalp, *J. Dermatol.* 36 (9) (2009) 518–521 ISSN03852407, 13468138 <https://doi.org/10.1111/j.1346-8138.2009.00692.x>.

[96] S. Prakash, S. Kumar, Fabrication of micro channels : a review, *Proc. IME B J. Eng. Manufact.* 229 (8) (2015) 1273–1288 ISSN0954-4054,2041-2975 <https://doi.org/10.1177/0954405414535581>.

[97] C. S. Haak, W. A. Farinelli, J. Tam, A. G. Doukas, R. R. Anderson, M. Haedersdal, Fractional laser-assisted delivery of methylaminolevulinate : impact of laser channel depth and incubation time, *Lasers Surg. Med.* 44 (10) (2012) 787–795 ISSN01968092 <https://doi.org/10.1002/lsm.22102>.

[98] M. Haedersdal, F. H. Sakamoto, W. A. Farinelli, A. G. Doukas, J. Tam, R. R. Anderson, Pretreatment with ablative fractional laser changes kinetics and biodistribution of topical 5-aminolevulinic acid (ALA) and methylaminolevulinate (MAL) : PRETREATMENT WITH AFXL CHANGES OF ALA AND MAL, *Lasers Surg. Med.* 46 (6) (2014) 462–469 ISSN01968092 <https://doi.org/10.1002/lsm.22259>.

[99] T. Omi, K. Numano, The role of the CO₂ laser and fractional CO₂ laser in dermatology, *Laser Ther.* 23 (1) (2014) 49–60.

[100] E. H. Taudorf, C. S. Haak, A. M. Erlendsson, P. A. Philipsen, R. R. Anderson, U. Paasch, M. Haedersdal, Fractional ablative erbium YAG laser : histological characterization of relationships between laser settings and micropore dimensions : FRACTIONAL ABLATIVE ERBIUM YAG LASER, *Lasers Surg. Med.* 46 (4) (2014) 281–289 ISSN01968092 <https://doi.org/10.1002/lsm.22228>.

[101] J. Preissig, K. Hamilton, R. Markus, Current laser resurfacing technologies : a review that delves beneath the surface, *Semin. Plast. Surg.* 26 (03) (2012) 109–116 ISSN1535-2188,1536-0067 <https://doi.org/10.1055/s-0032-1329413>.

[102] K. Togsverd-Bo, C. Haak, D. Thaysen-Petersen, H. Wulf, R. Anderson, M. Haedersdal, Intensified photodynamic therapy of actinic keratoses with fractional CO₂ laser : a randomized clinical trial : intensified PDT of AK with fractional CO₂ laser, *Br. J. Dermatol.* 166 (6) (2012) 1262–1269 ISSN00070963 <https://doi.org/10.1111/j.1365-2133.2012.10893.x>.

[103] H. S. Song, S.-E. Jung, Y.H. Jang, H.Y. Kang, E.-S. Lee, Y.C. Kim, Fractional carbon dioxide laser-assisted photodynamic therapy for patients with actinic keratosis, *Photodermatol.*

[104] C. Haak, K. Christiansen, A. Erlendsson, E. Taudorf, D. Thaysen-Petersen, H. Wulf, M. Haedersdal, Ablative fractional laser enhances MAL-induced PpIX accumulation : impact of laser channel density, incubation time and drug concentration, *J. Photochem. Photobiol. BBiol.* 159 (2016) 42–48ISSN10111344 <https://doi.org/10.1016/j.jphotobiol.2016.03.021>.

[105] D. V. Mc Allister, P. M. Wang, S. P. Davis, J.-H. Park, P. J. Canatella, M. G. Allen, M. R. Prausnitz, Micro fabricated needles for transdermal delivery of macromolecules and nanoparticles : fabrication methods and transport studies, *Proc. Natl. Acad. Sci.* 100 (24) (2003) 13755–13760.

[106] P. Mikolajewska, R. F. Donnelly, M. J. Garland, D. I. J. Morrow, T. R. R. Singh, V. Iani, J. Moan, A. Juzeniene, Microneedle pre-treatment of human skin improves 5 aminolevulinic acid (ALA) –and 5-aminolevulinic acid methylester (MAL)-induced ppix production for topical photodynamic therapy without increase in pain or erythema, *Pharm. Res.* 27 (10) (2010) 2213–2220ISSN0724-8741,1573-904X <https://doi.org/10.1007/s11095-010-0227-2>.

[107] H. S. Gill, D. D. Denson, B. A. Burris, M. R. Prausnitz, Effect of microneedle design on pain in human volunteers, *Clin. J. Pain* 24 (7) (2008) 585–594ISSN0749-8047 <https://doi.org/10.1097/AJP.0b013e31816778f9>.

[108] T. Miyano, Y. Tobinaga, T. Kanno, Y. Matsuzaki, H. Takeda, M. Wakui, K. Hanada, Sugar micro needles as transdermic drug delivery system, *Biomed. Microdevices* 7 (3) (2005) 185–188.

[109] J. W. Lee, J.-H. Park, M. R. Prausnitz, Dissolving micro needles for transdermal drug delivery, *Biomaterials* 29 (13) (2008) 2113–2124ISSN01429612 <https://doi.org/10.1016/j.biomaterials.2007.12.048>.

[110] J.-H. Park, M. G. Allen, M. R. Prausnitz, Polymer micro needles for controlled-release drug delivery, *Pharm. Res.* 23 (5) (2006) 1008–1019ISSN0724-8741,1573904X <https://doi.org/10.1007/s11095-006-0028-9>.

[111] A. L. Teo, C. Shearwood, K. C. Ng, J. Lu, S. Moochhala, Transdermal micro needles for drug delivery applications, *Mater. Sci. Eng. B* 132 (1–2) (2006) 151–154ISSN 09215107 <https://doi.org/10.1016/j.mseb.2006.02.008>.

[112] M. R. Prausnitz, R. Langer, Transdermal drug delivery, *Nat. Biotechnol.* 26 (11) (2008) 1261–1268ISSN1087-0156 <https://doi.org/10.1038/nbt.1504>.

[113] Y.-C. Kim, J.-H. Park, M. R. Prausnitz, Micro needles for drug and vaccine delivery, *Adv. Drug Deliv. Rev.* 64 (14) (2012) 1547–1568 ISSN0169409X <https://doi.org/10.1016/j.addr.2012.04.005>.

[114] M. R. Prausnitz, Micro needles for transdermal drug delivery, *Adv. Drug Deliv. Rev.* 56 (5) (2004) 581–587 ISSN0169409X <https://doi.org/10.1016/j.addr.2003.10.023>.

[115] T. A. Petukhova, L. A. Hassoun, N. Foolad, M. Barath, R. K. Sivamani, Effect of expedited micro needle-assisted photodynamic therapy for field treatment of actinic keratoses : a randomized clinical trial, *JAMA Dermatol.* 153 (7) (2017) 637 ISSN 2168-6068 <https://doi.org/10.1001/jamadermatol.2017.0849>.

[116] M. Badran, J. Kuntsche, A. Fahr, Skin penetration enhancement by a micro needle device (dermaroller) in vitro : dependency on needle size and applied formulation, *Eur. J. Pharm. Sci.* 36 (4-5) (2009) 511–523 ISSN09280987 <https://doi.org/10.1016/j.ejps.2008.12.008>.

[117] H. Lev-Tov, L. Larsen, R. Zackria, H. Chahal, D. Eisen, R. Sivamani, Micro needle assisted incubation during aminolaevulinic acid photodynamic therapy of actinic keratoses : a randomized controlled evaluator-blind trial, *Br. J. Dermatol.* 176 (2) (2017) 543–545 ISSN00070963 <https://doi.org/10.1111/bjd.15116>.

[118] M. T. Clementoni, M. B-Roscher, G. S. Munavalli, Photodynamic photorejuvenation of the face with a combination of micro needling, red light, and broadband pulsed light, *Lasers Surg. Med.* 42 (2) (2010) 150–159 ISSN01968092,10969101 <https://doi.org/10.1002/lsm.20905>.

[119] A. G. Harris, C. Naidoo, D. F. Murrell, Skin needling as a treatment for acnes carring : an up-to-date review of the literature, *Inte. J. Womens Dermatol.* 1 (2) (2015) 77–81 ISSN23526475 <https://doi.org/10.1016/j.ijwd.2015.03.004>.

[120] S. P. Sullivan, D. G. Koutsonanos, M. del Pilar Martin, J. W. Lee, V. Zarnitsyn, S. O. Choi, N. Murthy, R. W. Compans, I. Skountzou, M. R. Prausnitz, Dissolving polymer micro needle patches for influenza vaccination, *Nat. Med.* 16 (8) (2010) 915–920 ISSN1078-8956,1546-170X <https://doi.org/10.1038/nm.2182>.

[121] M. Alexiades, Randomized, controlled trial of fractional carbon dioxide laser resurfacing followed by ultra short incubation aminolevulinic acid blue light photodynamic therapy for

actinic keratosis, *Dermatol. Surg.* 43 (8) (2017) 1053–1064 ISSN1076-0512
<https://doi.org/10.1097/DSS.0000000000001117>.

[122] C. Haak, K. Togsverd-Bo, D. Thaysen-Petersen, H. Wulf, U. Paasch, R. Anderson, M. Haedersdal, Fractional laser-mediated photodynamic therapy of high-risk basal cell carcinomas—a randomized clinical trial, *Br. J. Dermatol.* 172 (1) (2015) 215–222 ISSN00070963
<https://doi.org/10.1111/bjd.13166>.

[123] S. Choi, K. Kim, K. Song, Er : YAG ablative fractional laser-primed photodynamic therapy with methylaminolevulinate as an alternative treatment option for patients with thin nodular basal cell carcinoma : 12-month follow-up results of a randomized, prospective, comparative trial, *J. Eur. Acad. Dermatol. Venereol.* 30 (5) (2016) 783–788 ISSN09269959
<https://doi.org/10.1111/jdv.13453>.

[124] L. Torezan, Y. Chaves, A. Niwa, J. A. Sanches, C. Festa-Neto, R.-M. Szeimies, A pilot split-face study comparing conventional methylaminolevulinate-photodynamic therapy (PDT) with micro needling-assisted PDT on actinically damaged skin, *Dermatol. Surg.* 39 (8) (2013) 1197–1201 ISSN1076-0512
<https://doi.org/10.1111/dsu.12233>.

[125] E. Christensen, T. Warloe, S. Kroon, J. Funk, P. Helsing, A. Soler, H. Stang, Ø. Vatne, C. Mørk, Guidelines for practical use of MAL-PDT in non-melanoma skin cancer, *J. Eur. Acad. Dermatol. Venereol.* 24 (5) (2010) 505–512 ISSN09269959, 14683083
<https://doi.org/10.1111/j.1468-3083.2009.03430.x>.

[126] E. Dika, P. A. Fanti, A. Ismaili, C. Misciali, S. Vaccari, A. Barisani, A. Patrizi, Basal cell carcinoma margin delineation : is curettage useful? A surgical and histological study, *J. Dermatol. Treat.* 24 (3) (2013) 238–242 ISSN0954-6634,1471-1753
<https://doi.org/10.3109/09546634.2012.756572>.

[127] J.-A. See, S. Shumack, D. F. Murrell, D. M. Rubel, P. Fernández-Peñas, R. Salmon, D. Hewitt, P. Foley, L. Spelman, Consensus recommendations on the use of daylight photodynamic therapy with methylaminolevulinate cream for actinic keratoses in australia : daylight PDT consensus recommendations, *Australas. J. Dermatol.* 57 (3) (2016) 167–174 ISSN00048380
<https://doi.org/10.1111/ajd.12354>.

[128] M. C. Fagnoli, K. Peris, Photodynamic therapy for basal cell carcinoma, *Future Oncol.* 11 (22) (2015) 2991–2996 ISSN1479-6694,1744-8301
<https://doi.org/10.2217/fon.15.208>.

[129] C. V. Nissen, S. R. Wiegell, P. A. Philipsen, H. C. Wulf, Short-term chemical pretreatment cannot replace curettage in photodynamic therapy, *Photodermatol. Photoimmunol. Photomed.* 32 (3) (2016) 146–152 ISSN09054383
<https://doi.org/10.1111/phpp.12236>.

[130] A. M. Soler, T. Warloe, A. Berner, K. E. Giercksky, A follow-up study of recurrence and cosmesis in completely responding superficial and nodular basal cell carcinomas treated with methyl 5-aminolaevulinate-based photodynamic therapy alone and with prior curettage, *Br. J. Dermatol.* 145 (3) (2001) 467–471.

[131] J.-Y. Fang, W.-R. Lee, S.-C. Shen, Y.-P. Fang, C.-H. Hu, Enhancement of topical 5 aminolaevulinic acid delivery by erbium : YAG laser and microdermabrasion : a comparison with iontophoresis and electroporation, *Br. J. Dermatol.* 151 (1) (2004) 132–140 ISSN0007-0963,1365-2133 <https://doi.org/10.1111/j.13652133.2004.06051.x>.

[132] D. J. Karimipour, G. Karimipour, J. S. Orringer, Micro dermabrasion : an evidence based review, *Plast. Reconstr. Surg.* 125 (1) (2010) 372–377 ISSN0032-1052 <https://doi.org/10.1097/PRS.0b013e3181c2a583>.

[133] M. Thissen, C. A. Schroeter, H. A. M. Neumann, Photodynamic therapy with delta aminolaevulinic acid for nodular basal cell carcinomas using a prior debulking technique, *Br. J. Dermatol.* 142 (2) (2000) 338–339.

[134] H. Dickel, A. Goulioumis, T. Gambichler, J. Fluhr, J. Kamphowe, P. Altmeyer, O. Kuss, standardized tape stripping : a practical and reproducible protocol to uniformly reduce the stratum corneum, *Skin Pharmacol. Physiol.* 23 (5) (2010) 259–265 ISSN1660-5535,1660-5527 <https://doi.org/10.1159/000314700>.

[135] K. Takahashi, T. Hasegawa, T. Ishii, A. Suzuki, M. Nakajima, K. Uno, I. Yasuda, A. Kishi, K. Sadamoto, F. Abe, Antitumor effect of combination of hyperthermotherapy and 5-aminolevulinic Acid (ALA), *Anticancer Res.* 33 (7) (2013) 2861–2866.

[136] A. Bettaieb, P. K. Wrzal, D. A. Averill-Bates, Hyperthermia : cancer treatment and beyond, in : L. Rangel (Ed.), *Cancer Treatment—Conventional and Innovative Approaches*, In Tech, 2013, pp. 257–282, <https://doi.org/10.5772/55795> ISBN 978-953-51-1098-9.

[137] J. T. Van den Akker, K. Boot, D. I. Vernon, S. B. Brown, L. Groenendijk, G. C. van Rhoon, H. J. Sterenborg, Effect of elevating the skin temperature during topical ALA application on in vitro ALA penetration through mouse skin and in vivo PpIX production in human skin, *Photochem. Photobiol. Sci.* 3 (3) (2004) 263–267.

[138] A. Willey, R. R. Anderson, F. H. Sakamoto, Temperature-modulated photodynamic therapy for the treatment of actinic keratosis on the extremities : a pilot study, *Dermatol. Surg.* 40 (10) (2014) 1094–1102 ISSN1076-0512 <https://doi.org/10.1097/01.DSS.0000452662.69539.57>.

- [139] A. Juzeniene, P. Juzenas, I. Bronshtein, A. Vorobey, J. Moan, The influence of temperature on photodynamic cell killing in vitro with 5-aminolevulinic acid, *J. Photochem. Photobiol. B Biol.* 84 (2) (2006) 161–166 ISSN10111344 <https://doi.org/10.1016/j.jphotobiol.2006.02.009>.
- [140] J. Yang, A. C.-H. Chen, Q. Wu, S. Jiang, X. Liu, L. Xiong, Y. Xia, The influence of temperature on 5-aminolevulinic acid-based photodynamic reaction in keratinocytes in vitro, *Photodermatol. Photoimmunol. Photomed.* 26 (2) (2010) 83–88.
- [141] S. Mordon, A commentary on the role of skin temperature on the effectiveness of ALA-PDT in dermatology, *Photodiagn. Photodyn. Ther.* 11(3)(2014)416–419 ISSN15721000 <https://doi.org/10.1016/j.pdpdt.2014.05.004>.
- [142] A. Mamalis, E. Koo, G. Sckisel, D. Siegel, J. Jagdeo, Temperature-dependent impact of thermal aminolaevulinic acid photodynamic therapy on apoptosis and reactive oxygen species generation in human dermal fibroblasts, *Br. J. Dermatol.* 175 (3) (2016) 512–519 ISSN00070963 <https://doi.org/10.1111/bjd.14509>.
- [143] A. Willey, R. R. Anderson, F. H. Sakamoto, Temperature-modulated photodynamic therapy for the treatment of actinic keratosis on the extremities : a one-year follow up study, *Dermatol. Surg.* 41 (11) (2015) 1290–1295 ISSN1076-0512 <https://doi.org/10.1097/DSS.0000000000000512>.
- [144] C. Morton, H. Wulf, R. Szeimies, Y. Gilaberte, N. Basset-Seguín, E. Sotiriou, S. Piaserico, R. Hunger, S. Baharlou, A. Sidoroff, L. R. Braathen, Practical approach to the use of daylight photodynamic therapy with topical methylaminolevulinate for actinic keratosis : a European consensus, *J. Eur. Acad. Dermatol. Venereol.* 29 (9) (2015) 1718–1723 ISSN09269959 <https://doi.org/10.1111/jdv.12974>.
- [145] H. C. Wulf, Photodynamic therapy in daylight for actinic keratoses, *JAMA Dermatol.* 152 (6) (2016) 631–632.
- [146] S. R. Wiegell, M. Haedersdal, P. A. Philipsen, P. Eriksen, C. D. Enk, H. C. Wulf, 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, *British Journal of Dermatology* 158 (4) (2008) 740–746.
- [147] S. Wiegell, M. Haedersdal, P. Eriksen, H. Wulf, Photodynamic therapy of actinic keratoses with 8% and 16% methylaminolaevulinate and home-based daylight exposure : a

double blinded randomized clinical trial, *Br. J. Dermatol.* 160 (6) (2009) 1308-1314 ISSN00070963,13652133 <https://doi.org/10.1111/j.13652133.2009.09119.x>.

[148] S. Wiegell, S. Fabricius, I. Stender, B. Berne, S. Kroon, B. Andersen, C. Mørk, C. Sandberg, G. Jemec, M. Mogensen, K. Brocks, P. Philipsen, J. Heydenreich, M. Haedersdal, H. Wulf, A randomized, multicentre study of directed daylight exposure times of 1 vs. 2 h in daylight-mediated photodynamic therapy with methyl aminolaevulinate in patients with multiple thin actinic keratoses of the face and scalp : daylight-mediated PDT of thin actinic keratoses, *Br. J. Dermatol.* 164 (5) (2011) 1083–1090 ISSN00070963 <https://doi.org/10.1111/j.1365-2133.2011.10209.x>.

[149] S. Wiegell, S. Fabricius, M. Gniadecka, I. Stender, B. Berne, S. Kroon, B. Andersen, C. Mørk, C. Sandberg, K. Ibler, G. Jemec, K. Brocks, P. Philipsen, J. Heydenreich, M. Haedersdal, H. Wulf, Daylight-mediated photodynamic therapy of moderate to thick actinic keratoses of the face and scalp : a randomized multicentre study : daylight-mediated PDT of moderate to thick actinic keratoses, *Br. J. Dermatol.* 166(6)(2012)1327-1332 ISSN00070963 <https://doi.org/10.1111/j.1365-2133.2012.10833.x>.

[150] B. Grinblat, G. Galimberti, G. Pantoja, G. Sanclemente, M. Lopez, D. Alcala, L. Torezan, D. Kerob, T. Pascual, E. Chouela, Feasibility of daylight-mediated photodynamic therapy for actinic keratosis throughout the year in Central and South America : a meteorological study, *Int. J. Dermatol.* 55 (9) (2016).

[151] C. N. Lemos, J. G. de Souza, P. S. Simão, R. F. V. Lopez, Iontophoresis improved growth reduction of invasive squamous cell carcinoma in topical photodynamic therapy, *PLoS One* 11 (1) (2016) e0145922 ISSN1932-6203 <https://doi.org/10.1371/journal.pone.0145922>.

[152] T. Gratieri, Y. N. Kalia, Topical iontophoresis for targeted local drug delivery to the eye and skin, in : A. J. Domb, W. Khan (Eds.), *Focal Controlled Drug Delivery*, Springer US, Boston, MA, 2014, pp. 263–284, https://doi.org/10.1007/978-14614-9434-8_12 ISBN978-1-4614-9433-1978-1-4614-9434-8.

[153] R. F. Lopez, M. V. L. Bentley, M. B. Delgado-Charro, R. H. Guy, Iontophoretic delivery of 5-aminolevulinic acid (ALA) : effect of pH, *Pharm. Res.* 18 (3) (2001) 311–315.

[154] J. D. Byrne, J. J. Yeh, J. M. De Simone, Use of iontophoresis for the treatment of cancer, *J. Control. Release* (2018), <https://doi.org/10.1016/j.jconrel.2018.06.020> ISSN01683659.

[155] H. Boddé, P. Roemelé, W. Star, Quantification of topically delivered 5-aminolevulinic acid by iontophoresis across ex vivo human stratum corneum, *Photochem. Photobiol.* 4 (75) (2002) 418–423.

[156] T. Omi, M. Akimoto, M. Miyazaki, S. Kawana, Iontophoresis-enhanced cutaneous absorption of 5-aminolevulinic acid shortens the incubation period in photodynamic therapy, *Laser Ther.* 18 (3) (2009) 143–149.

[157] S.-H. Choi, T.-H. Kim, K.-H. Song, Efficacy of iontophoresis-assisted ablative fractional laser photodynamic therapy with short incubation time for the treatment of actinic keratosis : 12-month follow-up results of a prospective, randomised, comparative trial, *Photodiagn. Photodyn. Ther.* 18(2017)105–110 ISSN15721000 <https://doi.org/10.1016/j.pdpdt.2017.01.184>.