Recent Advancements in Nanomaterials for Photodynamic Therapy of Cancers

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Abstract

Surgery, chemotherapy, and radiotherapy are the commonly used therapeutic interventions for cancerous tumors but they often cause deleterious side effects to the normal cells surrounding the tumor site leading to poor prognosis. In the past two decades, photodynamic therapy has emerged as one of the most investigated techniques for treating cancerous tumors due to minimum invasiveness, cancer cell selectivity, and high therapeutic effect. In photodynamic therapy, a photosensitizer is excited with a light source, resulting in a photochemical reaction within the cell's microenvironment that generates cytotoxic free radicals. However, the overall therapeutic efficacy of photodynamic therapy depends on several factors such as tumor location and microenvironment, photosensitizer molecule, and wavelength and intensity of the activation light. Most of the photosensitizers are highly hydrophobic which often leads to aggregation in an aqueous environment resulting in decreased singlet oxygen quantum yield. By using nanomaterials as delivery agents, photosensitizers can be delivered at the target site with high load and increased aqueous solubility leading to increased therapeutic efficacy. In this chapter, we review different types of nanomaterials as delivery agents for photosensitizers. We also summarize the application of nanomaterials as down-converting and up-converting photosensitizers, and their advantages over conventional photosensitizers.

Keywords

Photodynamic therapy · Photosensitizers · Nanoparticles · Reactive oxygen species

Introduction

As per the GLOBOCAN 2020 database from the International Agency for Research on Cancer, the global prevalence of cancer in 2020 was more than 19.3 million cases resulting in more than ten million deaths. Most cancers result from malignant tumors, which involve uncontrollable cell growth that eventually leads to the invasion of other body parts (Hejmadi 2014). One of the major reasons for cancer is genetic mutations inherited from parents at birth or acquired later due to environmental and lifestyle factors such as smoking, exposure to harmful chemicals and radiation, and obesity. Even though early detection, screening, and palliative care are a few ways to enhance the survival rate of the patients, there is a greater need for developing better treatment options that are efficient, affordable, and acceptable to people living with cancer. Current therapeutic intervention for cancer treatment includes surgery, radiation therapy, and chemotherapy. Surgery and radiation therapy

are more commonly used in treating localized and non-metastatic tumors, while chemotherapy is used for treating patients with tumors that have metastasized to distant organs in the body. The major drawback of chemotherapeutic agents is non-selectivity, which often results in the death of healthy cells and multiple drug resistance (Jing et al. 2019).

Over the years, photodynamic therapy (PDT) has emerged as one of the most efficient methods for treating both benign and malignant tumors due to its minimal invasiveness, high target selectivity, and high therapeutic effect. In PDT, a light-absorbing chemical, known as a photosensitizer, is administered, which upon light irradiation produces reactive oxygenated species (ROS), leading to the death of cancerous cells (Turubanova et al. 2019). The photophysical and photochemical properties of photosensitizers govern the extent of the death of cancer cells and can be tuned to produce efficient photosensitizers. The efficacy of PDT has been enhanced by using engineered nanomaterials for efficient ROS generation at the target site (Wang et al. 2004; Bruns et al. 2017). This chapter reviews the principle, different generations of the photosensitizers, their limitations, and the advantages of using engineered nanomaterials to increase the anti-cancerous efficacy of photosensitizers.

Principle of Photodynamic Therapy

The excitation of a photosensitizer by light is a physical process, leading to type I and type II photochemical processes producing reactive oxygen species (ROS) that result in cell death. Upon light irradiation, a photosensitizer gets excited from the ground state to the upper excited state from where it non-radiatively decays to the first-excited state through internal conversion. From the first excited state, it can undergo three different processes, which are (1) non-radiative decay back to the ground state (2), radiative decay to the ground state, i.e., fluorescence (3), or inter-system crossing to the long-lived triplet state (Lakowicz 2007). From the excited triplet state, it decays to the ground state by emitting radiative phosphorescence, but most importantly, it interacts directly with the cellular substrate to form radicals, which upon reacting with oxygen, produces reactive oxygen species (ROS) like hydrogen peroxides (H_2O_2), superoxide anion radicals (O_2^-), alpha-oxygen, and hydroxyl radicals (OH^*) known as the type I reactions. On the other hand, the energy from the excited photosensitizer is transferred to the molecular oxygen in the triplet ground state to form singlet oxygen with high reactivity, known as the type II reaction as shown in the Fig. 1. The hyperactive singlet oxygen reacts with the amino acids such as methionine, tryptophan, and histidine of the proteins and the unsaturated lipids present in the cellular and the nuclear membranes, leading to cell death. Photosensitizers can involve either type I or type II or both processes simultaneously to kill the cancer cells. Besides, photosensitizers have also been developed, which work by photo-induced electron transfer, fluorescence resonant energy transfer, and intramolecular charge transfer processes (Urano et al. 2009; Fan et al. 2013; Wu et al. 2017).

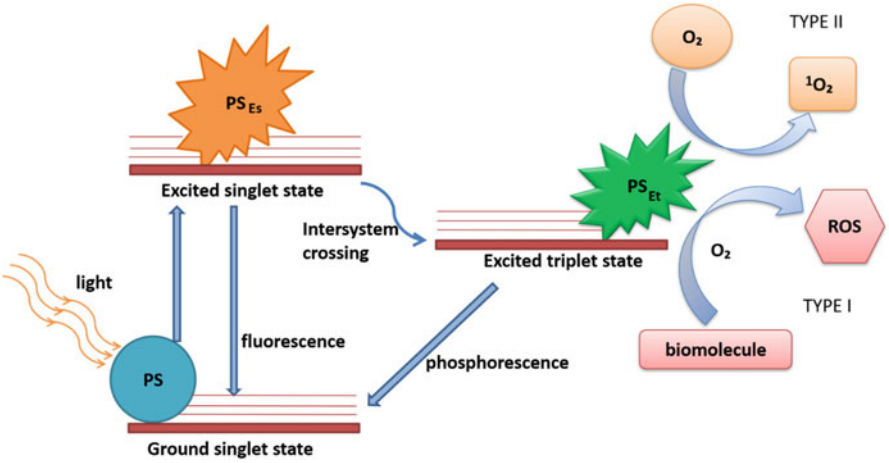

Fig. 1 Schematic representation of Type I and Type II reactions in PDT (photodynamic therapy). the PS reaches an excited singlet state after light absorption and reaches an excited singlet state. Following PS an excited singlet state falls to an intersystem crossing where PS in a triplet excited state. From triplet excited state, PS can react in two ways, i.e.., Type-I and Type-II. In type-I, PS reacts with biomolecules through a hydrogen atom (electron) transfer to form radicals, which react with molecular oxygen to generate ROS. Whereas Type-II reaction, PS in its triplet state, can directly reacts with oxygen through energy transfer, generating singlet oxygen. PS: photosensitizer; PSEs: PS excited singlet state; PSEt: PS excited triplet state; ROS: reactive oxygen species; $^1O_2$: singlet oxygen

Conventional Photosensitizers and their Limitations

The utilization of photosensitizers for diagnostic and therapeutic purposes began as early as the year 1903 when eosin combined with white light was used to treat skin cancers (Hamblin 2020). Based on their properties and the period in which they were developed, the photosensitizers are classified into different generations (Hamblin 2020), The first-generation photosensitizer developed in the nineteenth century were hematoporphyrins, which were devised from dried blood and constituted a mixture of porphyrins with specific properties (Maldonado-Carmona et al. 2020; Kou et al. 2017). Due to the heterotypic nature of the blood-derived photosensitizers, they were not ideal as fluorescent diagnostic tools. The hematoporphyrins on further processing led to the formation of a derivative of hematoporphyrin with better tumor localization, and on further purification resulted into a more efficient clinically used photosensitizer known as Photofrin® or porfimer sodium (Kwiatkowski et al. 2018). Although the first-generation photosensitizers showed promise in clinical setting, there were several disadvantages associated with them such as short wavelength (< 700 nm) light absorption, requirement of high intensity light, and high dosage, which often resulted in skin photosensitive toxicity (Kou et al. 2017; Gomer 1991). To overcomes the inefficiencies of the first-generation photosensitizers, a range of second-generation

photosensitizers were developed, which consisted of texaphyrins, pheophorbides, phthalocyanines, and bacteriopheophorbides. The second-generation photosensitizers were used to reduce the drug dosage, thereby minimizing the photosensitivity of the skin. The adjustments made in their optical properties led to the treatment of tumors in deep tissues. The advent and integration of nanotechnology and genetic engineering led to the development of the third-generation photosensitizers (Mfouo-Tynga et al. 2021). The third-generation photosensitizers include the second-generation photosensitizes modified through nanotechnology or genetic engineering for better target selectivity and high therapeutic effect.

First-Generation Photosensitizers

The first-generation photosensitizers constitute the hematoporphyrin, the hematoporphyrin derivatives, and Photofrin®. The advent of hematoporphyrin for tumor localization in cancer treatment led to the research of porphyrin-based photosensitizers. The complex mixture of porphyrin compounds present in the hematoporphyrins were chemically modified and purified to produce hematoporphyrin derivatives, comprising an exclusive mixture of porphyrin oligomers, dimers, and monomers (Hlapisi et al. 2019). The hematoporphyrin derivatives synthesized were known to produce less photosensitivity on the skin, and they also proved to be highly selective in the treatment of tumors compared to the hematoporphyrins. Photofrin® or porfimer sodium was produced by combining up to eight porphyrin oligomers and dimers derived from the hematoporphyrin derivatives (Josefsen and Boyle 2008). The advantages of using Photofrin® were that it exhibited minimal toxicity, led to the effective reduction of the tumor, and their low hydrophobicity property allowed intravenous delivery. Although hematoporphyrin oligomers were considered advantageous, the uncertainty in the prediction of whether they were esters or ethers and if the side chains consisted of either a vinyl group or a hydroxyethyl group proved to be difficult in determining the structure of the compound and identifying its components (Chilakamarthi and Giribabu 2017). Due to these uncertainties and significant variation in the individual component, the widespread use of hematoporphyrin derivatives declined. Although the photosensitivity reduced considerably in hematoporphyrin derivatives, it led to the accumulation in the normal tissue under the skin, and the patient had to avoid sunlight and other radiations for up to 6 – 8 weeks. The absence of a narrow absorption band, the administration of a high amount of the drug to induce the required phototherapeutic effect, and very low tumor localization led to the advent of novel second-generation photosensitizers.

Second-Generation Photosensitizers

In the late 1980s, the second-generation photosensitizers were developed with higher extinction coefficients at wavelengths longer than 630 nm to address the shortcomings of the first-generation photosensitizers (Dougherty et al. 1998). The second-generation

photosensitizers are categorized into porphyrinoid compounds and non-porphyrinoid compounds. Porphyrin and other macrocyclic porphyrin-based compounds such as phthalocyanines, chlorins, texaphyrins, pheophorbides, bacteriochlorins, and bacteriopheophorbides constitute the porphyrinoid compounds. In contrast, curcuminoids, anthraquinones, xanthenes, phenothiazines, cyanines, and metal-based derivatives such as tin ethyl etiopurpurin (SnET2), aluminum phthalocyanine tetrasulfonate [$AlPc(SO_3H)_4$], and Si(IV)-naphthalocyanine (SiNC) belong to the non-porphyrinoid class of photosensitizers (Lucky et al. 2015). In comparison with the hematoporphyrin derivatives, the second-generation photosensitizers exhibited a higher yield of the singlet oxygen and presented a higher tumor-to-normal tissue concentration, thus producing a maximum antitumor effect. They require shorter time to accumulate in the tissue, thereby reducing the duration of the treatment, as both administration of the drug and PDT are carried out on the same day, thus making it convenient for outpatient procedures (Jones 2016). Hence, due to a short treatment duration, less photosensitivity of the skin has been observed with second-generation photosensitizers. Some of the second-generation photosensitizers like the lutexaphyrin, tetraphenyl porphine (TPPS3, TPPS4), aminolevulinic acid (ALA), AlPcS4, and mono-L-aspartyl chlorin e6 (MACE) are hydrophilic. In contrast, photosensitizers which have a porphyrin ring such as Tookad®, meta-tetrahydroxy phenyl chlorin (mTHPP), SnET2, Chlorin e6 (Ce6), unsubstituted phthalocyanines, bacteriochlorophyll-a, and 2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH) are hydrophobic (Huang et al. 2018). The drug administration route and its pharmacokinetics depend largely on the degree of hydrophobicity of the photosensitizer. Hydrophobic photosensitizers exhibit a higher tumor to normal tissue localization at the ratio of 7:1 to 8:1. In contrast, the hydrophilic photosensitizer exhibit a low localization ratio of 2:1 (Lucky et al. 2015). Even though the hydrophobic property of the photosensitizer allows it to permeate inside the cell, it agglomerates in aqueous solutions, thereby limiting its quantum singlet oxygen production and its clinical application. Therefore, it is mandatory to maintain an equilibrium between the degree of lipophilicity and hydrophilicity for successful clinical application. Various polar hydrophilic substitutes like the hydroxyls, pyridinium substituents, carboxyl acid, carbonyl groups, sulfonic acid, and quaternary ammonium salts are attached to any one or more of the 12 positions in the porphyrin ring to enhance the aqueous solubility of the photosensitizer (Khadria et al. 2017). In addition to the side chain substitutions, the porphyrin ring can be modified to hold a central metal ion, thereby improving the pharmacological and photophysical properties of the drug (Kitanosono et al. 2018). We have tabulated various first-generation and second-generation photosensitizers used as PDT agents in cancer treatment are tabulated in Table 1 with their mechanisms of action.

Third-Generation Photosensitizers

The third-generation photosensitizers were developed to overcome the disadvantages of second-generation photosensitizers in terms of specificity, targeting, and delivery abilities (Mfouo-Tynga et al. 2021). The design of the third-generation photosensitizers is performed in two ways (Hejmadi 2014). By chemical

Table 1 List of first-generation and second-generation Photosensitizers used in PDT of cancer with Characteristics and Mechanism of action

Photosensitizer	Chemical family	Wavelength (nm)	Characteristics	Mechanism
Photofrin®	Porphyrin	630	First-generation photosensitizer. Intracellular localization in the mitochondria and the plasma membrane	Controls the vascular factors and mediates cell death
2-[1-hexyloxyethyl]-2-devinylpyropheophorbide-a (HPPH)	Chlorin	665	Second-generation photosensitizer. Intracellular localization in the lysosomes and mitochondria	Significantly reduces the tumor regrowth
5-aminolevulinic acid (5ALA)	Porphyrin Precursor	630	Second-generation photosensitizer. Intracellular localization in mitochondria	Induces cell apoptosis in the mitochondria
Rostaporfin, SnEt2: Tin ethyletiopurpurin I, or (Purlytin)	Chlorin	660	Second-generation photosensitizer. Intracellular localization in the lysosomes	Provides high retention time for the drug in the cell
Temoporfin, mTHPC: Meso tetrahydroxy phenyl chlorine (Foscan)	Chlorin	652	Second-generation photosensitizer. Intracellular localization in the mitochondria, Golgi apparatus and ER	Induces necrosis in the tumor tissue

encapsulation/conjugation of the photosensitizer with the delivery vehicles and carriers to efficiently improve the administration to the targeted tissue (Jing et al. 2019). By modifying the photosensitizer with the integration of biological conjugates such as peptides, antibodies, and sugar molecules to ensure tumor specificity. Even though several third-generation photosensitizers have been shown to possess high efficacy in *in vitro* models, only a few have been successful *in vivo*. Further research is required to produce highly targeted as well as effective third-generation photosensitizers which prove a greater efficacy in clinical studies than the previous-generation photosensitizers.

Current Limitations of Photodynamic Therapy

The potential of PDT to function as the primary treatment method for cancers is widely debated because of its inefficiency to completely eradicate bulky, solid, and

deep tumors. PDT is mostly useful only for the treatment abscesses and lesions that can be accessed through an endoscope or an adjuvant (Kim and Darafsheh 2020). With PDT, the treatment of a completely spread cancer has not been possible as of today because of unavailability of a technology which can irradiate the whole body with electromagnetic radiation in the visible to NIR region. Simultaneously, the dosage of light should reach every spot on the tumor for its effective elimination, and scare of residual tumor re-growth remains due to the underexposure of light, while overexposure leads to induction of toxicity in the surrounding normal tissues. Even though the successive generations of photosensitizer developed were successful to a certain extent, they failed in achieving overall selectivity, specificity, and efficiency in eradicating the tumors (Baskaran et al. 2018). To overcome the limitations of the conventional photosensitizers, engineered nanomaterials were introduced, which significantly increased the efficiency of PDT.

Nanoparticles in Photodynamic Therapy

Nanoparticles have shown immense promise to overcome the drawbacks of classic photosensitizers in PDT. Delivery of photosensitizers for PDT is classified as active or passive depending on the absence or presence of targeting molecule on the surface. The passive carriers consist of polymer-based biodegradable and non-biodegradable nanoparticles made of materials such as PLA, PLGA, gold, and polyacrylamide, while the active carriers include photosensitizer nanoparticles up-converting nanoparticles, and self-lighting nanoparticles. The primary advantages of nanoparticles in photodynamic therapy are

1. Highly target specific.
2. Large surface to volume ratio.
3. Surface modifications improve bio-distribution, cell uptake, pharmacokinetics.
4. Increased permeability and retention effect.
5. Prevent premature release of photosensitizer which may result into non-specific accumulation in normal tissues.

Nanoparticles as a Delivery Agent

Nanoparticles have reported to deliver vaccines in a controlled manner with better immune responses and target-specific delivery of photosensitizers (Fig. 2). Biodegradable nanoparticles undergo hydrolytic or enzymatic degradation, thereby reducing the bioaccumulation in the biological system and leading to clearance from the body. In contrast, non-biodegradable nanoparticles do not degrade in the biological system but can act as theragnostic agents with several functions.

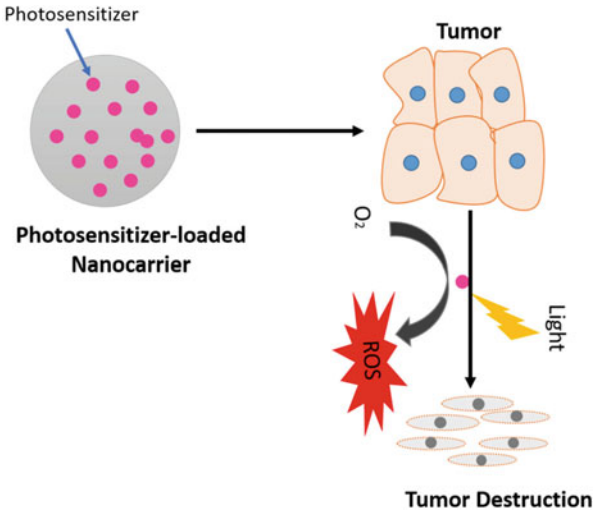

Fig. 2 The application of nanomaterial as carrier for photosensitizer drugs for significantly enhanced efficacy and tumor-selectivity of photodynamic therapy

Biodegradable Nanoparticles

(i) Lipid-based nanoparticles

Liposomes are spherically structured lipid bilayers made of natural or synthetic phospholipids of various sizes, compositions, and cholesterol to maintain their structural stability (Gopi and Balakrishnan 2021). Liposome-based nanoparticles are used as carriers to deliver photosensitizers with different molecular weights, hydrophobicity, and charge. They are biodegradable, have high drug loading and release capacity, and can be easily chemically modified. Unilamellar liposomes have only one lipid bilayer of size 20 – 100 nm and can be loaded with only a small amount of photosensitizer, whereas multilamellar liposomes consist of multiple lipid bilayers of size 100 – 5000 nm and can carry both hydrophilic and lipophilic photosensitizers (Mironov et al. 2018). Although multilamellar liposomes are highly stable, they exhibit a few limitations such as degradation of liposomes due to HDL lipid exchange, low target specificity, and opsonization by normal cells, which can lead to their rapid clearance from the system (Derycke and De Witte 2004). This can be overcome by the use of stealth liposomes in which the liposomes are modified with a low immunogenic compound with good aqueous solubility such as polyethylene glycol (PEG) to increase their half-life and to reduce the identification of the liposomes by the reticuloendothelial system (Shen et al. 2018).

The drug Visudyne, approved by the FDA in the United States in the year 2001 to treat vision impairment by age-related macular degeneration, is a formulation of lyophilized liposomes with encapsulation of Verteporfin, a second-generation photosensitizer(*Am J Ophthalmol* 2001). The pharmacokinetics of Visudyne has been improved by modifying the liposomes with PEG, folic acid, and antibodies. Another

drug Fospeg, a liposomal formulation of PEG carrying photosensitizer mTHPC, showed increased cellular permeability and photosensitizer distribution in the tumor cells, thereby reducing cytotoxicity and increasing the half-life stability and circulation time (Reshetov et al. 2012). Liposomes made of phosphatidylethanolamine, sodium stearate, and cholesterol are used to deliver photosensitizer 5-aminolevulinic acid (5ALA) for PDT of cancer (Fang et al. 2008).

Porphysomes are self-assembled structures of liposomes where photosensitizers are encapsulated inside the liposomal nanoparticles (Lovell et al. 2011). Porphysomes have unique photoacoustic and photothermal properties along with fluorescence quenching (Lovell et al. 2011). Triggered liposomes, based on the degradation of liposomes in response to the exogenous or endogenous stimuli employing temperature and irradiation or pH and enzymes help in the targeted release of photosensitizer in the tumor cells (Majumdar et al. 2014).

Vector molecules such as monoclonal antibodies, aptamers, glycoproteins, polysaccharides, peptides, ligands such as growth factors, and folic acid have been employed to increase the efficiency of liposomal photosensitizers (Chen et al. 2007). In rodents, a significant decrease of tumor size in ductal adenocarcinoma was observed when the animals were treated with liposomes carrying photosensitizers coated with monoclonal antibody Bevacizumab photosensitizer binds to the vascular endothelial growth factor (VEGF) to inhibit its activity (Spring et al. 2016). Similarly, ovarian carcinoma was treated effectively with liposomes containing Verteporfin and surface antibodies which targets the epidermal growth factor receptors (EGFG), and folate conjugated liposomes were used to treat cervical cancer co-loaded with C6 ceramide to target the folate receptors present in HeLa cells, A2780-ADR and H69-AR cells (Michy et al. 2019).

Micelles *Micelles* are biodegradable nanoparticles (5 – 100 nm) consisting of an inner hydrophobic core and outer hydrophilic surface. There are two types of micelles, the micelles consisting of conjugates of lipids with water-soluble polymers known as lipid micelles, and the micelles containing amphiphilic block copolymers known as polymeric micelles (Mironov et al. 2018). Phospholipids and photosensitizers are bound to each other by either covalent or non-covalent bonds to form lipid micelles. Lipophilic micelles are formed by the fabrication of phosphatidylcholine, thermosensitive phosphatidylethanolamine-poly (N-histidine), pH-sensitive phosphatidylethanolamine-poly(I-histidine), and other lipopolymers loaded with Chl-e_6. This system was found to increase the circulation time of the particles in the blood with increased stability, reduced cytotoxicity, and enhanced photodynamic property. The core of polymeric micelle nanoparticles contains hydrophobic polymer molecules, and the outer shell contains hydrophilic PEG molecules (Zhou et al. 2016). For instance, Pluronic PEG-PPO-PEG micelle has been used in the entrapment of Verteporfin photosensitizer for PDT of Human SKOV-3 ovarian cells while MCF-7/WT breast cancer cells have been treated with Photofrin II loaded with polymeric Pluronic P123/F127 mixed micelles (Managa et al. 2017).

(ii) **Lipoprotein-based nanoparticles**

Lipoprotein-based nanoparticles have triglycerides and cholesterol esters in their inner hydrophobic core and apoproteins, cholesterol, and phospholipids in their outer shell. Highly hydrophobic photosensitizers are incorporated into the inner core of the nanoparticles and transported to the tumor tissues in the body. As many types of LDL receptors are present on the surface of tumor cells, H_p complexed with low-density lipoproteins showed the target-specific killing of tumor cells since hydrophobic photosensitizers are easily conjugated with LDL, which is effectively internalized by cells. Kessel studied the delivery of nanoparticles by incorporating photosensitizers such as mono-, di-, and tetrasulfonated tetraphenyl porphyrins into LDL(Kessel 1986). Rapid thrombosis and necrosis of tumor cells were observed when benzoporphyrin was incorporated into LDL-based nanoparticles for PDT to treat choroidal melanoma in rabbits (Ng et al. 2011). Similarly, increased photodynamic activity was observed when third-generation photosensitizer bacteriochlorin e_6 bisoleate was loaded into HDL for the treatment of human HepG2 liver carcinoma in nude mice (Marotta et al. 2011).

(iii) **Polymeric nanoparticles**

Polymers are biodegradable materials in which the composition, morphology, and surface properties are optimized for the controlled release of photosensitizers and drugs with varying degrees of charge, molecular weight, and hydrophobicity. The first report on polymeric nanoparticles was observed in the early 1990s using polyalkylcyanate nanoparticles, which failed due to their low carrier capacity and rapid drug release. Increased circulation time of nanoparticles was observed when the surface was modified with polymers like polyethylene oxide and polyethylene glycol.

Polyester and Polyacrylamide-Based Nanoparticles PHAs are naturally occurring polymers, and poly (β-amino esters) (PbAE), poly (orthoesters), as well as poly (α-hydroxy esters), are synthetic polymers of polyesters. Nanoparticles and micelle prepared by polymers such as poly (glycolide) (PGA), poly(D, L lactide) (PLA), and poly(ε-caprolactone) (PCL) constitute the polyester and polyacrylamide based nanoparticles (Hejmadi 2014). Biopolymer-based nanoparticles can prevent premature leakages, are highly biodegradable, and possess increased solubility. FDA approved combinations of PLGA with PLA and PGA polymeric nanoparticles to encapsulate photosensitizer for photodynamic therapy in humans (Arroyo-Maya and McClements 2015). The polymeric nanoparticles are also easily degraded by hydrolysis rather than enzymatic degradation, and normal clearance mechanisms in the body easily remove the nontoxic degraded products. Zinc (II) phthalocyanine incorporated into the PLGA nanoparticle provides enhanced selectivity in targets, increases cytotoxicity by photogeneration of singlet oxygen, and therefore is considered as a promising drug delivery system (Ricci-Júnior and Marchetti 2006).

The limitations of immune recognition and sudden removal of macrophages by the immune system were overcome by using PEGylated polymeric nanoparticles, also called stealth nanoparticles. The stealth nanoparticles increased the circulation time in the blood, and the PEGylated PLGA were found to have an increased half-life of about 7 hours, whereas the PLGA without PEGylation has a half-life time of only about 13 to 35 seconds. The premature release of photosensitizer from the polymeric nanoparticles leading to lesser bioavailability in the target tissue is overcome by using bioresponsive polymeric systems such as pH-responsive systems to produce pH-responsive nanoparticles based on the difference of the pH in the normal cell and the tumor cell. For instance, 2- (diisopropylamine)ethyl methacrylate (DPA) based poly- (ethylene glycol) methacrylate-co-DPA (PEGMA-co-DPA) nanoparticles were able to release the hydrophobic photosensitizer m-THPC only at a pH below 6.89, leading to target specific release of photosensitizer (Peng et al. 2010).

Multifunctional PLGA nanoparticles in which PLGA coupled with methoxy-PEG (mPEG) or Ce6) are used to carry inorganic cargo-iron oxide (Fe_3O_4) for targeted photodynamic therapy of human nasopharyngeal epidermal carcinoma-KB in nude mice (Zoppellaro 2020). Enhanced killing of tumor cells were observed when the chemotherapeutic drug DOX along with photosensitizer hematoporphyrin monomethyl ether (HMME) [PEG− PDLLA-DOX-HMME] were loaded onto the polymeric nanovesicle poly(ethylene glycol)-block-poly(D, L-lactic acid) [PEG−PDLLA] for the treatment of human hepatocellular carcinoma (HepG2) cells (Xiang et al. 2013).

(iv) **Dendrimer-based nanoparticles**

Dendrimers are 3D tree-like branched macromolecules with functional groups at the peripheral side with inner cavities loaded with the drug molecules. They can be used as photosensitizer carriers due to the presence of several functional groups to which photosensitizers can be conjugated via encapsulation or covalent bonding. Photoexcitation and induction of $^1O_2$ are used to release photosensitizer molecules by breaking the covalent bond between the dendrimer and the photosensitizer, which prevents the premature release of photosensitizer and also reduces the toxicity. Hydrophobic silicon-based phthalocyanine (PcSi) has been encapsulated into PPI dendrimer, which has a drug encapsulation efficiency of ~20% w/w. This method has been shown to simplify the process of dendrimer synthesis and conjugation of the photosensitizer. Increased biocompatibility and targeted photosensitizer delivery have been achieved by forming a complex with PEG and luteinizing hormone-releasing hormone (LHRH) peptides (Taratula et al. 2013).

(v) **Natural macromolecule-based nanoparticles.**

Chitosan Chitosan, a natural polysaccharide found in the shells of insects and crustaceans, is made of repeating units of b-(1-4)-D-glucosamine and N-acetyl-D-glucosamine. Chitosan is a widely used polymer since it is biodegradable, biocompatible, and has low immunogenicity. Chitosan is soluble in the acidic medium,

whereas insoluble in water and organic solvents. Amphiphilic chitosan-based nanoparticles have been reported as carriers for hydrophobic anticancer drugs and photosensitizers in photodynamic therapy (Melchels et al. 2010). Chitosan nanoparticles are prepared by modification of hydrophobic groups such as fatty acids. The derivatives of chitosan molecules are self-assembled to form a nanoparticle by conjugating with a hydrophobic moiety. Chitosan has been modified with hydrophobic ursodeoxycholic acid (UDCA) to form a chitosan nanoparticles of size 200 – 400 nm for delivering Chlorin e_6 to the cholangiocarcinoma cells (HuCC-T1) (Lee et al. 2013). Increased phototoxicity and ROS generation were observed by enhancing the uptake of chlorin e_6 by the tumor cells. Vitamin-E-grafted chitosan nanoparticles added with cyclic RGD peptides have been used to carry Temoporfin and interact with $\alpha_v\beta_3$-integrin receptors in U87MG glioblastoma cells. Singlet oxygen species are released when irradiated with light and increased anti-tumorous activity, and less systemic cytotoxicity was produced by the chitosan nanoparticles modified with RGD peptides (Chen et al. 2017).

Albumin Nanoparticles made of albumin have been used as a lipophilic drug carrier for PDT (Jeong et al. 2011). The endogenous albumin pathway is exploited for the transport of chemotherapeutic drugs at the tumor's specific target, thus preventing the accumulation of drug nanoparticles at other parts of the body (Elzoghby et al. 2012). Metastatic breast cancer was treated with the paclitaxel-loaded albumin nanoparticles and was approved by the FDA in the U.S. under the trade name Abraxane (Gradishar 2006). To reduce limitations such as instability, complicated fabrication techniques, and unintended release of photosensitizer before reaching target cells, the nanoparticles modified by conjugating Ce6 to lysine residues within human serum albumin are promising. The drug is released at a specific target site by increasing the circulation time in the blood leading to increased therapeutic efficacy. The photosensitizer loaded in the albumin-based nanosphere with the DOX chemotherapeutic drug is a promising drug-delivering agent that has been reported to show necrosis in both the central and peripheral regions of the tumor with less toxic side effects (Quan et al. 2011).

Gelatin Gelatin is a water-soluble polymer obtained from porcine or bovine collagen by hydrolysis and is widely used as a food additive. Due to its high biocompatibility and low immunogenicity, it has been used as a drug carrier (Lee et al. 2021; Foox and Zilberman 2015). The surface of the gelatin nanoparticle has been modified with polyethylene glycol (PEG) to carry hypocrellin B (HB) for photodynamic therapy (Zhou et al. 2009). The drug loading and drug release efficiency was improved by adding polylactic acid with the PEG-modified gelatin nanoparticles. The phototheranostic agent (PTNA) has been developed by incorporating the photosensitizer trans-AB-porphyrin in gelatin nanomatrix (Kirar et al. 2019).

Hyaluronic Acid Hyaluronic acid is a biodegradable component of the extracellular matrix, and hyaluronic acid nanoparticles are widely used as a drug delivery vehicle in photodynamic therapy because of their low immunogenicity, less toxicity, high

biodegradability, and good biocompatibility (Lee et al. 2020). Hyaluronic acid nanoparticles can act as both carriers and targeting agents due to overexpressed hyaluronic acid receptors on the surface of tumor cells. Stable spherical nanostructures have been formed by covalent conjugates of Chl-e_6 and hyaluronic acid coupled via adipic dihydrazide (ADH). Hyaluronic acid nanoparticles are disassembled by the enzyme hyaluronidase and internalized by the target cells of lung cancer by CD44 receptors. Laser irradiation has been used to image the tumor tissues by photoacoustic imaging and near-infrared fluorescence, thereby producing high efficiency in suppression of the tumor cells than the free Chl-e_6 (Li et al. 2016). Self-assembled nanogels are based on fabrication by the conjugates of acetylated hyaluronic acid and pheophorbide *a* (Li et al. 2010).

Non-biodegradable Nanoparticles

Non-biodegradable nanoparticles are not often used for drug delivery due to their non-degradability even though they have multifunctional properties such as good optical properties and tenability into any shape and size. In non-biodegradable nanoparticle carrier systems, the photosensitizers do not directly kill the tumor cells but act like a catalyst converting non-toxic compounds into toxic products which kill the cancer cells. Ceramic-based nanoparticles have been coupled with photosensitizer HPPH (2-devinyl-2-(1-hexyloxyethyl) pyropheophorbide and anticancer drugs on irradiation by light with a wavelength of 650 nm, generated a singlet oxygen species for the treatment of HeLa and UCI-107 tumor cells (Chatterjee et al. 2008).

Silica-Based Nanoparticles such as organized modified silica (ORMOSIL), Stöber silica nanoparticles, and mesoporous silica nanoparticles are used in PDT due to their inert property, transparency, and porosity. The surfaces of silica nanoparticles have been modified with polymers like PEG for specific tumor targeting. Silica nanoparticles of size 50 nm to 1 μm can be synthesized by sol-gel procedure at low temperature, and their pore sizes can be controlled by surfactants such as C12-trimethylammonium bromide or C16-trimethylammonium bromide. The two strategies of loading photosensitizers into the nanoparticles are covalent and non-covalent bonding. Covalent coupling is used to reduce the effect of premature release of photosensitizer from the vehicle. ORMOSIL nanoparticles with the pore size of 0.5 – 1 nm diameter have been used to prevent the leakage of photosensitizer HPPH. Highly monodispersed silica nanoparticles of size about 25 nm having large surface area have been used to deliver photosensitizer PpIX to the targeted HeLa cells. On irradiation at 532 nm resulting in necrosis of cancer cells (Qian et al. 2012).

Gold Nanoparticles have unique properties, such as producing heat for photodynamic therapy and surface plasmon resonance (SPR). The three-component system of PS/gold/phase transfer reagent (PTR) of 2 – 4 nm is constructed in which PTR tetraoctylammonium bromide (TOAB) is used to stabilize the photosensitizer phthalocyanines, which generate the singlet oxygen, and TOAB has increased the triplet energy transfer to the oxygen molecule. This system have been shown to be taken up

by HeLa cells effectively, and 57% of cell death was observed (Wieder et al. 2006). Covalent attachment of silicon phthalocyanine 4 (SiPc4) on PEGylated gold nanoparticles showed less drug release efficiency than the non-covalent attachment of photosensitizer into the gold nanoparticles. Multifunctional gold nanoparticles have been used in photothermal and photodynamic therapy by using two different wavelengths. Gold nanorod AlPcS4 complex is used to reduce 95% of the tumor growth by excitation at wavelengths of 810 and 670 nm lasers.(Wang et al. 2014).

Active Nanoparticles

Photosensitizer Nanoparticles Quantum dots have high photostability and fluorescence quantum yields, and their properties can be tuned based on their sizes. Quantum dots are water-soluble and can transfer energy to surrounding oxygen atoms because of which they can act as photosensitizers of their own. Traditionally, quantum dots cannot generate singlet oxygen, so several attempts were attempted to make it possible, and one such attempt was to covalently conjugate photosensitizers to the CdSe/ZnS via organic bridges (Sewid et al. 2021). All attempts made to achieve singlet oxygen from quantum dots alone suffered from low aqueous solubility and also toxic oxygen generated by quantum dots was not utilized.

Self-Lighting Nanoparticles Self-Lighting photodynamic therapy (SLPDT) is a combination of radiation therapy and PDT described as a new approach in cancer treatment. The *in vivo* agents used in the PDTs, like porphyrins, are attached to the photosensitizers thereby producing scintillation or persistent luminescence (Chen 2009). In this process, the cancer cells are killed by the production of singlet oxygen, by ionizing radiation such as x-rays. Conventional radiation therapy damages the health, and in combination with PDT gives lower levels of radiation. However, no studies have been reported to show its clinical efficacy.

We have enlisted various biodegradable and non-biodegradable nanoparticles used as nano-carriers for photosensitizers in photodynamic therapy in Table 2.

Nanoparticles As Down-Converting Photosensitizers Agent

Few nanoparticles can act as photosensitizers by themselves due to their unique optical absorption properties and their ability to generate ROS. For example, TiO_2 nanoparticles, ZnO nanoparticles, and fullerenes.

Zinc Oxide Nanoparticle Zinc oxide (ZnO) is a nanosized photocatalyst that is often found in cosmetics, paints, and medical materials and has similar bandgap energy as TiO_2. Zinc oxide nanoparticles have been reported to show antibacterial and anticancer activities, which could be attributed to mediated oxidative stress due to generation of reactive oxygen species (ROS). The oxidative stress can cause detrimental side effects and thus raise concerns over their application as drug delivery systems or therapeutic agents (Applerot et al. 2009). Hence, it is essential

Table 2 List of biodegradable and non-biodegradable nanoparticles used as drug carriers for photosensitizer for photodynamic therapy

Type of Nanoparticle	Model	Photosensitizer	Target	Advantages
Poly-lactic gluconic acid	*In vitro*	Verteporfin	EMT-6 Mammary Tumor cells	Increased intracellular Uptake and high Photocytotoxic effect.
Cationic nanolipoplexes	*In vivo*	(Chl-e_6), 1,2-dioleoyl-sn-glycero −3-ethylphosphocholine	SCC7 tumor Cells	Higher uptake and complete degradation of The tumor.
Micelle	*In vivo*	p N-(2-hydroxypropyl) Methacrylamide Polymer conjugated With zinc protoporphyrin IX complex	S-180	Longer half-life And targeted loading into Tumor cells.
Thermosensitive Liposomes	*In vivo*	Chl-e_6 and folic acid	Folic acid Receptor	Cancer significant Inhibition of Tumor without any Adverse effects.
Hyaluronic-based Nanoparticles	*In vitro*	Chlorin Chl-e_6	MCF-7	Enhanced cellular intake, Improved phototoxicity, Biocompatibility.
Chitosan nanoparticles	*In vivo*	Protoporphyrin IX	SCC-7	Higher uptake by tumor cells.
PEGylated gold Nanorods	*In vivo* *In vitro*	Ce6 molecules with PEG	MDA-MB-435	Significant reduction in Tumor volume than free Ce6 and enhanced Anti-cancer efficacy

to find a strategy such that undesired toxicity of ZnO nanomaterials to normal cells/tissue may be reduced without significantly affecting its therapeutic properties. One of the most common strategies is to functionalize the nanoparticles with biopolymers/biomolecules. Mahanta et al. have demonstrated that functionalization of zinc oxide materials using bovine α-lactalbumin (BLA) show enhanced toxicity to breast cancer cells MCF-7 and MDAMB231 along with increased cellular uptake and minimal effect to normal cells as compared to unfunctionalized ZnO nanomaterials (Mahanta et al. 2017). ZnO has been used as delivery agents for anti-cancer drugs for combination therapy as the majority of pharmaceutically active molecules do not interact with zinc or ZnO nanoparticle (Mishra et al. 2017). Green synthesized zinc oxide nanoparticles using casein as a reducing agent has been reported to demonstrate excellent loading of curcumin of 302.2 mg/g as well as higher cytotoxicity to multiple cancer cell lines (breast, cervical, osteosarcoma, and myeloma), thereby providing an opportunity for combination therapy (Somu and Paul 2019).

Titanium Dioxide Nanoparticle is also called titania, a naturally available oxide of titanium. It was discovered to form a photo-induced decomposition of water on TiO_2 electrode under UV light. In the recent past, TiO_2 has been used as a photosensitizing agent for PDT due to physiological inertness, reduced toxicity, and unique photocatalytic activity. They also find application in killing cancer cells when irradiated in UV light by forming various ROS species. In addition to this, a detailed mechanism of the phototoxic effect of TiO_2 inducing UV was reported on a series of human cancer cells such as bladder, colon, and breast epithelial carcinoma cells (Lucky et al. 2015).

Fullerene Buckminster fullerenes or bucky-ball is an allotrope of carbon. It has a spherical shape and is mainly composed of 60 (C_{60}) or 70 (C_{70}) carbon atoms of approximately 7 – 10 Å diameter with a hollow caged and fused structure arranged in pentagon or hexagon. Their π-conjugation structure allows them to absorb light in the UV or blue region to form a long-lived triplet state and generate ROS, making them act as photosensitizers (Sharma et al. 2011). Fullerenes are more photostable and not easily susceptible to photobleaching which makes them advantageous over other photosensitizers. The optical absorption property is its main disadvantage as it absorbs light where the tissue penetration depth is lowest. Its insolubility in water makes it weak to apply in biological conditions, and surface modification of supramolecular approaches have been used to overcome these disadvantages. Functionalized fullerenes have been shown to develop an anti-tumor PDT effect under the irradiation of light over GD^{3+} chelated C60-PEG-DTPA-GD with significant accumulation and reduction in tumor cells (Mroz et al. 2008). Functionalized fullerenes have been shown to target the cancer cells, and their potential is being investigated for PDT. Similarly, bovine α-lactalbumin functionalized graphene oxide nano-sheets demonstrated dose-dependent toxicity against breast cancer cells MCF-7 and MDA-MB-231 due to the generation of reactive oxygen species (ROS) without affecting normal cells (HaCaT and 3 T3) in comparison to unfunctionalized graphene oxide nano-sheets (Mahanta and Paul 2015a).

Nanoparticles As up-Converting Photosensitizers

Up-converting materials convert low light energy into high energy light by using multiple photons via sequential excitation. Initial absorption of energy from the NIR region promotes electrons from the lowest metastable state to the first metastable state (S1). The second NIR absorption passes electrons from S1 to the higher metastable state (S3). The electrons can also be returned from S3 to S2 and the ground energy state by relaxation and internal conversion emitting high energy photons (Xu and Tanabe 2019). The properties of sharp emission bandwidth, tunable emission, increased photostability, and high anti-stokes shift of up-converting nanoparticles have enormous biological applications in photodynamic therapy. The up-conversion nanoparticles have advantages such as acting as carriers for photosensitizers and converting deep tissue penetrating NIR light into visible light for phototherapy.

The three critical components of constructing up-converting nanoparticles are host, dopants, and photosensitizer. The host provides a crystalline lattice space that accommodates the dopants ions. Some of the host materials used are transition metals (Zr^{4+} and Ti^{4+}), trivalent rare-earth ions (Gd^{3+}, Y^{3+}, and La^{3+}), and alkaline earth ions (Ba^{2+}, Sr^{2+}, and Ca^{2+}) (Boyer et al. 2006). The crystal structure of host materials determines the optical properties of up-converting nanoparticles and the effect of the PDT. The phase-purity and cationic size of the host also influence minimal energy loss and enhances the efficiency of the up-converting nanoparticles by providing a uniform crystal field around the dopants. Dopants absorb and emit photons in the process of up-conversion nanoparticles. Rare earth metals, such as lanthanides, are dopants except Ca, Lu, La, and Yb. Sensitizer and activator are the two dopant ions used in the process of up-conversion in PDT. Up-converting nanoparticles such as $NaYF_4$:Yb^{3+}, Er^{3+} are used with silica dopants carrying lipophilic photosensitizer merocyanine-540 (MC-540) for PDT (Traul and Sieber 2015). Different types of photosensitizers are used along with up-converting nanoparticles such as TPP, pheophorbide-a, zinc phthalocyanine (ZnPC), hypocrellin A, Ce6, HP, silicon phthalocyanine dihydroxide. Photosensitizer selection is very critical such that there should be a close match between the light absorption maxima of photosensitizer and the emission of UCNP for efficient PDT.

The surface chemistry and modification are important for up-converting nanoparticles for determining their stability, solubility, druggable properties, and ability to attach with target functional groups, ligands, and photosensitizers. They have been modified using PEG (polyethylene glycol), chitosan, PEI (polyethyleneimine), BSA, and silica. Covalent modification of photosensitizer to the surface to up-converting nanoparticles helps prevent photosensitizer leakage during circulation and increases the half-life of the nanoparticles. Photosensitizers can also be attached to the surface of up-converting nanoparticles by non-covalent modifications such as physical adsorption. The up-converting nanoparticle $NaYF_4$:Yb^{3+}, Er^{3+} of size 53 nm, was synthesized by a one-pot hydrothermal method and was surface modified with O-carboxyl methylated chitosan covalent modification. It was loaded with photosensitizer pheophorbide-a through covalent attachment to the chitosan 2.8:527- pheophorbide-a:up-converting nanoparticle and targeted into the c(RGDyk) intro PDT. It showed the specific killing of tumor cells; about 80% of cells were killed, and stable but less photosensitizer loading was observed (Zhou et al. 2012). The thermal decomposition method is used to synthesize the up-converting nanoparticle $NaGdF_4$:Yb^{3+}, Er^{3+} of size 60 nm, and it was surface modified with polyacrylic acid by surfactant exchange method. The photosensitizer Rose Bengal has been attached to the up-converting nanoparticle through the physical adsorption method and used for *in vitro* photodynamic therapy. The up-converting nanoparticle $NaYF_4$:Yb^{3+}, Er^{3+} of size 90 nm has been synthesized by solvent, thermal method, and its surface was modified with mesoporous silica coating by calcination and silica shell by microemulsion. The photosensitizers ZnPC and MC-540 were encapsulated in mesoporous silica and targeted into the folic acid receptor *in vitro* and *in vivo* PDT/UCL imaging. The reduction in tumor growth was observed when two different photosensitizers were

co-doped in PDT (Idris et al. 2012). We have enlisted the various up-conversion nanoparticles reported in literature in Table 3 used as Photosensitizers in cancer treatment by PDT.

Apart from these metal/metal nanoparticles, there are several reports of protein-based nanoparticles with altered conformation states of the proteins showing anticancer activity. Mahanta et al. have prepared a self-assembled lysozyme nanostructure using desolvation and cross-linking using glutaraldehyde and demonstrated its excellent antiproliferative activity against breast cancer cells (Mahanta et al. 2015). Similarly, self-assembled bovine α lactoalbumin nanostructure also prepared using desolvation and cross-linking using glutaraldehyde exhibited excellent antiproliferative activity against breast cancer and HeLa cells via reactive oxygen species (ROS) mediated cytotoxicity (Mahanta and Paul 2015b). Further, self-assembled lysozyme nanostructures have been reported to be used as a drug carrier for curcumin, thereby providing an opportunity for combination therapy (Somu and Paul 2021).

Conclusion and Future Perspectives

PDT has widely been used to treat cancer and other diseases by using different carriers for photosensitizers. Localized photosensitizers are photo-excited to generate cytotoxic singlet oxygen species ($^1O_2$), peroxides, and free radicals thus providing a therapeutic effect by specifically killing the tumor cells without affecting the neighboring healthy cells. The limitations of the first- and second-generation photosensitizers such as poor aqueous solubility, aggregation of hydrophobic photosensitizers, and lesser generation of singlet oxygen have been overcome by third-generation photosensitizers. The successful application of photosensitizers depends on effective drug delivery systems. Various delivery systems have been developed for targeted drug delivery into the tumor sites. Nanoparticles carrying photosensitizers by covalent binding or adsorption methods provide hydrophilicity and enhanced permeability and retention for passive targeting of tumor cells. Targeted accumulation of photosensitizer have also been achieved by modifying the surface of nanoparticles with monoclonal antibodies and other molecules such as PEG. The ligand-based targeting approach is promising to deliver the photosensitizers in the specific tumor cells, and a deeper understanding of molecular biology and pathways helps in selecting ligand molecules for targeted drug delivery.

Similarly, exogenous and endogenous stimuli-responsive nano vehicles also develop research areas with increased release of photosensitizers into the cancer cells. However, there are several clinical limitations of PDT, such as photosensitizers that absorb light below 700 nm can only be superficial lesions. This has been overcome by using second-generation nanoparticles or active nanoparticles which work by fiber-optic technology that can penetrate body cavities. Engineered nanoparticles can act as photosensitizers or can facilitate excitation organic photosensitizers by acting as transducers of NIR or X-ray radiation. Second and

Table 3 List of up-converting nanoparticles as photosensitizers in PDT

Upconverting nanoparticle	Surface modification	PS	Target	Mode of PDT	Remarks
$NaYF_4:Yb^{3+}$, Er^{3+}	PEI	ZnPC	FA	*In vitro*/ UCL Imaging	Low photosensitizer loading And cytotoxic
$NaYF_4:Yb^{3+}$, Er^{3+}	Amino Functionalized And PEG	RB	FA	*In vitro*/ UCL Imaging	High photosensitizer loading
$NaYF_4:Yb^{3+}$, Er^{3+}	(OQPGA)-PEG Lipid Micelles	ZnPC	RGD And TAT Peptide	*In vitro*/ UCL Imaging	TAT peptides were first used In UCNs for membrane Crossing
$NaLuF_4:Gd^{3+}$ Yb^{3+}, Er^{3+}	PEI	TMPy P_4	G4 Aptamer (sgc8)	*In vitro*/ UCL Imaging	Photosensitizer loaded on UCN conjugated with G4-aptamer and exhibits Increased specificity
$NaYF_4:Yb^{3+}$, Er^{3+}	3-aminophenyl-Boronic acid (APBA)	HA-C_{60}	APBA And HA	*In vitro*/ UCL Imaging/ Fluorescence Imaging	No additional step required For conjugating targeting Moiety UCN and photosensitizer Surface stabilizers Itself tumor cell targeting
$NaYF_4:Yb^{3+}$, Tm^{3+}	Oleic acid	CPE	cRGD	*In vitro* UCL imaging & combined Chemotherapy	Self-assembling conjugated CPEs-DOX conjugates Utilized for NIR Mediated synergistic PDT and chemotherapy
$NaYF_4:Yb^{3+}$, Er^{3+}	PAAm PEG-succinimidyl Carbonate	ZnPC	FA	*In vitro*/ UCL imaging	Yb^{3+} doping enhanced the Upconversion emission FRET Efficiency

third-generation nanoparticles for PDT are promising for cancer treatment; however, more clinical trials and research is required for PDT to replace existing techniques such as chemotherapy and surgery.

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