Photodynamic Therapy in Cancer

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An Overview

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Abstract

Photodynamic therapy (PDT) is recently gaining importance as an alternative to conventional clinical modalities like chemotherapy and radiation therapy protocols for cancer due to its efficacy in targeting cancer cells, enhanced cytotoxicity, and improved delivery. PDT is the therapeutic approach that deals with the generation of reactive oxygen species (ROS) by the use of light, a photosensitizer

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S. Chakraborti (ed.), *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects*,
https://doi.org/10.1007/978-981-16-5422-0_232

(PS), and oxygen. The efficiency and targeted delivery of the PS can be augmented by entwining PDT with nanotechnology. Conjugation of organic or inorganic nanomaterials enhances the solubilizing property of PS that aids in its accumulation at the target site. Encapsulation is the major strategy employed for targeted delivery of PS. PS molecules may be encapsulated with nanocarriers like liposomes, polymeric micelles, and polymeric nanoparticles. The mechanism of PDT in addressing the killing of cancer cells with particular reference to the advantages of nanotechnology-based PS has been discussed.

Keywords

Photodynamic therapy · Photosensitizers · Nanoparticles · Cancer

Introduction

Understanding the molecular basis of diseases has led to better management of dreaded maladies like cancer. The severity of cancer has been managed to a greater extent with the commonly available strategies such as radiation therapy, surgical removal, and chemotherapy. Even though these traditional methods helped to cure different types of cancers to a certain level, none are without their limitations. One of the important challenges is that of targeted drug delivery. Along with this, severe side effects associated with the treatment protocols have promoted the search for newer therapeutic strategies.

Photodynamic therapy (PDT) is an old concept that has been gaining ground due to its efficacy in targeting it to the appropriate site. PDT uses a photosensitizer (PS) along with a light of a specific wavelength. In the presence of oxygen, the excited PS can result in the generation of reactive oxygen species (ROS), which oxidizes cellular macromolecules that result in cytotoxicity of the cancer cells (Ibbotson 2010). PDT has its own advantages in comparison with other conventional treatments; it poses little toxic risk to the organisms compared to other chemotherapeutic drugs and radiation, which can cause serious damages to surrounding normal cells. PDT has been found to be highly effective in the treatment of lung, skin, head, and neck cancer. There are, however, issues in the formulation of the PS, and lack of perfect PS has been a deterrent in its gaining approval as a first-line cancer treatment modality. In an attempt to resolve the issues faced in PDT, nanoparticles have been associated with the conventional PS.

Mechanism of Photodynamic Action

Photodynamic action requires the simultaneous combination of a PS, specific wavelength of light, and molecular oxygen. The reaction begins with the absorption of light by the PS accumulated in the target tissue. The absorbed light then triggers a sequence of photochemical reactions that leads to the production of ROS. ROS in the form of

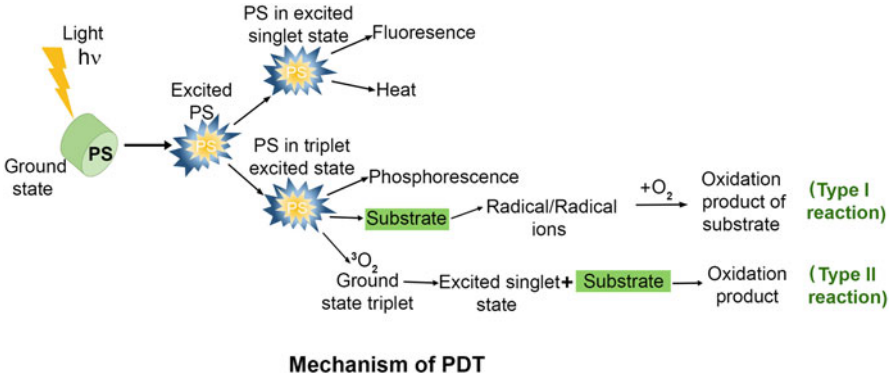

Fig. 1 Mechanism of PDT

singlet oxygen ($^1O_2$) has the capability to cause severe oxidative damage to cellular structures and biomolecules leading to the death of cells (Konan et al. 2002). During the process, other ROS, such as hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and superoxide ions ($O_2^{-\bullet}$) are also produced. Figure 1 demonstrates the basic reaction mechanism of photodynamic action. After the absorption of light, the PS gets excited from the ground state to an electronically excited state with a short lifetime varying between a few nanoseconds or even less. From the excited state, it can either revert back to the ground state by emitting fluorescence or it can go to the triplet state through intersystem crossing over. The higher the lifetime of the triplet state, the longer the time for the interaction of the excited PS with the molecular oxygen or with the other substrates present in the tissues (Castano et al. 2004). Direct interaction of the excited triplet PS with a substrate can result in a proton transfer leading to the formation of radical cation or anion. These radical ions reacting with oxygen can lead to the formation of oxygenated products like hydroxide radicals, superoxide anion radicals, and hydrogen peroxide. This process is termed type I reaction. In type II reaction, the energy from the excited PS can be transferred directly to molecular oxygen to form $^1O_2$. The therapeutic benefits of photodynamic action depend on the products that are formed in type I and II reactions. Both type I and II reactions can take place simultaneously in a photodynamic reaction. The ratio of occurrence of type I and type II reactions varies according to the type of PS, the concentration of molecular oxygen, and the amount of substrate present in the tissues. Most studies have indicated the occurrence of the type II reaction, and hence, it is believed that $^1O_2$ plays a significant role in the photodynamic reaction involved in PDT.

Tumor Destruction Mechanism

In PDT, the amount of photo-induced destruction of the tumor depends on several factors such as the type of the PS, the concentration of the PS, its localization in the tumor site, the time gap between administrations of the PS, and light used for

irradiation, the tumor type, and the level of oxygen present in the tumor. The ROS produced during the PDT reaction is the key component that causes irreversible damage to the microvasculature and tumor cells, thereby eliciting a sequence of immune and inflammatory response, and this combination assists in achieving a long-term control of the tumor.

Direct Killing of the Tumor Cells

The photodamage occurs at the site where the PS has accumulated. This is ensured by the short half-life of $^1O_2$ that is formed near the PS present and is unable to diffuse away much further. This damage occurs in the cells that have taken up the PS. It oxidizes DNA, proteins, and membranes to induce severe photodamage to the important subcellular targets of the cells such as the Golgi apparatus, endoplasmic reticulum, lysosomes, and mitochondria (Mroz et al. 2011). The pro-death signal is often activated through the protein unfolding response cascade (Hetz and Feroz 2018). In membrane, peroxidation can occur through type I and II reactions (Bacellar and Mauricio 2019). In most cases, PDT does not target DNA; depending on the PS used, the location of the drug in cells, and also cell type, the damage is different, and the mechanism of cell death also differs (Mroz et al. 2011). The classical PUVA (psoralen and ultraviolet A used in photochemotherapy) therapy is used in different skin disorders to target DNA (Bulat et al. 2011). One acridine derivative, 9-phenylacridine, has been found to bind to DNA and act as a PS. Its efficacy in cell killing has been demonstrated in different cell lines (Hansda et al. 2020; Hansda et al. 2021). Other PSs are known to target DNA (Wang et al. 2019), while some other PSs target proteins, lipid membranes, or mitochondria (Jiang et al. 2017; Jiménez-Munguía et al. 2019; Chakrabortty et al. 2017).

The location of the PS in the tumor site depends on the structural features of the PS such as its solubility, its net charge, and the type of symmetry present. PSs that are hydrophobic in nature with lower negative charges tend to be largely taken up by the tumor cells by diffusing through the plasma membrane, while those that are less hydrophobic are taken up by the process of endocytosis since they are too polar to diffuse through the plasma membrane (Buytaert et al. 2007). PDT induces death in cells through autophagy, apoptosis, or necrosis without depending on the cell cycle phase (Castano et al. 2004). Location, type, and dosage of the PS are more important for determining the mode of death. Damage to mitochondria results in apoptosis and paraptosis (Chen et al. 2014). Recently, the role of autophagy in PDT is recognized. Autophagy occurs in cells where the PS is targeted to the endoplasmic reticulum, mitochondria, or both (Liang et al. 2016). Necrosis occurs when the PS accumulates in the plasma membrane. This is characterized by the swollen plasma that triggers the release of the intracellular contents (Baluk et al. 2005).

Cell Killing Mechanisms in PDT

During PDT, cell death can occur through any one or a combination of the different death mechanisms. Induction of death process depends on the cell type, light dosage, and PS; the initial site of PDT-related damage also determines which cell death pathway is initially activated. In most instances, undesired cells are eliminated during PDT essentially through the conserved and irreversible apoptotic pathway, which is an energy-dependent cell death process characterized by a distinct morphological and biochemical alteration in cells (Goldar et al. 2015); but in some instances, cell death also occurs either through the other programmed death pathway, namely, autophagy, parapoptosis, or through necrosis. In bovine retinal capillary endothelial cells, PDT using the PS, lutetium texaphyrin, induced apoptotic cell death (Mroz et al. 2011). Photodamage leading to cleavage of genomic DNA can lead to rapid apoptotic death in cells (Kessel and Oleinick 2018). Mitochondrial photodamage triggered apoptotic response that led to the destruction of the antiapoptotic proteins, Bcl-2 and Bcl-xL (Aniogo et al. 2020). Upregulation of Bcl-2 and downregulation of Bax level led to resistance to PDT in HT29 human colon adenocarcinoma cell line (Mroz et al. 2011). Lysosomal proteases can result in the formation of proapoptotic fragment tBid from the cytosolic protein Bid. The interaction between mitochondria and tBid also triggers apoptotic response (Kessel and Oleinick 2018). The lysosome-mediated photodamage is less direct compared to mitochondria or ER-related apoptosis. PDT utilizing red diode laser and Ag NPs induced apoptosis in MCF-7 and A549 cell lines (El-Hussein et al. 2015). NpAuPpIX conjugate with 630 nm light-mediated PDT resulted in apoptotic death in HeLa cell line (Juárez et al. 2019). Iron-based NPs (FeNPs) directly induced apoptosis by inducing oxidative stress (Chizenga and Heidi 2020). The PS 9-phenyl acridine with UVA could exert its photodynamic action in A375 cells mainly through apoptosis, along with some autophagic cell death (Hansda et al. 2020).

Autophagy is a dynamic process; it brings about cell death through transport of different cellular organelles and proteins to the lysosomes and their subsequent degradation therein (Glick et al. 2010). Lysosomes are a good target for the initiation of lethal photodamage. Lysosomal photodamage causes the release of the lysosomal proteases by creating large gaps in the membrane. The enzyme calpain cleaves the autophagy-associated protein, ATG5. ATG7 and ATG5 proteins are believed to be integral to this pathway. ATG5 has a dual role; it also has the effect of promoting apoptosis in PDT (Kessel and Oleinick 2018). PDT mediated by nanoparticles encapsulating chlorin e6 (UCNPs-Ce6) was responsible for the induction of autophagy through ROS production (Han et al. 2017). Fe@Au-NPs influenced a cancer specific cytotoxicity through mitochondria-mediated autophagy in OECM1 oral cancer cells. Iron oxide (FeO) NPs displayed cytotoxic action through mitochondrial membrane alteration-mediated autophagy in cancer cells. A good number of NPs including GNP-Chl, magnetic NPs (C225-NPs), C60(Nd)-NPs, FeO-NPs, α-Al2O3-NPs and such others were shown to mediate autophagic death in cancer cell by PDT

(Panzarini et al. 2013). Metal nanoparticles can directly modulate the apoptotic and autophagy pathways. Autophagic death was induced by magnetic FeNPs in murine alveolar macrophage and RAW264.7 cells (Chizenga and Heidi 2020).

Paraptosis is another type of programmed cell death that is morphologically distinct from other forms of cell death. It lacks apoptotic morphology, cytoplasmic vacuolation, unconstrained caspase activation, and inhibition. Paraptotic responses were evident in photodamage to the Endoplasmic Reticulum (ER). Here, vacuolization is mainly mediated through the ER and perhaps mitochondria. It is reported that photodamage of DNA caused cell death at the G_2/M phase by massive vacuolization in a synchronized cell culture. The benzoporphyrin derivative, verteporfin, mediated PDT demonstrated paraptosis in A549, NSCLC, and 1c1c7 murine hepatoma cells (Kessel and Oleinick 2018).

Necrosis is the most common consequence of external physical or chemical injury leading to death from damages that results from cytoplasmic swelling, disintegration of cytoplasmic membranes, and cellular fragmentation. It is characterized by loss of membrane integrity and cell lysis (Syntichaki and Nektarios 2002). Necrosis is associated with adverse effects and nonspecific effect on normal cells and tissues. Therefore, the criteria of PDT do not rely on necrosis death for treatment of cancer (Kessel and Oleinick 2018). However, in the presence of PS, direct photodamage can occur to damage the plasma membrane leading to necrotic death. During PDT, high concentration of PS or a high fluence of light or both tend to induce cell death by necrosis. Such effects have been found with the PS phthalocyanine; but damage also occurs in the other organelles like the lysosomes and endosomes that are directly involved in the autophagic process (Mroz et al. 2011). In PDT using CQ-conjugated gold NPs (GNP-Chl), cytotoxicity was triggered in MCF-7 cells through autophagy, but necrotic cell death was also observed (Panzarini et al. 2013).

Vascular Damage

In general, the growth of any solid tumor primarily depends upon its ability to form new blood vessels through a process called angiogenesis. The incomplete and poor cellular borders present in these abnormal cells may facilitate the accumulation of PS in the tumor site through the leaky vasculature (Chen et al. 2006). PSs that are bound with carrier molecules have a higher affinity toward the tumor cells, as these cells have a larger number of specific receptors on their surface. When the PS gets activated in the endothelial cells, it destroys the tight junctions present between the cells and the vascular basement membranes. This leads to the formation of thrombogenic sites in the tumor cells and stirs up a sequence of reactions like vessel constriction, aggregation of the platelets, and increased vascular permeability (Chen et al. 2006). All these changes lead to the shutdown of the vascular system, tissue hemorrhages, and stasis of blood flow, which ultimately leads to the control of the tumor (Beck et al. 2007).

Immune and Inflammatory Response

The initial ablation of the tumor mainly depends on the direct killing of tumor cells and vascular damage. Initiation of secondary cytotoxicity in the tumor cells may occur that depends on the immune process and its enhancement, which could assist in the response of the tumor to PDT. The release of inflammatory mediators like cytokines, proteinases, peroxides, growth factors, and others from the treated site is the main characteristic feature of the inflammatory process. This process induces the components of white blood cells to converge on the treated region. The damaged cancer cells are removed by the process of phagocytosis. Thus, PDT possesses the advantage of acting as a modality that is immune-stimulatory in contrast to surgery and chemotherapy, which are immunosuppressive (Allison and Moghissi 2013).

Photosensitizers

In general, a PS should have the capability to get accumulated in the tumor site and should get cleared from the normal tissue easily. The PS has to be amphiphilic in nature so that the PS can travel to the targeted site without any hindrance. For this purpose, the PS needs to be hydrophilic so that it binds to its target in the cell; the PS also requires lipophilicity to a certain extent to cross the membrane barrier for entering inside the cells. Further, the PS needs to exhibit high quantum yield, negligible dark toxicity, and long triplet lifetime to facilitate the interaction of the PS with the reactant (Zhao et al. 2013). To ensure the deeper penetration of light in the biological tissues, PSs that are activated by a larger wavelength of the light, above 700 nm, would be preferred. This prevents the absorption of light by the endogenous molecules whose absorption is below 700 nm. The process of developing an ideal PS with all the requirements is rather difficult. Yet many PSs have been approved for use in the clinical section, which does not satisfy all the requirements of an ideal PS. These PSs mostly belong to the first generation and second generation. Few of the PSs are also being approved for clinical trials.

First-Generation Photosensitizers

The PSs that are based on porphyrins come under the first-generation PSs; they are effective against brain, laryngeal, lung, esophageal, gastric, and skin carcinoma. The examples of the first-generation PSs are the derivatives of hematoporphyrin (HpD), which contains a mixture of monomers, dimers, and oligomers of porphyrin or porfimer sodium. The HpD PSs were the earliest PSs used in clinical trials. Porfimer sodium has several advantages like the effective destruction of the tumor cells, negligible dark toxicity, and the ability to formulate the PS as a water-soluble preparation and is still in use in the treatment of different kinds of cancer. The relatively weak absorption of light of these PSs in the red portion of the

electromagnetic spectrum significantly reduces the depth of penetration of the light, which in turn reduces the efficacy of the treatment. The lower extinction coefficients of PSs require a larger amount of the drug to be administered to ensure a satisfying phototherapeutic response. This often results in aggregation of the PS. Metal ions are often included to prevent the aggregation and to increase the stability of the PS. The position and type of substitution can influence its lipophilicity (McFarland et al. 2020). During the drug-light interval (DLI) (48–72 h), the patient has to be protected from light exposure. Another issue with the PSs is the accumulation and retention of the PSs in the skin and normal tissue for a prolonged time, which leads to the problem of severe photosensitivity after the PDT treatment. These issues can be managed by preventing exposure to sunlight and high energy light, or protective glasses and clothes can be worn after the PDT treatment. The first-generation PSs thus suffered from unfavorable biodistribution, less bioavailability, and prolonged photosensitivity during the beginning of the clinical trials.

Second-Generation Photosensitizers

The second-generation PSs were developed mainly to overcome the limitations faced by the first-generation PSs. Second-generation PSs comprise porphyrinoid compounds and non-porphyrinoid compounds. The former consists of the macrocyclic structures of porphyrin such as bacteriochlorins, bacteriopheophorbides, chlorins, pheophorbides, texaphyrins, and phthalocyanines (Fig. 2). Non-porphyrinoid compounds consist of anthraquinones, xanthenes, curcuminoids, phenothiazines, and cyanines (Fig. 3) (Ormond and Freeman 2013).

A limited number of these drugs have been approved for clinical treatment of cancer. These PSs in contrast to the first-generation PSs have higher absorption maxima that are longer than 630 nm and also have high extinction coefficients. The metalated derivatives such as Si(IV)-naphthalocyanine, tin ethyl etiopurpurin (SnET2), and phthalocyanine tetrasulfonate (AlPcS4) are included in the category of second-generation PSs (Josefsen and Boyle 2008). The second-generation PS exhibits a better cell killing effect by presenting higher quantum yields and greater concentration of tumor-to-normal tissue in comparison to HpD. The shorter accumulation time of these PS makes it possible to carry out the treatment on the same day of drug administration. It provides the opportunity to perform PDT in an outpatient setup, thus making it more acceptable and convenient for the patients. Apart from the rapid treatment time, the second-generation PSs also exhibit a shorter period of cutaneous photosensitivity. These features of the PSs mainly depend upon the physical and chemical parameters such as the type of the charged groups, lipophilicity, number and type of the ring, and core substituents (Peng et al. 1996). Few of the second-generation PSs like mono-L-aspartyl chlorin e6 (MACE), AIPcS4, and aminolevulinic acid (ALA) are comparatively hydrophilic in nature, especially those compounds that are composed of porphyrin ring structures (chlorin e6, bacteriochlorophyll a, and SnET2). Few unsubstituted phthalocyanine compounds have higher hydrophobicity. ALA is directly not a PS, but when it is taken up by cells, it is metabolized by protoporphyrin IX.

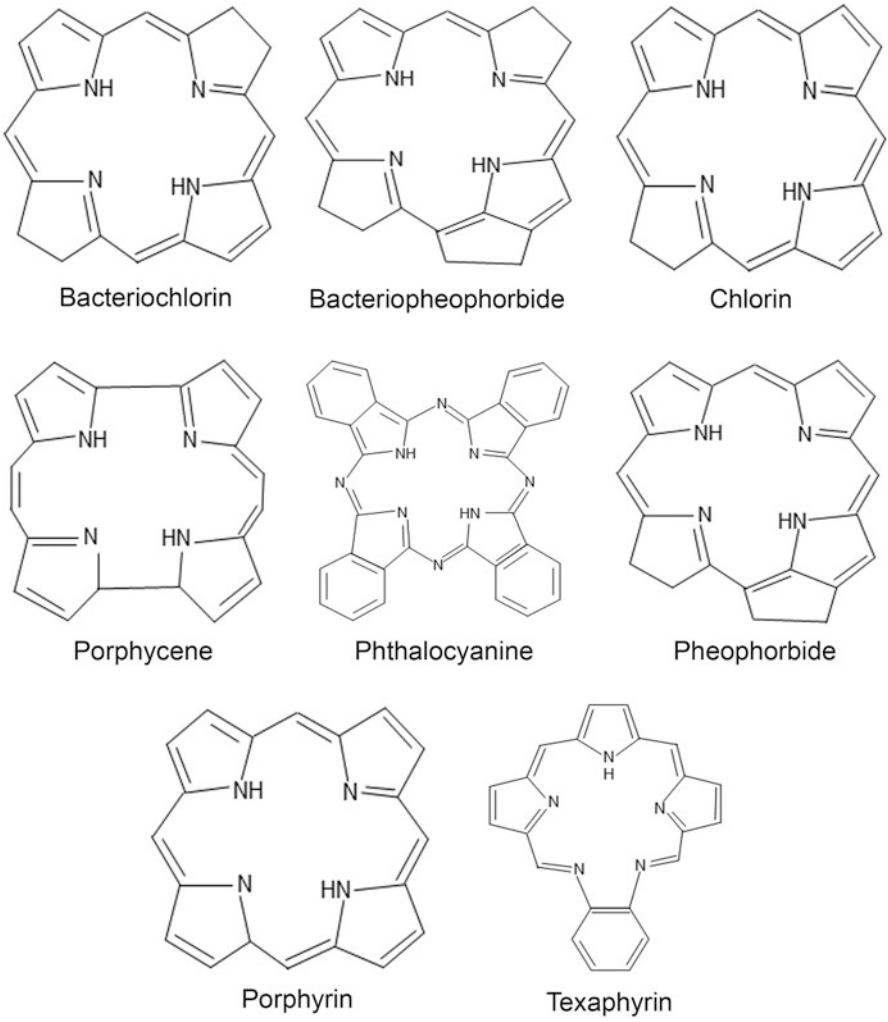

Fig. 2 Basic structures of porphyrin-based photosensitizers

The Photofrin derivative, ALA, is a second-generation porphyrin. Some of these second-generation photoporphyrins target new vasculature (Taquet et al. 2007). When the second-generation photoporphyrins are conjugated with biological motifs like antibodies or with any other synthetic material such as liposomes, they are categorized as third-generation porphyrins (Mfouo-Tynga et al. 2021). ALA and some of its derivatives that are in use are shown in Fig. 4. 5-Aminolevulinic acid (ALA) and ALA esters and their derivatives have been used in PDT in human glioma spheroids. The cell killing capacity of the compounds benzyl-ALA (b-ALA) and hexyl-ALA (h-ALA) was similar to that of the parent compound, ALA, but they were required at 10–20 times lower concentrations for eliciting similar response. The

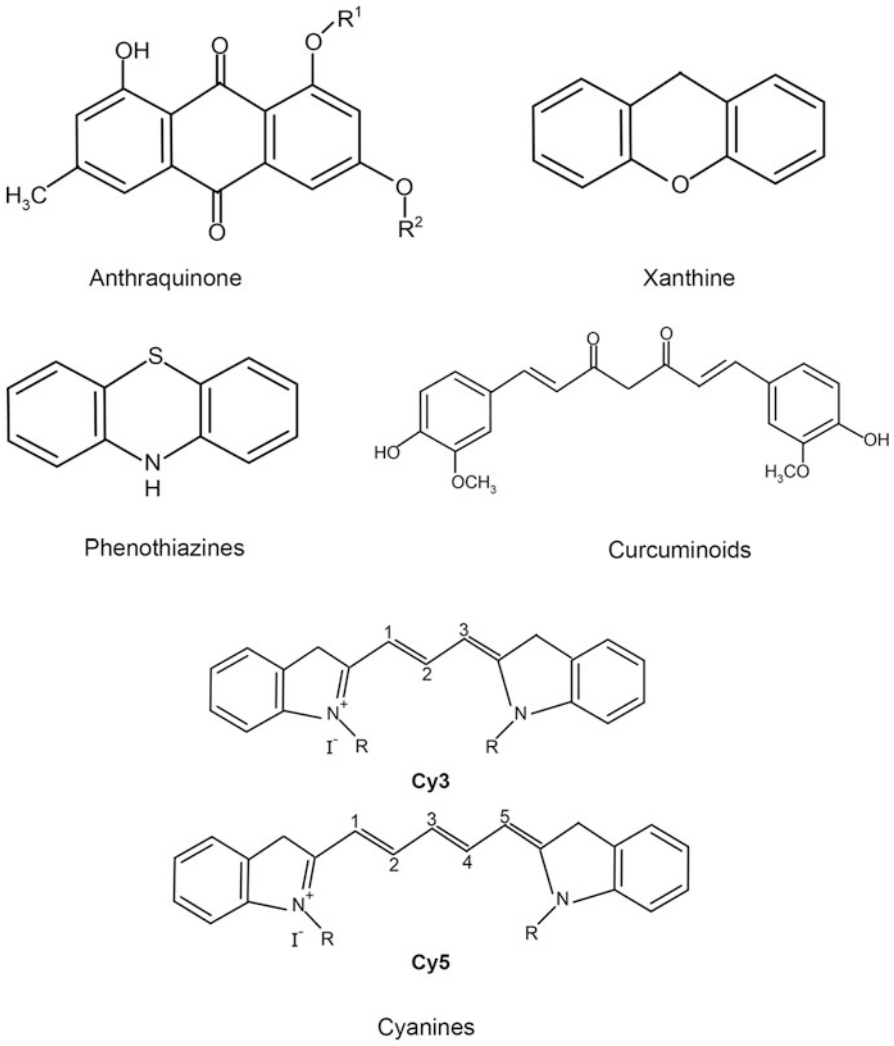

Fig. 3 Basic structures of non-porphyrinoid-based photosensitizers

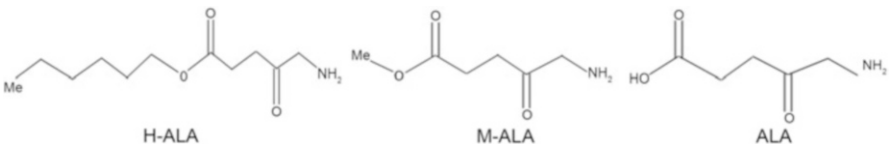

Fig. 4 ALA and its derivatives

derivatives of ALA also exerted their photosensitizing action on being metabolized to protoporphyrin IX. The improved capacity of cell killing of these esters of ALA was attributed to its enhanced penetration to the cell membrane exerting its activity at

low doses (Hirschberg et al. 2002). It was found in another study that the efficient delivery of hydrochloric salts of ALA and its methyl ester (m-ALA) could be achieved by solubilizing them in the lipid sponge phase made from propylene glycol, monoolein, and an aqueous buffer. Monoolein is a monoglyceride, which is produced during the digestion of oil in the upper intestine. During PDT, the m-ALA or ALA-loaded sponges were applied over the tumor surface to deliver them to the basal cell carcinoma and then irradiated with visible light. This initiated the production of singlet oxygen, inducing toxicity and resulting in tumor cell death (Merclin et al. 2004). PDT is an approved treatment modality by the US Food and Drug Administration (FDA) for premalignant as well as malignant diseases like Barrett's esophagus, esophageal cancers, keratosis, endobronchial non-small cell lung cancers, and choroidal neovascularization. High-grade gliomas (HGGs) and other types of brain tumors could be managed using ALA as the PS or precursors for PDT. Clinical trials are in progress for considering ALA for the therapy of intraoperative resection cavity and interstitial PDT in case of inoperable HGGs (Mahmoudi et al. 2019).

The hydrophobic and hydrophilic nature of the PS affects the drug administration route and influences the biodistribution and pharmacokinetic profile of the drug (Castano et al. 2004). It has been observed that the PSs that are hydrophobic in nature exhibit a higher tumor to normal accumulation ratio in comparison to the hydrophilic PSs. Even though the hydrophobic nature of the administered PSs assists them to penetrate into the cell membrane and to get located into the subcellular compartments, they form aggregates under the physiological environment that affects their ROS generation efficiency and cytotoxic property (Allison 2014). In addition to this, the hydrophobic nature of the PSs interferes with their solubility in the physiological pH, which limits their use in clinical applications. Therefore, it is essential to provide a stringent balance between its hydrophobicity and solubility in various solvents.

In an attempt to enhance the degree of solubility of the PSs and to make them amphiphilic, different types of polar substituents were functionalized into the PS structure. The porphyrin ring system with its inherent 12 positions offers the possibility to substitute them with various other functional groups such as carboxylic acid, quaternary ammonium salts, sulfonic acid, and carbonyl groups, thus leading to the synthesis of a countless number of porphyrin derivatives. Porphyrin ring systems also offer the possibility of oxidizing, extending, and modifying the ring to load a central ion so that the properties related to pharmacology and photophysiology can be altered. The same modification also works for the second-generation PSs such as phenothiazines, dyes, and perylenequinones. The nature of the solubility of the PS depends on the charge of the substituent groups. PSs with no charged terminal groups are hydrophobic, and the PS compounds with three or more charged substituent groups are hydrophilic; those PS compounds with two charged groups will probably be amphiphilic in nature (Hudson et al. 2005).

Besides this, the PS compounds with anionic substituents have been found to localize selectively in the cytoplasm, and those PS compounds with cationic groups get accumulated in the mitochondria. The exact mechanism behind this localization

and distribution is still unclear, and so the question of maximizing the tumor selectivity still remains.

Third-Generation Photosensitizers

Currently, much focus was given in the area of the third-generation PSs, which would have the ability to be activated by light of longer wavelength with much-reduced photosensitivity and better tumor selectivity. To achieve this, two different strategies were followed. Targeted distribution can be achieved to include improved efficacy and reduced adverse effects by using ligands like biotin, folate, peptide, and such others for delivery (Zhang et al. 2018; Gierlich et al. 2020). One such method is the modification of the existing PS with different biologic conjugates to actively target the tumor site (Taquet et al. 2007). The second method is the conjugation of the PS to a delivery vehicle or carrier that can efficiently transport the employed carrier from the site of administration to the tumor site. The most commonly used targeting ligands are folate (FA) and transferrin, and reports exist on the FA conjugation to a platinum porphyrin complex using an ethylenediamine linker. When carboxylic acids get activated from both platinum porphyrin complex and FA, they form amide bonds with the linker that gives rise to a new FA-targeted PS selective for FRα-positive cells. The endocytosis of this engineered PS was confirmed by confocal microscopy inside the FRα-positive HeLa cells in comparison to FRα-negative A549 cells, where there was no endocytosis seen. The cell killing was 78% for the FRα-positive cells, whereas the FRα-negative cells showed only 25% cell killing (Yang et al. 2019a). Similar result was also obtained for the FA-targeted π-extended diketopyrrolopyrrole-porphyrin that exhibited its selectivity for the FRα-positive HeLa cells (Jenni et al. 2019). In vivo studies in mice with induced nasopharyngeal epidermoid carcinoma showed some promising result when they were treated with FA-conjugated pyropheophorbide with 1 kDa polyethylene glycol (PEG) spacer. The accumulation of this PS was superior in the tumor compared to free/non-targeted controls without the spacer PEG. A reduced dose of the PEGylated FA-targeted PS was sufficient to eradicate the subcutaneous KB tumors induced in BALB/c nude mice with no recurrences even after 90 days of treatment with the PEGylated FA-targeted PS compared to non-targeted PS and non-PEGylated PSs (Liu et al. 2019). Another type of ligand that is regularly used to conjugate with the PSs is small peptides, which usually aid in improving their aqueous solubility, leading to enhanced phototoxicity to improve the therapeutic efficacy. GE11 is one such small peptide that was generated by phage display against EGFR and has been utilized by researchers to conjugate with PSs. The in vitro phototoxicity of a GE11-targeted 1,4-bis(triethylene glycol)-substituted carboxyl ZnPc against EGFR-positive A431 cells was detected. This was attributed to the improved internalization and such effect was, however, derided in low-EGFR expressing MCF7 cells. In vivo fluorescence imaging was also utilized to study the biodistribution of such peptide-conjugated PS (Yu et al. 2019).

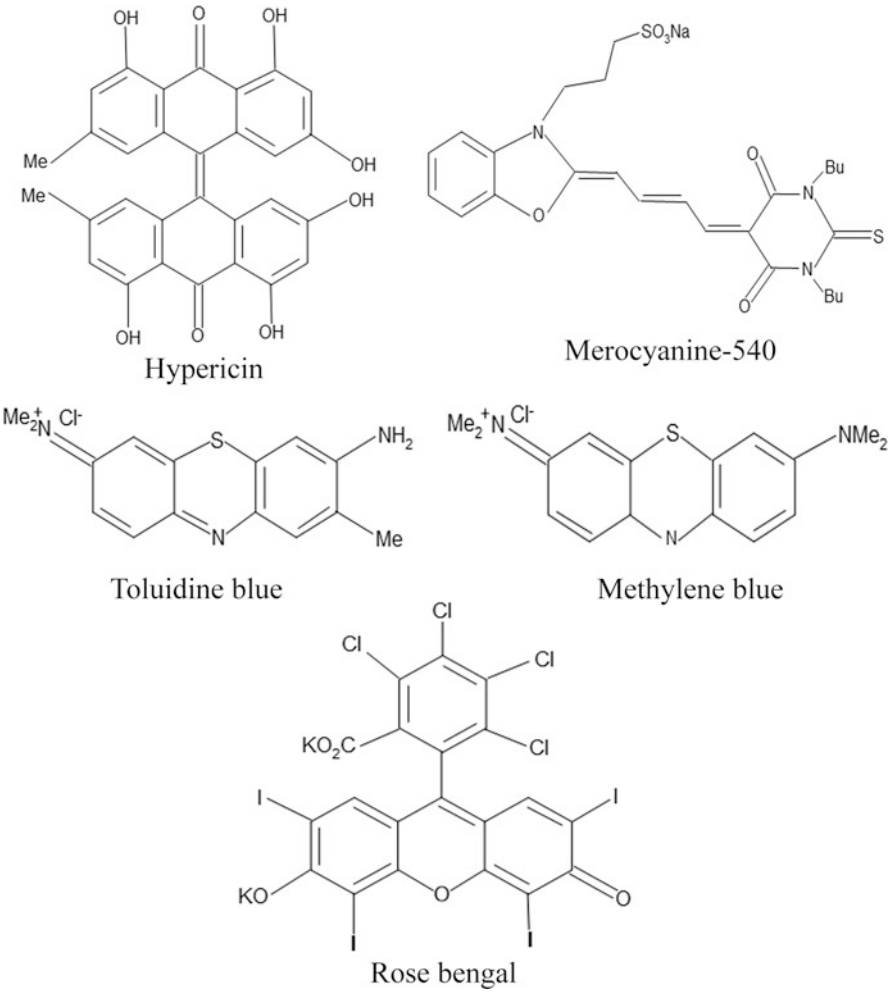

Fig. 5 Basic structures of third-generation PSs

To be precise, third-generation PSs will be an improved version of the previous two generations in view of targeted delivery and biodistribution. Even though most of the third-generation PSs have been widely considered for the PDT study (Fig. 5), very few PSs have been examined for applications in the clinical side as they lack in vivo selectivity (Allison 2014).

Limitations of PDT

Despite the potential ability of the PDT to be used as a stand-alone treatment modality, PDT is currently used only for the treatment of superficial lesions and for those that can be accessed by endoscopes. The use of PDT is limited by its

inability to treat the solid tumors and the tumors that are situated deep inside the tissues. The efficiency of the treatment with PDT to treat the solid and deep-seated tumors is hindered by the tissue penetration depth of the visible light, which is essential for the PS to get activated. The red light can penetrate through the living tissue only for a depth of 1–3 mm. When the target area will be illuminated by the light, energy associated with the incident light will be reduced as most of the light energy will be absorbed by the chromophores present in the tissues. Thus, PDT efficacy greatly depends on the thickness of the tissues. Hence, to treat the deep-seated tumors and to penetrate tissues to a greater depth, near-infrared reflectance (NIR) beams have to be used (Deng et al. 2017). Even though various works have been done to utilize PSs that can absorb light in the NIR range, in order to improve the depth of penetration of the PSs, the low energy of the NIR beam is not sufficient enough to excite the PSs to generate ROS. This limits the use of NIR beams in PDT.

There are a number of challenges faced for the effective use of PDT as a treatment modality. One of the features includes wavelength of light to be used for effective absorption to a great depth. The advancement in optical technologies can greatly benefit the transport and delivery of light. PDT is gaining popularity after the use of lasers (600–800 nm) and coupling it with optical fibers. Thus, delivery of the light without the requirement for a straight-line path is an added benefit. Development of light delivery system in different geometry is an active area of research. The optical properties of the light often vary on penetration in different tissue depths, so the exact dose delivered needs to be quantified accurately, which is also a challenge. In case of over-illumination, PDT can cause damage to the normal tissues surrounding the tumor site (Allison 2014). The concentration of PS is another factor, where photo-bleaching is also to be considered, as it can result in the destruction of the PS. Furthermore, it is not possible to treat the whole body with radiation in case of cancer metastasis with the technologies that are currently available. The lack of knowledge about the light dose that has to be delivered to the tumor environment without posing a potential threat to the normal tissue makes it difficult for the clinicians in planning the treatment. Moreover, being a localized treatment method, PDT requires the right dose of light to be delivered so that it can reach the deeper tumor sites to eradicate their growth. The major drawback of PDT is the failure to control the tumor recurrence due to poor illumination. PDT is an oxygen-consuming modality; it depends mainly on the presence of oxygen in the tumor tissues. If the tumor tissues are deprived of oxygen, the cell killing effect of PDT will be severely hampered. Tumor hypoxia can occur due to fast tumor growth or rapid depletion in the supply of oxygen. If the rate of oxygen consumption is higher than the rate of vascularization, transient hypoxemia can occur. It is reported that the solid tumor with levels of hypoxic cells shows less effectiveness in PDT treatment as it is PDT resistant (Larue et al. 2019).

The issue of generalized photosensitivity linked with the earlier generation PSs makes it uncomfortable for the patients to make changes in their lifestyle and to stay indoors for longer periods. These reasons keep most of the patients to give consent to PDT. Although some of the PSs get selectively accumulated in the tumor tissues but the mechanism behind the selectivity is not clearly known yet. The abnormal tumor

microenvironment such as poor lymphatic drainage, acidic pH, and the larger number of receptors that are overexpressed on the tumor cells are considered to be responsible for the accumulation of the PS, particularly at the tumor site (Beck et al. 2007). The tumor selectivity of the PS could be enhanced by the targeting moieties that can transport the PS from the site of administration to the site where the tumor is located for selective accumulation in the tumor region. This strategy could allow the avoidance of unfavorable biodistribution to increase the circulation time of the PS. With these modifications, it is possible to reduce the side effects such as longer exposure and damage to the surrounding normal tissues.

Formulation of Photosensitizer

For the formulation of a clinically successful PS, the following properties are essential.

1. Chemically pure to obtain the regulatory approval
2. Easy and convenient synthetic procedure so that the PS can be produced on a large scale
3. Not degradable upon activation by the light
4. Longer lifetime of PS in triplet state for efficient energy transfer
5. Chemically stable for long-term storage and transport inside the biological system
6. Amphiphilic to penetrate the tissue
7. High absorption value
8. Soluble in the body's fluids
9. Ability to target the cancer cells
10. Beneficial half-life in tissues
11. Excellent photostability
12. Rapid clearance from the normal tissues and minimal skin photosensitivity

The potentiality of the PDT treatment largely depends on the photochemical and photobiological properties of the PS. Most of the PSs that have high capability contain aromatic π electron systems that are highly delocalized. This electron system provides the PSs to absorb light in an effective way. These PSs are vulnerable to form aggregates when they are introduced into an aqueous medium possibly due to the hydrophobic interactions and the π-π stacking. In one way, the hydrophobic nature of the PSs could be considered as an essential characteristic since the PS solubilization is found to have been one of the main reasons for the efficacy of the PS. But this characteristic also leads to the formation of aggregates in an aqueous solution, which will affect the ROS generation efficacy of the PDT. The hydrophobic nature of the PS also makes it difficult to prepare pharmaceutical formulations to be administered via the parenteral route. This issue could be resolved by preparing the PS formulation in a suitable carrier system that can efficiently transport the PS in a stable monomeric form. This prevents the altering of the spectroscopic and

functional properties of the PS. To be used as a therapeutic modality, PS formulation has to be selectively internalized by the tumor cells so that it can facilitate convenient and standard dosing. It is also essential that the carrier employed to transport the PS has to be biodegradable and nontoxic.

Nanoparticles in PDT

Nanoparticles have gained their immense applications in the field of targeted drug delivery (Haribabu et al. 2019; Girigoswami et al. 2018), imaging (Haribabu et al. 2020; Amsaveni et al. 2013), designing of biosensors (Metkar and Girigoswami 2019; Akhtar et al. 2017), as well as theranostics (Haribabu et al. 2021). The fascinating features of nanoparticles can be exploited to overcome the limitations faced by the classic PS. Nanoparticles can be synthesized from materials that are of natural or synthetic origin and can be fabricated to serve multiple functions, thus functioning as a theranostic agent. Based on the nanocarrier type and the method in which the PS is loaded in it, PDT in conjunction with nanoparticles has the following advantages.

1. The large surface area of the nanoparticles can prompt an increased amount of PS delivery to the target site.
2. Nanoparticles can help in preventing the premature release of the loaded PS.
3. Nanoparticles by utilizing the enhanced permeability and retention (EPR) effect facilitate the diffusion and retention of PS carriers into the tumor tissue.
4. The easily modifiable surface of the nanoparticles allows PS carriers to be modified with functional groups or targeting agents to improve the biodistribution, enhanced cellular uptake, and targeted delivery of the PS nanocarriers.
5. Nanoparticles can be fabricated as platforms that can carry multiple components to function as theranostic agents. For example, PS nanocarriers can also carry imaging agents, chemotherapeutic drugs, and targeting ligands (Amsaveni et al. 2013; Haribabu et al. 2021).

Two methods are followed to develop nano-based carriers for PDT. They are as follows.

Biodegradable Nanoparticles

Biodegradable nanoparticles are synthesized from naturally occurring or synthetic polymers to carry the PS payload; these nanocarriers can either undergo enzymatic degradation or hydrolytic degradation and can be excreted from the biological system, thus minimizing the accumulation of the nanocarriers. Biodegradable nanoparticles used as PS nanocarriers are polymeric liposomes, polymeric nanospheres, polymeric nanocapsules, dendrimer-based nanoparticles, and nanoparticles that are

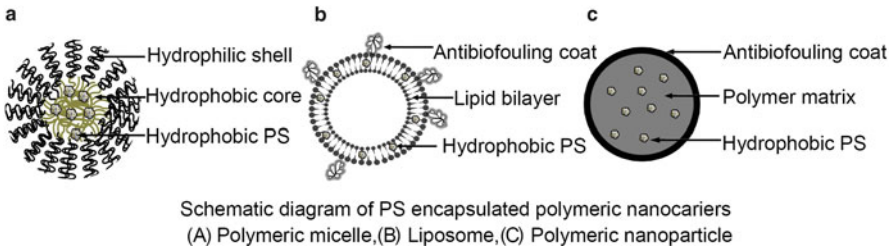

Fig. 6 The different types of polymeric nanocarriers commonly used in PDT: (**a**) polymeric micelle, (**b**) liposome, and (**c**) polymeric nanoparticle

based on natural macromolecules. Some of the biodegradable nanostructures that can carry the payload in PDT are shown in Fig. 6.

The water solubility and accumulation of PSs at targeted site can be enhanced by encapsulating the PSs inside the macromolecular nanostructures, such as liposomes (Broekgaarden et al. 2014; Bovis et al. 2012), polymeric nanoparticles (Chung et al. 2013), and polymeric micelles (Gibot et al. 2014). These nanostructures share some common characteristics: accumulation inside the tumor tissues selectively due to the improved microvascular permeability and compromised lymphatic drainage inside the tumor tissue, which is termed EPR effect. Thus, the delivery of PSs using polymeric nanocarriers will provide enhanced water solubility along with the control of biodistribution of hydrophobic PSs by intravenous administration with delivery of PSs selectively to the target tissues.

Liposomal encapsulation of the PSs is also demonstrated by encapsulating hydrophobic PS such as phthalocyanine derivatives (Love et al. 1996) and porphyrin (Ben-Dror et al. 2006) in the phospholipid bilayer pocket and water-soluble components such as ALA, the prodrug (Casas and Batlle 2006), that were encapsulated inside the hydrophilic core of the liposomes. There are many advantages of liposomal carriers of the delivery of PSs that are beneficial compared to the PS aqueous dispersions. pH-sensitive liposomes, target-sensitive liposomes, light-sensitive liposomes, thermo-sensitive liposomes, and fusogenic liposomes are the different types of liposomes used for triggered release of PSs in PDT. Antibody-modified liposomes and ligand-modified liposomes are the actively targeted liposomes used for PDT. Passive targeting was explored in PDT for long circulating liposomes toward tumor tissue. Monoacid ring A (BPD-MA) is a benzoporphyrin derivative that was incorporated inside glucuronide-modified liposomes (PGlcUA-liposomes). The study demonstrated that the subcutaneous sarcoma-bearing mice showed a significant regression in the tumor size with 80% rate of cure after the intravenous injection of the PS followed by tumor illumination. On the other hand, there was only 20% cure observed for the animals that were treated with the conventional liposome encapsulated BPD-MA (DPPG-liposomes) (Oku et al. 1997). In another study, a dually loaded hybrid liposomes were designed to improve the tumor therapy, where the aqueous core contained iron oxide nanoparticles and the lipid bilayer was loaded with the PS (meta-tetrahydroxyphenylchlorin, m-THPC). This designed double

cargo demonstrated double functionality by generating singlet oxygen after exposure to laser excitation as well as production of heat when exposed to alternating magnetic field. These two methods have coupled the PDT with magnetic hyperthermia (MHT), and the combined PDT/MHT showed cancer cell death completely in vitro and ablation of solid tumor completely in rodent in vivo model (Di Corato et al. 2015). Yang et al. have recently demonstrated an aggregation-induced PS (AIE-PS) strategy encapsulated in the liposomes for eliciting the photosensitization in a controlled manner. When the AIE-PSs are carried to the tumor sites entrapped into the liposomes, the liposomes get degraded and the AIE-PSs are released. A controlled photosensitivity was achieved against killing of tumors both in vitro and in vivo without the need of a dark room (Yang et al. 2019b).

Later, polymeric nanoparticles were introduced as an alternative to liposomes to deliver payloads of PSs in PDT. The advantage was that the size of the polymeric particles can be manipulated, which plays an important role in PS formulation delivery to the site of tumor via EPR effect. This inhibits the recognition of PSs by the macrophages and the proteins, which also enhanced the circulation time in blood. A step forward, using the "stealth" coating by polyethylene glycol (PEG), the nanoformulations can easily circumvent the RES uptake post intravenous injection, and the blood circulation time can be increased (Veronese and Pasut 2005). The PSs that are hydrophobic in nature can be entrapped physically inside the nanoparticles through electrostatic or hydrophobic interactions in between the polymer and the PS. The different kinds of biodegradable polymers that have been used for PS delivery are polylactic acid (PLA), polyglycolide (PGA), and poly(D,L-lactide-co-glycolide) (PLGA) (Kumari et al. 2010). The advantages of polymeric nanoparticles for the delivery of PS payloads are their physical robustness, high capacity of loading, versatility, and their surface properties that could be controlled for its degradation and release of PSs. Earlier studies have elaborately described the applications, basic mechanisms, and challenges of PDT with the different types of nanoparticle-based PDT agents, especially the polymeric nanoparticle-based cargos. The PDT involving polymers used for cancer therapy has been described earlier, where polysaccharides, proteins, polyesters, polyacrylamide, and pluronic micelles used for encapsulation and delivery of PSs have been elaborately discussed. Pluronics is a commercially available FDA-approved polymer that contains a central poly(propylene oxide) which is flanked on both sides by two blocks of poly(ethylene oxide). It exists in variable molecular weights, and it can form nanosized micelles immediately when dissolved in aqueous media (Conte et al. 2016).

Polymeric micelles are another mode of delivery of PSs, which are formed when amphiphilic graft or block copolymers get dispersed in any aqueous solution beyond the critical micelle concentration (CMC), and the process is spontaneous. Since the aqueous compatibility of the hydrophobic amphiphilic copolymers is poor, they get readily assembled to make a core structure that can incorporate the hydrophobic drugs. A stabilizing interface is maintained by the hydrophilic segments between the hydrophobic compartment and the hydrophilic environment through a shell region. This allows the solubilization of hydrophobic PSs as well as controlled release of PSs at specific sites through diffusion followed by dissociation of polymer or the

micelle. The building blocks of the core of lipophilic polymers are polyesters and polyamino acids, but the corona usually consists of PEG due to its biocompatibility, high solubility in water, and nonfouling properties (Nishiyama and Kataoka 2006). The advantages of polymeric micelles in PDT include simple methods of preparation, controlled release, and efficient loading of the drugs without any chemical modification. They also show long blood-circulation time and selectively target the tumors through EPR effect and reduce adverse side effects such as skin photosensitivity. The drug loading, studies on biodistribution, and therapeutic efficiency of different polymers used for encapsulation of PSs such as PEG-lipid conjugates, pluronics, and the polyion complex (PIC) micelles or the pH-sensitive poly(N-isopropylacrylamide)-based micelles were discussed earlier. In a previous study, amphiphilic block copolymeric micelles were engineered using poly(ethylene oxide-b-D, L-lactide), poly(ethylene oxide-b-ε-caprolactone), and poly(ethylene oxide-b-styrene). The synthesized micelles were characterized using different photophysical tools like dynamic light scattering, asymmetrical flow field-flow fractionation, and electron microscopy, which showed a size of 20 nm. The stability of the micelles upon dilution was investigated to find their capacity to be used as carriers in the presence or absence of blood proteins. The results demonstrated good stability for more than 48 h in all the systems, and they released the load slowly. Pheophorbide was used as sensitizer, and the PDT efficacy to kill cancer cells in 2D and 3D systems was assessed. The 3D system used was spheroid, which was compared for its killing by micelle-loaded PSs with 2D cell culture (HCT-116 cells), and the results showed a huge increase in spheroid photocytotoxicity (Gibot et al. 2014). Nanoencapsulated rhodamine has also been used for killing the multidrug-resistant bacteria present in sewage treatment plants using photodynamic therapy (Vimaladevi et al. 2016).

Nonbiodegradable Nanoparticles

Ceramic-based nanoparticles and metal-based nanoparticles that do not undergo degradation in the biological system come under the category of nonbiodegradable nanoparticles. Regardless of their nonbiodegradable nature, these nanoparticles have attracted much attention in the PDT field owing to the excellent characteristics such as tunable size, shape, and porosity. Various nanoparticles such as silica nanoparticles, magnetic nanoparticles, gold nanoparticles, zinc oxide nanoparticles, and quantum dots are being used as nanocarriers for PS (Allémann et al. 1995).

Noble metal nanoparticles are highly stable under irradiation compared to the organic dyes used in PDT. The very high extinction coefficient of metal nanoparticles like gold and silver becomes another advantage over the conventional PS. It is possible to tune the plasmon band of metal nanoparticles, and that can be optimized in biological transparency window where the radiation has its maximum depth of tissue penetration (Ghosh et al. 2011). For example, the plasmon band of gold nanorods can be shifted to 950 nm by changing the aspect ratio. Studies have shown that the molecular oxygen can be absorbed on the metal nanoparticle surface to promote rapid energy transfer from nanoparticles to oxygen for the generation of

singlet oxygen (Krajczewski et al. 2019). In addition to the generation of ROS, gold nanoparticles induce temperature increase in the local tissues on the basis of photothermal therapy (PTT) that can suppress the tumor growth (Vankayala et al. 2014). Nanocomposites are another set of nanoparticles that are widely used in PDT. The organic dye methylene blue encapsulated in a silica shell around the gold nanostars exhibits better PDT and PTT efficacy than the bare methylene blue (Fales et al. 2011). ALA immobilized on gold and silver nanoparticles is widely used as PS in clinical applications (Yazdi et al. 2018). Similarly, the quantum yield of ROS from riboflavin functionalized on the surface of the silver nanoparticles increased 1.8-fold compared to riboflavin alone (Rivas Aiello et al. 2018). Semiconductor nanoparticles or quantum dots with higher band gap than that of singlet oxygen are also widely used as PS nowadays. In vitro cytotoxicity assay on HeLa cells showed micromolar concentration of graphene quantum dots (400–800 nm) that can kill 60% cells after 10 min of irradiation (Ge et al. 2014). Surface-functionalized CdTe quantum dots using meso-tetra(4-sulfonatophenyl)porphine dihydrochloride led to the shift of luminescence maxima to the blue region. The quantum yield of the system was estimated to be very high, whereas no generation of singlet oxygen was observed without surface functionalization (Shi et al. 2006). Zinc oxide quantum dots with an average diameter of 11.6 showed better efficacy in producing ROS like hydroxyl radical and superoxide anion after irradiation at 400–500 nm (Yang et al. 2020).

Conclusion

PDT as a major alternative for the treatment of cancers has a huge potential for the future. In the small number of patients who have received this mode of treatment, healing was excellent without scarring, which is an additional benefit of this therapeutic modality. Improvement in optical technology provides for ease of illumination in deep-seated tumors besides the ease of topical application in the case of superficial lesions. Finding ideal PS molecules has proven to be a major challenge. Porphyrin-related structures have been a popular choice, although a number of nonporphyrin PSs have attracted attention. Parameters like shorter activation time, longer activation wavelength, and higher yield of $^1O_2$ have been some of the important criteria. Apart from the improved photophysical properties, emphasis has also been put on better delivery through the use of receptors or biomolecules like small peptides and folic acid as ligands. One of the major advantages is the recent improvement in PSs with a short period of cutaneous photosensitivity that imparts convenience of treatment in outpatient and daycare settings. Uses of nanostructures to enhance activity and delivery have been gaining consideration in recent times. Besides the therapeutic activity that comes from their photo-activating capacity, the auto-fluorescence characteristics of the PSs impart them the additional benefit that can be exploited for detection. The selective accumulation property can be utilized for imaging purposes to detect the precancerous or early malignant lesions that aid in identifying the tumor margins.

Acknowledgments The authors (P.P., A.G., and K.G.) are grateful to Chettinad Academy of Research and Education for the infrastructural support; the authors (S.H. and R.G.) acknowledge the University of Kalyani, DST-PURSE (GoI), and UGC-SAP (GoI) for supporting this work.

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