Chapter 9
Photomedicine

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Abstract This chapter discusses the various modalities of photomedicine, an interdisciplinary branch of medicine that involves the study and application of light with respect to health and disease. The following main concepts are covered: Photodynamic Therapy (PDT) for the treatment of cancer, PDT for bacterial infections, vascular PDT, photochemical internalisation, photochemical tissue bonding and the use of lasers in medicine.

9.1 Photomedicine: An Introduction

The notion that light can cure diseases goes back to ancient civilisations, when people worshipped the Sun and believed in the health-giving and protective properties of sunlight. It was not, however, until the late part of the nineteenth century, that the scientific discipline of 'photomedicine' was born. The official birth of photomedicine began with the discovery by the Danish physician Niels Finsen that artificial UV light from a carbon arc lamp could efficiently cure facial lesions (*lupus vulgaris*), which commonly develop on tuberculosis sufferers. Finsen consequently received the Nobel Prize in Physiology or Medicine in 1903 *'in recognition of his contribution to the treatment of diseases, especially lupus vulgaris, with concentrated light radiation, whereby he has opened a new avenue*

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for medical science' [1]. Attention to the beneficial properties of light continued after a chance discovery in 1900 by Oscar Raab, a medical student in Munich, Germany, that the combination of the drug *acridine red* and light killed microorganisms. Raab was able to establish [2] that the acridine drug alone or light alone were not able to cause any damage to *Infusoria* and thus a prototype for contemporary Photodynamic Therapy (PDT) treatment (*vide infra*) was first documented. Indeed, based on Raab's findings about photodynamic effects, his advisor Professor Tappeiner in collaboration with Jesionek performed the first PDT treatment of a patient with skin cancer, using eosin as the photosensitiser [2].

Today, photomedicine is an interdisciplinary branch of medicine that involves the study and application of light with respect to health and disease. Photomedicine is closely connected with medical practice in various fields including oncology, dermatology, ophthalmology, surgery, dentistry, optical diagnostics, cardiology and many others.

The following topics are of interest to practical photomedicine:

(1) the effect of light upon skin;
(2) the diagnostic uses of light, by luminescence;
(3) the therapeutic uses of light.

Ultraviolet (UV) radiation is the part of the electromagnetic spectrum emitted by the sun which is of major importance to human health. The higher energy UV-C radiation (100–280 nm) is absorbed efficiently by atmospheric constituents (ozone, water vapour, oxygen and carbon dioxide), however most radiation in the UV-A range (315–400 nm) and about 10 % of UV-B rays (280–315 nm) reach the surface of the Earth. Small amounts of UV radiation are essential for human health, e.g. for the production of vitamin D (see below), yet overexposure may result in acute and chronic health effects, in particular on the human skin. The most harmful consequence of the overexposure to sunlight is skin cancer, both carcinomas and malignant melanoma. We will not dwell on this aspect of photomedicine here, as the various harmful effects of light upon skin along with currently available prevention strategies are discussed in Chap. 4. Likewise, photodiagnosis is discussed in detail in Chap. 10.

In the present Chapter we will mainly focus on the beneficial effects light can make on human health and on its many therapeutic applications. In order to follow a historical timeline, we will start by discussing the only beneficial effect of UV radiation from sunlight on human health: vitamin D synthesis.

9.2 Vitamin D Synthesis

Vitamin D is essential in the body's calcium metabolism and is formed in the skin from previtamin D, which is in turn synthesised from 7-dehydrocholesterol under exposure to UV light. The deficiency of vitamin D causes rickets, which manifests itself as deformities of the skeleton, enlargements of the head, bending of the spine

and legs, which become unable to sustain body weight. This collection of symptoms was very common in developed countries in the late nineteenth century, and up to 90 % children in cities suffered from this condition. Fortunately, in the early twentieth century the importance of UV light in avoiding rickets was recognised and by 1920 artificial UV light was used to cure rickets and it is now rare [3]. (The use of light in the industrial synthesis of vitamin D is discussed in Chap. 2.)

9.3 Phototherapy of Hyperbilirubinemia

Another impressive example of the beneficial properties of UV/blue visible light is the phototherapy of jaundice. About 50 % of new born babies suffer from hyperbilirubinemia, which is caused by the inability of an infant to excrete the substance bilirubin as fast as it is being produced by the body. In an adult, bilirubin is bound by albumin in the blood stream, and is transported into the liver, where it is conjugated to glucuronic acid with the help of the enzyme uridine diphosphate glucuronyl transferase. The conjugate is then excreted into the bile. In a new born baby this process can be inefficient due to the delayed maturation of the enzyme. This leaves bilirubin to circulate in the blood stream and above a certain concentration it appears as a yellow pigmentation in the skin due to the deposition of a lipid soluble form. This pigmentation is known as *jaundice*. As bilirubin is toxic, in particular to the cells of the central nervous system, hyperbilirubinemia has to be treated promptly, to avoid irreversible brain damage in infants.

It turns out that the treatment of this condition is simple. Exposure of newly born infants to the UV light reduces jaundice and bilirubin levels in the blood. This was first reported by Cremer in *Lancet* in 1958 [4], and since this time the treatment has been used successfully to treat hyperbilirubinemia world wide.

The chemistry behind this UV light treatment of jaundice, long debated, was finally unravelled in 1979 by McDonagh *et al.* [5]. The team demonstrated that irradiation with UV light causes *cis–trans* isomerisation in bilirubin and thus renders the products of photoisomerisation water-soluble. This is all that is required to make bilirubin excretable.

9.4 PDT of Cancer

Perhaps the most widely applied technique in photomedicine, and the one responsible for most research effort, both fundamental and applied, is PDT. We will now discuss the principles, mechanism and application of PDT, as well as the techniques of photochemical tissue bonding, photochemical internalisation and PUVA therapy, which share photochemical principles with PDT. The reader can refer to the following recent reviews [6, 7] and monographs [8, 9] for more detailed information on the PDT mechanism and its applications.

PDT can be broadly defined as administration to a patient of a non-toxic drug called a photosensitiser, followed by the illumination of the lesion/diseased tissue with visible light. These actions lead to the generation of cytotoxic species resulting in cell death and destruction of the lesion. PDT is a minimally invasive procedure and has been successfully applied to the treatment of a range of cancerous diseases and, more recently, in ophthalmology to treat the wet form of AMD. In many cases the PDT treatment is a viable alternative to surgery and is characterised by an excellent cosmetic outcome with minimal scarring.

Photodynamic action relies on the fact that the photosensitising drug is non-toxic to cells and tissues in the dark, but upon activation with visible light of the appropriate wavelength in the presence of molecular oxygen, the photosensitiser generates reactive oxygen species (ROS). A schematic Jablonski diagram visualising all the processes leading to ROS production is shown in Fig. 9.1. Primarily, ROS comprise of the reactive singlet state of molecular oxygen, $O_2(^1\Delta_g)$, obtained through energy transfer from the triplet state of the photosensitiser to the ground state triplet oxygen, $O_2(^3\Sigma_g^-)$. This process is known as the Type II mechanism of PDT and is considered to be the major route of phototoxicity. Other photochemical products can originate from the triplet state of the photosensitiser through an electron transfer pathway (Type I mechanism of PDT). The Type I mechanism produces reactive species containing an unpaired electron, e.g. superoxide anion O_2^- and hydroxyl radical $OH^\bullet$. The generation and photochemical reactions of ROS are discussed in more detail in Chap. 8.

Both singlet oxygen, $O_2(^1\Delta_g)$, and radical intermediates are characterised by short lifetimes, are unstable and can react efficiently with cellular components such as proteins, lipids and DNA, thus causing irreversible damage to the affected cells. This damage leads to cell death through apoptosis or necrosis and results in eradication of unwanted tissues, e.g. cancerous lesions or unwanted vasculature (in tumour treatments or in AMD).

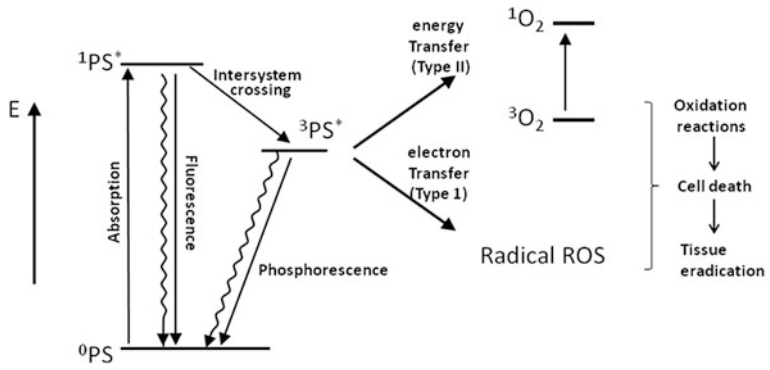

Fig. 9.1 Simplified Jablonski diagram visualising the processes leading to production of reactive oxygen species (ROS) following photosensitisation

The key components of PDT are the photosensitiser, light and molecular oxygen. Oxygen is the most likely acceptor of either energy or electrons, generating either singlet oxygen $O_2(^1\Delta_g)$ or oxygen-containing radicals. Therefore a high concentration and constant supply of oxygen in tissues under treatment is essential for efficient PDT. The immediate consequence of this requirement is that large hypoxic tumours are not suitable for PDT treatment. Another immediate implication is that the light dosimetry, as well as the fluency of light in PDT protocols need to be considered carefully to allow proper tissue re-oxygenation to achieve maximum clinical outcome.

The PDT photosensitiser has to possess a number of key properties, which include absorption in the red region of the spectrum (600–800 nm), allowing photoactivation within deeper tissues, the ability to generate efficiently singlet oxygen, selective uptake by malignant cells and minimal dark toxicity. We will consider each of these properties in turn.

Deep light penetration into tissue is extremely important, in order to achieve treatment of thicker segments of tissue as well as deeper lying lesions. The depth of light penetration is dependent upon the optical properties of the tissue and the wavelength of the light used. Water in biological tissues starts to absorb at wavelengths longer than 900 nm, whereas at wavelengths less than 650 nm absorption by endogenous chromophores such as haemoglobin and scattering by macromolecules becomes dominant. Thus, an ideal PDT photosensitiser should be activated at wavelengths where the absorbance of biological tissues is minimal, that is in the range from 700–950 nm, the so-called 'tissue therapeutic window' (Fig. 9.2).

The first clinical photosensitiser, called Photofrin® (Fig. 9.3), was approved in 1990. While it is still used in a number of PDT applications, it has a number of

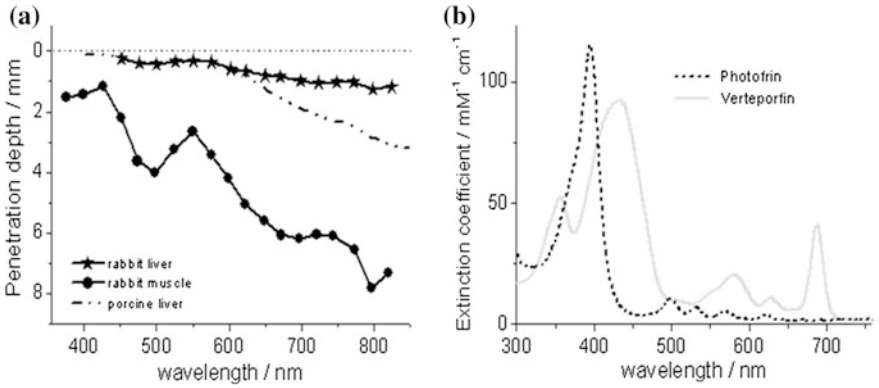

Fig. 9.2 **a** The depth of light penetration into tissue as a function of irradiation wavelength, data taken from [10, 11]. The range from 700–950 nm is called 'tissue therapeutic window' due to the minimal absorbance of biological tissues in this range. **b** The absorption spectra of the first generation photosensitiser Photofrin® as compared to the second generation photosensitiser Verteporfin

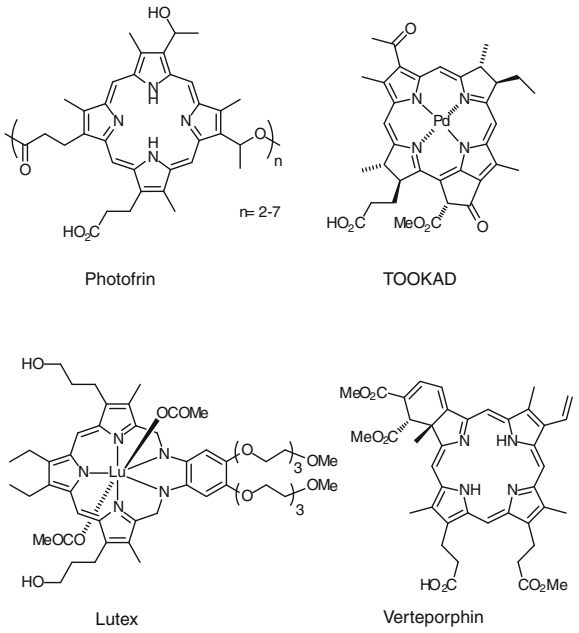

Fig. 9.3 The structures of clinical PDT photosensitisers Photofrin®, TOOKAD®, Lutex® and Visudyne®

limitations including poor absorption in the red (the lowest energy absorption maximum is at 630 nm, Fig. 9.2b, and this transition has a very low molar absorption coefficient). In recent years efforts have concentrated on so-called second generation photosensitisers with substantially improved properties over Photofrin®. The majority of these second generation photosensitisers are based on modified tetrapyrrolic macrocyles (porphyrinoids) with excellent absorption profiles at longer wavelengths. These include both naturally derived and synthetic chlorins/bacteriochlorins, benzoporphyrin derivatives and (na)phthalocyanines. Figure 9.2b shows the absorption spectrum of Photofrin® compared to that of the second generation photosensitiser Visudyne®. The benefit of using the latter drug to achieve both efficient tissue penetration and the efficient absorption of light by the drug are obvious, especially considering the low molar absorption coefficient of Photofrin® ($\varepsilon = 3200$ mol^{-1} dm^3 cm^{-1} at 630 nm *vs* $\varepsilon = 4 \times 10^4$ mol^{-1} dm^3 cm^{-1} for Visudyne® at 690 nm).

Several promising photosensitisers with activation wavelengths in the far-red or near infrared regions are currently in clinical trials. The structures of several clinical PDT photosensitisers are shown in Fig. 9.3. TOOKAD®, a palladium bacteriochlorophyll derivative with excitation at 763 nm is currently in phase II clinical trials for the treatment of prostate cancer (*via* vascular targeted PDT, *vide infra*). Lutetium texaphyrin (motexafin lutetium, Lutex®) which combines the advantages of water solubility, selective localisation, and the ability to be activated

by deeply penetrating far-red light ($\lambda_{ex} = 732$ nm) is currently in phase I/II clinical trials, while the chlorin derivative verteporfin (Visudyne®) is clinically approved for the treatment of AMD.

Singlet oxygen is considered to be the main cytotoxic intermediate in PDT, although contributions from the Type I electron transfer mechanism, generating oxygen radicals cannot be excluded. The ultimate proof for the involvement of either species in PDT *in vivo* would be the direct detection of these intermediates in the treated area, however due to their short lifetimes and high reactivity, such detection is a daunting task. It is a common practice to assess the potency of PDT photosensitisers by measuring their quantum yield of singlet oxygen production, ϕ_{Δ}. It has to be noted, however, that the photosensitisers' efficiency *in vitro* and *in vivo* does not always correlate with the simple trend derived from the photophysical properties, and *in vivo* properties depend crucially on the intracellular localisation of the drug.

The singlet oxygen quantum yields of clinical photosensitisers in solution vary from 0.1 to 1.0 and are strongly solvent dependent [12]. The latter phenomenon is often due to aggregation or self-association of hydrophobic photosensitisers, in particular in aqueous environment, and can cause complications in designing PDT photosensitisers. Association is governed by the hydrophobic/hydrophilic balance and is promoted for amphiphilic molecules with asymmetrically located polar or charged groups. In general, the fluorescence and most frequently the singlet oxygen quantum yields of aggregated species are much lower than those of monomers, thus reducing their PDT efficiency. Most crucially, the formation of such aggregates might also have an effect on subcellular localisation and pharmacokinetics of the photosensitiser. Since aggregation can be affected by binding to serum proteins or lipids, the photosensitiser behaviour is even more convoluted and sometimes poorly understood *in vitro* and *in vivo*.

An important advantage of PDT as a treatment modality is its dual selectivity towards lesions *vs* normal tissues, which is achieved through (i) preferential uptake of the photosensitiser by diseased cells and (ii) the selective application of light. We will now discuss both of these localisation strategies in turn.

Preferential uptake of the photosensitiser by diseased tissue is desirable in PDT and, if achieved, can overcome the main problem of this treatment modality, *i.e.* the enhanced skin photosensitivity in patients long after the treatment is complete. Photosensitivity occurs as a result of the low concentration of the photosensitising drug present in the skin of a patient, which causes painful effects upon exposure to sunlight. As a result, many patients have to remain in the dark until the photosensitiser has completely cleared from their body, which can take up to 1 month in the case of Photofrin®. Preferential or exclusive uptake of the PDT drug to diseased tissue will leave the patient free to be exposed to sunlight after the treatment, as no photosensitiser will be found in the skin. In principle, specific uptake can be achieved in several ways. Most commonly, tumourous tissues have poorer lymphatic drainage than healthy tissues and this leads to a somewhat higher concentration of the drug at the target lesion site (typically 4:1 tumour *vs* the healthy tissue). To improve on this rather low (passive) selectivity, more potent targeting

strategies are being currently developed, including attachment of photosensitisers to ligands showing high specificity for the tissues of interest [13, 14]. The possible ligands include monoclonal antibodies and antibody fragments, as well as cell penetrating peptides, which specifically recognise cancer markers. By displaying remarkably high specificity to a certain receptor, which is either over expressed in the tumour or is only present in specific tumour types, these photosensitiser/ligand constructs ensure targeted delivery of the light sensitive drug. These photosensitisers, which have favourable photophysical properties, but are also suitable for conjugation to biomolecules are known as third generation PDT photosensitisers.

On the subject of skin photosensitivity it is fitting to mention porphyria, which belongs to a group of inherited or acquired disorders of certain enzymes in the heme biosynthetic pathway. The main problem with porphyria is the accumulation of porphyrins, which are the heme precursors in the body. While porphyrins are non-toxic at biologically relevant concentrations, they become toxic to tissue at high concentration. Deficiency in the enzymes of the porphyrin biosynthetic pathway leads to insufficient production of heme, which plays a central role in cellular metabolism, and accumulation of porphyrins. The high concentration of porphyrins manifests itself by either neurological complications or by skin problems. The presence of porphyrins in the skin in porphyria patients is therefore akin to skin photosensitivity in PDT.

There is one case, however, where accumulation of a porphyrin from precursors can be used for therapeutic purposes, namely aminolevulinic acid (ALA) PDT [15, 16]. ALA is an endogenous precursor of protoporphyrin IX, which is converted into heme by the cellular machinery. When exogenous ALA is provided to the cell through topical application, protoporphyrin IX accumulates in high amounts and can be used successfully as a photosensitiser in PDT treatment. ALA PDT is particularly successful in treating of skin tumours and actinic keratoses.

The second mechanism for achieving selectivity in PDT lies in the fact that the photosensitiser is harmless to cells in the absence of light and thus cell death can be localised exclusively at the irradiated site, where the ROS are produced. Since ROS have a very short lifetime their diffusion, and therefore the spatial domain of activity, is restricted. For example, the lifetime of the most important ROS, singlet oxygen, in an aqueous environment is 3.5 μs [17], which is expected to shorten further in a cellular environment due to its reactions with intracellular targets. Other potent ROS of radical nature display even shorter lifetimes. This means that the diffusion distance of ROS is on the order of 100 nm or less (compared with the typical cell size of 10–100 μm) and thus the primary damage through photodynamic action only occurs at the intracellular level. An important consequence of such short diffusion distances of ROS is that the subcellular localisation (i.e. targeting vulnerable organelles within cells) as well as the selective accumulation of photosensitisers in diseased cells are important factors in determining PDT efficacy. Photosensitiser/ligand constructs, discussed above, can play an important role in targeting PDT action to a vulnerable intracellular domain, as well as to the tissue/organ of interest.

The other important development, based on the fact that the ROS diffusion distance is limited to hundreds of nanometres, utilises the fact that the irradiated volume in PDT can be as small as 1 μm^3, when femtosecond pulsed lasers are used as an excitation source. Precise localisation of excitation light should, in principle, allow the treatment of tissues without any damage to surrounding structures, which is crucial in the treatment of sensitive tissues such as those found in the eye and the brain of the patient. See Sect. 9.11 for more details of this emerging treatment modality: two-photon excited PDT (TPE PDT).

9.5 Vascular Targeted PDT and PDT of AMD

The vasculature of the tumour is an important target for PDT [18]. As tumours grow, they typically develop an extended vascular network, which enables efficient nutrient delivery to the tumour and the trafficking of metabolic waste away from the tumour. Disruptions of this network can serve as a very efficient way to 'starve' the tumour and stop its proliferation, *i.e.* instead of directly killing the malignant cells, PDT might be used efficiently to indirectly induce tumour death by damage to tumour stroma. An additional benefit stems from the PDT-induced inflammation activated by the damage to the vasculature, which is capable of activating the body's immune system, important in maintaining long-term tumour control.

This vascular targeting strategy has so far shown very promising results. The vascular targeting of the photosensitiser can be achieved by active or passive means, the former using the ligands and vectors over-expressed by the tumour, as discussed above. The ligands to endothelial cell markers (VEGF receptors, tumour endothelial markers) are commonly used for active vascular targeting. Passive delivery uses the fact that following an intravenous injection, the concentration of the photosensitiser in the blood plasma remains high for some time, typically < 60 min. Selective irradiation of affected tissue shortly after the drug injection will thus result in destruction of blood vessels carrying the drug and the desired tumour vascular shutdown. Several drugs are currently in stage I or II clinical trials for vascular PDT, primarily TOOKAD®, shown in Fig. 9.3.

Perhaps the most important application of vascular PDT is the treatment of the wet form of Age-related Macular Degeneration (wet AMD) [19, 20], the disease characterised by overproliferation of the blood vessels in the macula, behind the retina. This abnormal growth of blood vessels causes bleeding and scarring, and leads to loss of central vision in patients. Wet AMD is currently the most common source of blindness in the Caucasian popoulation over 60. The photosensitiser verteporfin (trade name Visudyne®), Fig. 9.3, is clinically approved for the treatment of wet AMD, and several other sensitisers (e.g. lutetium texaphyrin known as Lutex®) are currently in clinical trials.

It has been recognised that in many cases central and peripheral blood vessels of the tumour respond differently to vascular PDT, with peripheral vessels being

less susceptible to the treatment. This trait might have dangerous consequences and cause tumour recurrence. As often is the case with the treatment of tumours, a promising solution might be combination therapy, e.g. combining vascular PDT with antiangiogenic therapy (e.g. medication-driven approach limiting the growth of the new blood vessels), to target survival and repair pathways for endothelial cells in the vasculature. Such strategies employing PDT in combination with VEGF antibodies or with COX inhibitors have shown very promising therapeutic results.

9.6 Bacterial PDT

A variety of infective diseases can be treated using PDT [21, 22]. The use of PDT effectively overcomes a major problem associated with antibiotics, *i.e.* the development of resistance of microorganisms to many classes of antibiotics.

Photodynamic inactivation of microbial cells can be achieved upon irradiation of a suitable photosensitiser with visible light and effectively circumvents the mechanisms for resistance. The photosensitiser is typically administered topically, e.g. by spray formulation, and is expected to interact closely with the bacterial wall, to enable the most potent killing action through destruction of the bacterial membrane. As such, most photosensitisers designed for bacterial PDT are positively charged (targeting gram-positive bacteria) or contain targeting moieties such as poly-charged peptides.

Light irradiation of the photosensitiser produces either radical intermediates (through the Type I process) or singlet molecular oxygen (through the Type II process). In the case of the Type I process, the most common species formed is the superoxide radical $O_2^{-\bullet}$, which can be further converted to $OH^{\bullet}$ through the Fenton reaction (Chap. 6). Similarly to the case of PDT of cancer, it is generally accepted that singlet oxygen sensitisation is the most important mechanism of bacterial inactivation; however there is some evidence to the contrary. For example, it has been demonstrated that the Type I mechanism is predominant in bacterial PDT using sulphonated aluminium phthalocyanines as photosensitisers [23].

Since no resistance can be developed by bacteria to either singlet oxygen, or reactive radicals, photodynamic inactivation of microbial cells may provide an alternative where antibiotics are no longer working. This may be vital for patients undergoing cancer therapy, or HIV patients who demonstrate resistance to antibiotics. A very successful case for bacterial PDT has been made in dentistry for treatment of oral infections, in particular in the elderly, showing persistent oral infections. PDT treatments are being developed for a variety of infections, including tuberculosis and leishmaniasis.

9.7 Photochemical Internalisation

The utilisation of macromolecules in the therapy of cancer and other diseases is becoming increasingly important. In many cases the targets of such macromolecular therapeutics are intracellular, however many of these drugs can only enter the cell through the endocytotic pathway. In this case, degradation of macromolecules in endocytotic vesicles after uptake by endocytosis is a major problem for therapeutic application. Photochemical internalisation is an emerging technique for efficient light-directed delivery of endocytosed macromolecules and/or drugs into the cytosol, based on light-assisted rupture of the vesicles containing the drug, once inside the cell [24, 25].

Photochemical internalisation uses the fact that following the activation by light of photosensitisers, located in endocytotic vesicles, ROS assist in breaking of the vesicular membranes and thus the therapeutic macromolecules can be released from the endocytic vesicles. They are then free to reach their target of action before being degraded in lysosomes. Photochemical internalisation has been shown to stimulate intracellular delivery of a large variety of macromolecules and other drugs that do not readily penetrate the plasma membrane. Examples include DNA delivered as gene-encoding plasmids or by means of viruses, peptide nucleic acids and chemotherapeutic agents such as bleomycin and doxorubicin. The efficacy and specificity of photochemical internalisation can be further improved by combining the macromolecules with targeting moieties, such as the epidermal growth factor.

9.8 Photochemical Wound Healing

Photochemical tissue bonding (PTB) is a light-activated method for tissue repair, where photodynamic action of the drug applied to tissue surfaces, particularly on the surfaces of the wounds, induces covalent crosslinking of proteins across the surfaces [26, 27]. The thus formed *nanosutures*, a result of a photochemical process, probably mediated by singlet oxygen, create an immediate water-tight seal. PTB has distinct advantages over conventional sutures, staples and glues and is suitable for wide variety of surgical applications, including sealing corneal and skin incisions and reconnecting peripheral nerves, blood vessels and tendons. A pilot clinical study at the Wellman Center for Photomedicine (Massachusetts General Hospital) has compared the novel photochemical wound healing technique and traditional sutures for closure of skin wounds and has shown the process to be safe and to cause less scarring than sutured closure. The representative image, Fig. 9.4, shows closure of a skin wound that was made to remove a skin cancer, either using common interrupted sutures on one half of the wound (on the right) or photochemical tissue bonding (on the left). The redness on the right half is

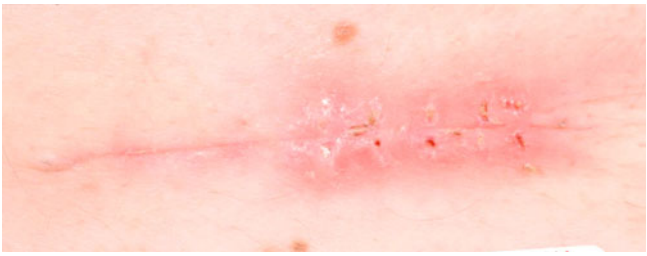

Fig. 9.4 The closure of a skin wound (an elliptical excision) that was made to remove a skin cancer. The closure of this type of wound includes two steps. First the sides of the wound were brought together with deep sutures. Then superficial sealing was done by using interrupted sutures on one half (*right*) and photochemical tissue bonding using Rose Bengal as a photosensitiser (532 nm irradiation) on the other half (*left*). The image shows the appearance of the wound 2 weeks after surgery just after the sutures were removed. The image courtesy of Prof. Irene E. Kochevar, the Wellman Center for Photomedicine (Massachusetts General Hospital)

caused by reaction to the sutures. The left half shows only a thin line where the wound sides were sealed with photochemical tissue bonding.

9.9 PUVA Therapy

PUVA is a treatment for eczema, psoriasis and vitiligo, which uses psoralen (furocoumarin molecule) as a photosensitiser, excited with UVA irradiation. The mechanism of PUVA action is similar to that of PDT, with photodynamic action utilising either a Type I or Type II mechanism. Psoralens are typically found in plants. They were known as early as ancient Egypt, but were only synthesised in a pure form in the 1970s. For PUVA therapy, psoralen can be taken orally or can be applied directly to the skin. PUVA therapy is highly effective at clearing skin problems such as psoriasis.

9.10 Use of Lasers in Surgery

The discussion of photomedicine would not be complete without several words on light sources. In PDT the selection of a light source is of utmost importance and is normally satisfied by using a laser. The ideal light source will deliver the correct wavelength showing good overlap with the absorption spectrum of the photosensitiser and sufficient power of visible light, resulting in reasonable treatment times. However there are several noteworthy applications of lasers in photomedicine which do not require the use of a photosensitiser. This section lists some of these applications.

Kidney stone removal using laser lithotripsy was invented in the 1980s and has revolutionised the treatment. Laser pulses delivered through a fibre optic were used to pulverise the stones and thus effectively remove them from the urinary tract. Laser lithotripsy allows kidney stone removal avoiding surgery.

Selective photothermolysis is the modality of skin treatment with lasers through lesion destruction by photo-thermal mechanisms. The unwanted structure or tissue is targeted using a specific laser wavelength of light, with the intention of absorbing light into the target area alone. The energy directed into the target area produces sufficient heat to damage the target while allowing the surrounding area to remain relatively untouched.

Safe removal of vascular and pigmented birthmarks can be achieved by selective photothermolysis. Selective absorption of high-power laser pulses causes selective removal of the abnormal vessels or pigment cells, without damaging other structures and without scarring. These treatments are now widely used in dermatology. Likewise, permanent laser hair removal and tattoo removal also uses the principles of selective photothermolysis.

Selective laser trabeculoplasty, a non-destructive laser treatment, has been developed on the basis of laser thermolysis, to treat glaucoma. Glaucoma is a common eye disease causing progressive, irreversible loss of vision, which is often associated with increased pressure of the fluid in the eye. Selective laser trabeculoplasty uses a 50 μm laser spot, aimed at the trabecular meshwork in the eye, to stimulate the opening of the mesh to allow more outflow of aqueous fluid. While a cure for glaucoma is not available, selective laser trabeculoplasty offers a good maintenance treatment and can be repeated as required several times without the damage to the eye.

9.11 New and Developing Treatment Modalities: Two Photon Activation

We have discussed earlier in this chapter how the efficiency of PDT can be improved by targeting photosensitisers to a specific cellular target. Precise localisation of the drug has the potential to improve on the treatment efficacy by delivering more molecules to the place where they will be most effective and to reduce the damage to healthy tissues. In this section we will discuss the emerging strategies for targeting in PDT and laser ablation based on a different irradiation regime.

9.11.1 Two-Photon PDT

The increasing availability of short-pulsed lasers is stimulating much research activity into novel ways of utilising the non-linear optical properties of materials.

Of particular interest for us here is the utilisation of multiphoton processes requiring high energy pulses. Such multiphoton processes have caused much excitement in the last decade and offered novel solutions for biological and medical applications, in particular, multiphoton imaging and two-photon excited PDT (TPE PDT) [28, 29].

As discussed in earlier chapters, the absorption of a single photon of appropriate wavelength excites the molecule from the ground state (S_0) to the first excited singlet state (S_1), from which it can undergo a series of photochemical processes. In PDT these photochemical reactions ultimately result in production of ROS and in ensuing cell death. In simultaneous two photon excitation (TPE), near-infrared light of twice the wavelength required for the S_0-S_1 transition can be used to produce the excited state of the photosensitiser. The sensitiser is then deactivated in the normal way by either luminescence (which may be utilised in imaging applications) or by photophysical or photochemical processes to produce cytotoxic species, which eradicate cells and tissue (as utilised in PDT).

A clear benefit of TPE PDT over conventional one photon PDT is that it provides the means to excite chromophores in the near-infrared spectral region (700–900 nm), enabling deeper penetration of useful light, due to minimised tissue absorption and scattering in the *tissue optical window* (Fig. 9.2a). However the major advantage of TPE PDT stems from the fact that biphotonic absorption depends on the square of the light intensity, so it is confined to the focal volume of the laser where the intensity is the highest. The latter factor yields considerably better spatial resolution for TPE imaging, compared to monophotonic confocal imaging, due to reduced out-of-focus blur. Likewise, TPE PDT has potential advantages in the treatment of sensitive tissues such as found in the wet form of age related macular degeneration (wet AMD) in the eye by reducing out-of-focus damage to adjacent healthy tissue.

Naturally, for TPE PDT and imaging applications to be successful, photosensitisers must be created which combine both desirable biological and photophysical properties with high two-photon absorption cross-sections to enable the efficient use of biphotonic excitation. In recent years several classes of efficient TPE PDT sensitisers have been reported [30–33]. For example, it has been demonstrated that TPE PDT using a conjugated porphyrin dimer and 900 nm pulsed excitation from a Ti–Sapphire laser (150 fs) can efficiently occlude a single blood vessel in an animal model, avoiding any damage to surrounding blood vessels in a 3D sample [33].

9.11.2 Nanosurgery

Similarly to TPE PDT, intense laser light can be used in two-photon laser ablation of tissues, termed *nanosurgery*. The major benefit of using femtosecond laser pulses for nanosurgery is high peak intensities that reduce the energy threshold for tissue removal (ablation) and enable laser ablation to proceed with a low-energy

source. With this method, single axons inside the nematode *Caenorhabditis elegans* (*C. elegans*) were cut successfully by using near-infrared laser pulses with relatively low pulse energies of 10–40 nJ at the specimen (200 fs pulses) [34].

In nanosurgery no specific photosensitiser is added to achieve the nanoscale tissue removal. The minimal energy used is consistent with measured optical breakdown thresholds in transparent materials. At these low energies, mechanical effects due to plasma expansion and shock waves are also significantly reduced with respect to other laser ablation techniques using nanosecond pulsed lasers, that require much higher energies. Thus nanosurgery using femtosecond pulsed lasers utilises multiphoton processes to evaporate very small volumes (1 μm^3) of a tissue with no heat accumulation and thermal damage to the environment. This for example enables the surgeon to cut axons at the nano-scale resolution with minimal damage to the micro-environment and no damage to neighbouring axons [35].

9.12 Conclusions

In this Chapter we aimed to demonstrate that photomedicine is a vibrant and actively developing branch of medicine that involves the study and practical applications of light-initiated processes, with respect to health and disease. While the early medical treatments involving light have been in use since the late nineteenth century, the new concepts and modalities continue to emerge today. With a significant amount of research conducted in theoretical and practical aspects of light-induced medicine and adjoining fields today, from basic chemistry of photosensitisation and biochemistry of cell death to optimisation of PDT procedures in clinical trials, we believe that the future of photomedicine is bright.

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References

1. The Nobel Prize in Physiology or Medicine 1903. http://nobelprize.org/nobel_prizes/medicine/laureates/1903/index.html
2. Raab C (1900) Ber die wirkung fluoreszierender stoffe auf infu-soria. Z Biol 39:524–546
3. Tappeiner H, Jesionek H (1903) Therapeutische versuche mit fluo-reszierenden stoffen. Munch Med Wschr 50:2042–2044
4. Rajakumar K (2003) Vitamin D, cod-liver oil, sunlight, and rickets: a historical perspective. Pediatrics 112:e132–e135
5. Cremer RJ, Perryman PW, Richards DH (1958) Influence of light on the hyperbilirubinaemia of infants. Lancet 1:1094

6. McDonagh AF, Lightner DA, Woolridge A (1979) Geometric isomerization of bilirubin-IX and its dimethyl ester. J Chem Soc Chem Commun 3:110
7. Dolmans DEJGJ, Fukumura D, Jain RK (2003) Photodynamic therapy for cancer. Nat Rev Cancer 3:380–387
8. Wilson BC, Patterson MS (2008) The physics, biophysics, and technology of photodynamic therapy. Phys Med Biol 53:R61–R109
9. Advances in photodynamic therapy: basic, translational and clinical (2008) Hamblin MR, Mróz P (eds), Artech House, London
10. Photodynamic therapy methods and protocols; series: methods in molecular biology (2010) In: Gomer CJ (ed), vol. 635. A product of Humana press, p 294
11. Wilson BC, Jeeves WP, Lowe DM, Adam G (1984) Light propagation in animal tissues in the wavelength range 375–825 nanometers. Progr Clin Biol Res 170:115–132
12. Ritz J-P, Roggan A, Isbert C, Müller G, Buhr HJ, Germer CT (2001) Optical properties of native and coagulated porcine liver tissue between 400 and 2400 nm. Lasers Surg Med 29:205–212
13. Wilkinson F, Helman WP, Ross AB (1995) Quantum yields for the photosensitized formation of the lowest electronically excited singlet state of molecular oxygen in solution. J Phys Chem Ref Data 24:663
14. Hudson H, Boyle RW (2004) Strategies for selective delivery of photodynamic sensitisers to biological targets. J Porphyrins Phthalocyanines 8:954–975
15. Sharmon WM, van Lier JE, Allen CM (2004) Targeted photodynamic therapy via receptor mediated delivery systems. Adv Drug Deliv Rev 56:53–76
16. Hongcharu W, Taylor CR, Chang Y et al (2000) Topical ALA-photodynamic therapy for the treatment of acne vulgaris. J Investigative Dermatol 115:183–192
17. Peng Q, Warloe T, Berg K et al (1997) 5-Aminolevulinic acid-based photodynamic therapy—clinical research and future challenges. Cancer 79:2282–2308
18. Egorov SY, Kamalov VF, Koroteev NI et al (1989) The lifetime of singlet oxygen. Chem Phys Lett 163:421–424
19. Chen B, Pogue BW, Hoopes PJ, Hasan T (2006) Vascular and cellular targeting for photodynamic therapy. Critical Rev Eukariotic Gene Express 16:279–305
20. Arnold J, Kilmartin D, Olson J et al (2001) Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: Two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization—verteporfin in photodynamic therapy report 2. Am J Ophthalmol 131:541–560
21. Arnold J, Kilmartin D, Olson J et al (2001) Photodynamic therapy of subfoveal choroidal neovascularization in pathologic myopia with verteporfin—1-year results of a randomized clinical trial—VIP report no. 1. Ophthalmology 108:841–852
22. Hamblin MR, Hasan T (2004) Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci 3:436–450
23. Jori G, Fabris C, Soncin M et al (2006) Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications. Lasers Surg Med 38:468–481
24. Phillips D (1997) Chemical mechanisms in photodynamic therapy with phthalocyanines. Prog React Kinetics 22:175–300
25. Berg K, Selbo PK, Prasmickaite L et al (1999) Photochemical internalization: A novel technology for delivery of macromolecules into cytosol. Cancer Res 59:1180–1183
26. Hogset A, Prasmickaite L, Selbo PK et al (2004) Photochemical internalisation in drug and gene delivery. Adv Drug Delivery Rev 56:95–115
27. Kamegaya Y, Farinelli WA, Echague AVV et al (2005) Evaluation of photochemical tissue bonding for closure of skin incisions and excisions. Lasers Surg Med 37:264–270
28. Tsao S, Yao M, Henry FP et al (2010) A phase I/II trial of photoactivated tissue bonding ("nanosuturing") for excisional wound closure. J Investig Dermatol 130:S42–S42
29. Bhawalkar JD, Kumar ND, Zhao CF, Prasad PN (1997) Two-photon photodynamic therapy. J Clin Laser Med Surg 15:201

30. Fisher WG, Partridge WP, Dees C, Wachter EA (1997) Simultaneous two-photon activation of type-I photodynamic therapy agents. Photochem Photobiol 66:141–155
31. Dy JT, Ogawa K, Satake A, Ishizumi A, Kobuke Y (2007) Water-soluble self-assembled butadiyne-bridged bisporphyrin: a potential two-photon-absorbing photosensitizer for photodynamic therapy. Chem Eur J 13:3491–3500
32. Balaz M, Collins HA, Dahlstedt E, Anderson HL (2009) Synthesis of hydrophilic conjugated porphyrin dimers for one-photon and two-photon photodynamic therapy at NIR wavelengths. Org Biomol Chem 7:874–888
33. Arnbjerg J, Jimenez-Banzo A, Paterson MJ et al (2007) Two-photon ab-sorption in tetraphenylporphycenes: are porphycenes better candidates than porphyrins for providing optimal optical properties for two-photon photodynamic therapy? J Am Chem Soc 129:5188–5199
34. Collins HA, Khurana M, Moriyama EH et al (2008) Blood-vessel closure using photosensitizers engineered for two-photon excitation. Nat Photonics 7:420–424
35. Yanik MF, Cinar H, Cinar HN et al (2004) Functional regeneration after laser axotomy. Nature 432:882