Targeted and effective photodynamic therapy for cancer using functionalized nanomaterials

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Received 15 December 2015; received in revised form 2 February 2016; accepted 22 February 2016

Abstract Photodynamic therapy (PDT) is an emerging, non-invasive therapeutic strategy that involves photosensitizer (PS) drugs and external light for the treatment of diseases. Despite the great progress in PS-mediated PDT, their clinical applications are still hampered by poor water solubility and tissue/cell specificity of conventional PS drugs. Therefore, great efforts have been made towards the development of nanomaterials that can tackle fundamental challenges in conventional PS drug-mediated PDT for cancer treatment. This review highlights recent advances in the development of nano-platforms, in which various functionalized organic and inorganic nanomaterials are integrated with PS drugs, for significantly enhanced efficacy and tumor-selectivity of PDT.

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1. Introduction

Photodynamic therapy (PDT) is a treatment option in which activation of photosensitizer (PS) drugs with specific wavelengths of light leads to energy transfer to oxygen molecules or other substrates in the surrounding areas, generating cytotoxic reactive oxygen species (ROS) which can trigger apoptotic and necrotic cell death. In the absence of external photo-activating light, the PS drugs are minimally toxic. Therefore, PDT provides a safe and effective way to selectively eradicate target cells/tissues such as cancerous cells while avoiding systemic toxicity and side effects on healthy tissues. Compared to traditional chemotherapy and radiotherapy, PDT-based cancer treatment significantly reduces side effects and improves target specificity because only the lesion under light irradiation is treated. Moreover, PDT-based cancer therapy is more beneficial to patients in which location or size of lesions limits the acceptability of conventional therapy.

Despite the many positive features of PDT on cancer therapy, PDT is still not fully adapted in the clinical settings because of some inherent properties of PS drugs. Most existing PS drugs are hydrophobic with poor solubility in water. Therefore, they are easily aggregated under physiological conditions, drastically lowering the quantum yields of ROS production. Even in the case of some modified PS drugs for increased water solubility, their accumulation selectivity at target tissues/cells remains insufficient for successful clinical use. In this regard, development of effective delivery systems that incorporate PS drugs and transfer them into target tissues/cells, addressing critical biological barriers for the conventional PS delivery, is indispensable.

Recently, nanomaterials in combination with PS drugs find considerable attention in PDT because they can overcome critical limitations of conventional PS drugs. Nanomaterials can significantly enhance the solubility of PS drugs in water through hydrophilic properties and thus increase their cellular uptake. When formulated as nanoparticles with nanomaterials, PS drugs can achieve passive targeting to tumor by the enhanced permeability and retention (EPR) effect, which is attributed to the leaky tumor vasculature and poor lymphatic drainage of tumor tissues. Moreover, cell-specificity of PS drugs can be significantly increased by surface modification of the nanoparticles to bind active targeting moieties, such as antibodies, peptides, and aptamers. This also improves bioavailability of PS drugs and reduces undesirable side effects of PS drugs to surrounding health tissues.

To date, numerous nano-platforms using a variety of organic and inorganic nanomaterials have been investigated for efficient and targeted PS delivery. Organic nanomaterials for PDT, such as liposomes and polymeric nanoparticles, have achieved safe and controlled delivery of PS drugs by using biodegradable/biocompatible materials and tailoring chemical compositions of the materials. Flexibility for versatile formulations is another benefit of using organic nanomaterials. Inorganic nanomaterials hold high potential for PDT due to tunable optoelectronic properties by tailoring their shape and size. Therefore, they can offer additional functionalities to PS drugs such as diagnosis and imaging. In addition to the benefits of each material, they both provide an effective solution to overcome the drawbacks of current PS drugs associated with stability in physiological conditions and selective delivery to the target sites by further surface functionalization. PS drugs have been generally combined with organic/inorganic nanocarriers via both physical methods using hydrophobic or electrostatic interactions between PS and the nanocarriers and chemical methods using various conjugation reactions. Here, we introduce various combinations of nanomaterials and PS drugs that have demonstrated effective PDT both in vitro and pre-clinical animal studies. We mainly concentrate on innovative formulations, molecular designs, and modifications that have been utilized for targeted and effective PDT while categorizing them into organic and inorganic nanomaterials.

2. Mechanism of PDT using PS

Mechanism of PDT using PS has been elucidated in several studies. Briefly, PS in the ground state absorbs a photon and is activated to an excited singlet state upon irradiation with suitable light. The excited singlet state can convert into the triplet state via intersystem crossing caused by a change in the spin of electrons. The PS in the triplet state interacts with surrounding molecules and thus produces ROS through Type I and Type II reactions. The Type I reaction involves the transfer of either hydrogen atom or an electron between the excited PS and the substrates, leading to the generation of free radicals. These radicals then react with oxygen, resulting in the production of ROS such as superoxide and hydroxyl radicals. The Type II reaction involves the energy transfer between the excited PS and the molecular oxygen in the ground state (O2), resulting in the formation of highly reactive state of oxygen known as singlet oxygen (1O2). The resulting ROS can cause irreversible damage to target tissues/cells.

3. Functionalized nanomaterials for effective and targeted PDT

For effective and targeted PDT, functionalized nanomaterials are required to efficiently incorporate and deliver hydrophobic PS drugs only into target tissues/cells and to activate them to produce ROS. In addition, functionalized nanomaterials need to be biocompatible and to have sufficient PS-loading capacity. They may need active targeting moieties to enhance the accumulation selectivity of PS drugs in the target tissue/cells. To achieve these requirements, various functionalized organic/inorganic nanomaterials have been developed, which will be reviewed in the following sections.

3.1. Organic nanomaterials for PDT

To improve the water solubility of PS drugs and their specific accumulation at the target site, a general strategy is encapsulation of the PS drugs to polymeric or lipid-based nanocarriers. In this respect, liposomes, polymeric micelles, and polymeric nanoparticles have been extensively explored for serving as PS carriers in PDT.

3.1.1. Liposomes

Liposomes are one of the first nano-platforms to be applied in drug delivery systems. Their unique ability to contain hydrophilic drugs in their aqueous core and hydrophobic agents within their lipid bilayers renders them excellent delivery vehicles. 5-Aminolevulinic acid (ALA) prodrugs for PDT were encapsulated in dipalmitoyl-phosphatidyl choline–based liposomes. ALA was used as a precursor of phototoxic protoporphyrin IX (PpIX) for PDT. The chemical structure and molar extinction coefficient of PpIX are represented in Table 1. In vitro experiments...
demonstrated that the ALA-loaded liposomes significantly increased the uptake efficiency into human cholangiocarcinoma HuCC-T1 cells compared to ALA itself. As a result, the photocytotoxic effect of the liposomes was substantially higher than the effect of the ALA alone because of their higher intracellular generation of PpIX.

Light-activatable PDT was achieved by NIR light-absorbing dye/PS-loaded liposomes. To develop the NIR light-activatable PDT, chlorin e6 (Ce6) as a PS (Table 1) was encapsulated into lecithin-based liposomes with indocyanine green (ICG), an NIR light-absorbing agent approved by Food and Drug Administration (FDA) (Fig. 1). Upon irradiation at 660 nm, singlet oxygen generation by Ce6 was effectively inhibited because Ce6 is located near the ICG. Interestingly, the phototoxicity of Ce6 was recovered when the ICG was degraded upon exposure to NIR light (808 nm). In addition, ICG could generate heat upon NIR irradiation to eradicate cancer cells, serving as a photothermal agent. In vitro work demonstrated that the liposomes containing Ce6 and ICG significantly increased the phototoxicity in cancer cells under photo-irradiation.

Magnetoliposomes (MLs) loaded with zinc phthalocyanine (ZnPc)/cucurbituril (CB) complexes (CB:ZnPc-MLs) were synthesized for combined PDT and magnetohyperthermia in malignant melanoma cells. The chemical structure and molar extinction coefficient of ZnPc are presented in Table 1. Significant reduction in cell viability was observed with melanoma cells treated with CB:ZnPc-MLs after application of both 670 nm light and AC magnetic field. The combined PDT and magnetohyperthermia by CB:ZnPc-MLs was much more effective than each therapy alone.

### Table 1 Chemical structures, activation wavelengths, and molar extinction coefficients of various photosensitizers.

| Photosensitizer | Chemical structure | Activation wavelength (nm) | Molar extinction coefficient (M⁻¹ cm⁻¹) | Ref. |
|----------------|--------------------|-----------------------------|----------------------------------------|------|
| Protoporphyrin IX (PpIX) | ![Protoporphyrin IX](image) | 408 | 275,000 | 23 |
| Chlorin e6 (Ce6) | ![Chlorin e6](image) | 667 | 55,000 | 24 |
| Zinc phthalocyanine (ZnPc) | ![Zinc phthalocyanine](image) | 674 | 281,800 | 25 |
| Pyropheophorbide a (PPa) | ![Pyropheophorbide a](image) | 669 | 45,000 | 26 |
| Pheophorbide a (PhA) | ![Pheophorbide a](image) | 667 | 44,500 | 27 |

3.1.2. Polymeric nanoparticles/micelles

Usually, hydrophobic PS drugs can be efficiently entrapped into nanoparticles by interaction between PS and hydrophobic polymers. Biodegradable polymers such as poly(lactide-co-glycolide) (PLGA) have been popularly employed as matrix materials for PS...
In vivo for PpIX-mediated PDT. PpIX was encapsulated by the graft A549 cells. Therefore, the PpIX-loaded micelles exhibited substantial growth upon laser irradiation 24 h after administration of the micelles. Tumors and then generated cytotoxic ROS that inhibited the tumor growth.

Fluorescent polymeric nanoparticles using 4-arm PEG functionalized with a coumarin fluorophore and chlorambucil showed higher cytotoxicity in HeLa cells compared to polymeric nanoparticles without the chlorambucil, demonstrating the synergistic effect of PDT and chemotherapy.

Effective tumor-targeting PDT using Ce6-loaded hyaluronic acid (HA) nanoparticles (Ce6-HANPs) was investigated as described in Fig. 2. The resulting Ce6-HANPs showed stable structural integrity in aqueous condition and rapid cellular uptake into cancer cells. Moreover, rapid biodegradation of Ce6-HANPs by hyaluronidases abundant in cytosol of cancer cells was documented, implying efficient intracellular release of Ce6 at the tumor tissues. Intravenously injected Ce6-HANPs into tumor-bearing mice efficiently targeted the tumor tissue via the EPR effect and readily entered tumor cells through HA receptor-mediated endocytosis. It was observed that Ce6 released from the HANPs could generate singlet oxygen inside tumor cells under 671 nm light irradiation for PDT, simultaneously generating fluorescence for in vivo imaging.

Lipopolymer hybrid-based, dual-stimuli-responsive nanoparticles encapsulating Ce6 were utilized for targeted PDT. Lipopolymer hybrids comprised of soybean lecithin-derived phosphatidylcholine, phosphatidylethanolamine-poly-(L-histidine) [PE-p(His)], and FA-conjugated phosphatidylethanolamine-poly(N-isopropylacrylamide) [PE-p(NIPAM)-FA]. pH and temperature responsiveness, respectively, resulted in dual-stimuli-responsive Ce6 release. The positively charged Ce6-loaded nanoparticles exhibited higher phototoxicity against KB tumor cells compared to free Ce6. In addition, the FA-conjugated, lipopolymer hybrid-based nanoparticles showed more efficient cancer-targeted PDT compared to free Ce6.

**3.1.3. Carbon nanomaterials**

Carbon nanomaterials with unique structures have a great potential as PDT agents by attaching PS drugs on the functionalized carbon nanomaterials via either covalent or non-covalent manner. Biocompatibility and water-solubility of the carbon nanomaterials are also achieved by conjugation with various functional polymers. Most commonly utilized carbon nanomaterials for PDT include fullerene, carbon nanotube, and graphene. Fullerene cages such as C60 have found their use in PDT because they can act as efficient PS drugs participating in the cascade of energy transfer that triggers the generation of ROS due to their abundant pi-bond electrons. Water soluble, cationic C60 fullerene cages functionalized with quaternary alkylammonium multi-salts were synthesized for effective PDT. Most cationic C60 fullerene cages rapidly bound to bacterial cell walls and retained high water-solubility, resulting in efficient antimicrobial effects. N-methylpyrrolidinum-fullerene in micelles composed of Cremophor EL was developed to treat intraperitoneally disseminated colorectal cancer in a mouse model. Intraperitoneal injection of the micelles combined with white-light illumination led to significant phototocytotoxicity in the mouse colon carcinoma model, destroying intraperitoneal tumors. As a result, the tumor-bearing mice subjected to white light irradiation after administration of the micelles maintained a higher survival rate compared with control mice that received Cremophor only.

Carbon nanotubes can also act as either photosensitizers themselves or carriers for exogenous photosensitizers. Single-walled carbon nanotubes (SWNTs) covalently functionalized with 80% of the chlorambucil after being exposed to UV/Vis light. The nanoparticles demonstrated selective accumulation in HeLa cells than in noncancerous L929 cells. The polymeric nanoparticles with a coumarin fluorophore and chlorambucil showed higher cytotoxicity in HeLa cells compared to polymeric nanoparticles without the chlorambucil, demonstrating the synergistic effect of PDT and chemotherapy.

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polyethylenimine (SWNT–PEI) and non-covalently functionalized with polyvinylpyrrolidone (SWNT–PVP) were developed for PDT agents. They showed the photocytotoxic effect against mice melanoma B16F10 cells under visible light illumination. SWNT–PEI showed stronger photocytotoxic effect compared to SWNT–PVP in vitro and in vivo, indicating that the photodynamic effect is dependent on the modification method of SWNT.

By taking advantage of the ultrahigh loading of molecules through π–π stacking, nano-sized graphene oxide (GO) has been used to deliver photosensitizers in vitro at high concentrations. Branched PEG-functionalized GO (GO-PEG) loaded with PS drugs was developed for PDT. Ce6, a PS drug, was loaded on the GO-PEG via π–π stacking. The resulting GO-PEG-Ce6 complex shows high water solubility and is able to generate cytotoxic singlet oxygen under light excitation (660 nm or 808 nm) for PDT. In addition, GO under NIR irradiation (808 nm) at a low power density could trigger local heating, enhancing the cellular uptake of GO-PEG-Ce6 by approximately 2-fold. As a result, GO-PEG-Ce6 complex achieved remarkable destruction of tumor under NIR irradiation compared to free Ce6. These results show the promise of using nano-sized GO in combined photothermal and photodynamic cancer therapy with synergistic effects.

Antibody-conjugated GO was synthesized for subcellular targeting PDT. GO was modified with integrin αvβ3 monoclonal antibody (mAb), and pyropheophorbide a (PPa) (Table 1) as a PS was incorporated into the GO, resulting in PPa-GO-mAb. PPa-GO-mAb was able to effectively target αvβ3-positive tumor cells due to receptor recognition by the antibody. It was observed that PPa-GO-mAb was localized into mitochondria after endocytosis due to an electronic reaction between the mitochondria membrane potential and the polarized GO. The mitochondria targeting of PPa-NGO-mAb significantly enhanced mitochondria-mediated apoptosis of PDT after irradiation with 633 nm light.

Functionalized graphene/C60 nanohybrid with targeting moieties was developed for targeted PDT and photothermal therapy (PTT) (Fig. 3). GO was used as a photothermal agent that induces hyperthermia under exposure to NIR light (808 nm). FA (i.e., cancer targeting moiety) and C60 (i.e., PS) were conjugated onto PEGylated graphene oxide (FA-GO-PEG/C60) for enhanced cellular uptake of C60 in cancer cells. The hybridization process increased the light absorption properties and inhibited the aggregation of C60, thus increasing the PDT efficacy of C60 against cancer cells. FA-GO-PEG/C60 nanohybrid under light irradiation (808 and 532 nm wavelength) showed higher cell apoptosis and death compared with PDT or PTT alone, demonstrating a synergistic effect of combined PDT and PTT.

3.2. Inorganic nanomaterials

Inorganic nanoparticles, covalently or non-covalently linked with photosensitizers, hold several advantages over organic nanoparticles, including high stability, and precise control over size and shape, and tunable optical properties. Moreover, the surface of the inorganic nanoparticles can be easily functionalized for high biocompatibility and selective targeting.

3.2.1. Gold nanoparticles

Gold nanoparticles possess high surface areas and biocompatibility and enable facile surface functionalization through gold-thiol chemistry. Moreover, since gold nanoparticles show tunable optical scattering and absorption, they have been extensively explored for diagnostic applications. Maximum absorption and scattering wavelengths of gold nanoparticles can vary based on their size and shape. Particular structures of gold nanoparticles such as gold nanorods and nanoshells have been extensively utilized for PTT due to their strong absorption in the NIR region. Because of their high biocompatibility and facile surface functionalization, gold nanoparticles have recently gained attention as suitable drug delivery vehicles both in diagnostics, bioimaging, and cancer therapy. Gold nanoparticles have also been utilized to deliver PS drugs to the target region, both passively and actively.

Heparin-coated gold nanoparticles (AuNPs) as glutathione (GSH)-responsive PS drug carriers were developed for efficient PDT. Heparin was used for increasing gold nanoparticles’ watersolubility, biocompatibility, and colloidal stability. Pheophorbide a...
(PhA) was used as a PS drug (Table 1). The hybrid gold nanoparticles were formulated by incorporation of PhA-conjugated heparins via gold–thiol interaction. The hybrid PhA/AuNPs made PhA non-fluorescent and photo-inactive because efficient energy transfer from PhA to AuNP occurs on the surface. However, photoactivity of PhA was recovered under GSH-rich intracellular environments via cleavage of gold–thiol linkage, generating singlet oxygen species following 670 nm light treatment. The hybrid PhA/AuNPs showed high phototoxicity and internalization efficiency in A549 cells in comparison with free PhA. The PhA/AuNPs also indicated prolonged blood circulation and increased tumor specificity in tumor-bearing mice, thus resulting in improved PDT efficacy compared with free PhA. This study demonstrated that GSH-responsive PhA/AuNPs are activatable and efficient PDT agents for cancer treatment due to their enhanced tumor specificity and phototoxicity.

Aptamer-conjugated gold nanorods (AuNRs)/Ce6 complex was developed for targeted cancer therapy. Aptamer Sgc8, which specifically targets leukemia T cells, was conjugated to an AuNR by a thiol–Au covalent bond. Then Ce6-labeled short DNA sequences were hybridized with the aptamer on the AuNR surface, resulting in fluorescence quenching due to close proximity of the Ce6 to the gold surface. When the aptamer binds to target cancer cells, Ce6 is released due to disruption of double-stranded DNA structure of the aptamer and serves as PDT agents upon irradiation with NIR light (812 nm). Furthermore, aptamer–AuNR conjugates killed the cancer cells dramatically by combined PDT and PTT upon NIR irradiation, demonstrating their feasibility for targeted multimodal cancer therapy.

PS-conjugated AuNRs, in which PS was linked to the surface of AuNRs via a matrix metalloproteinase-2–cleavable peptide linker (MMP2P), were prepared for cancer-targeted PDT. PPa was used as a PS, and its fluorescence and phototoxicity were suppressed when it was bound to the surface of the AuNRs. However, when MMP2P–AuNRs incorporating PPa were incubated with human fibrosarcoma cells (HT1080) over-expressing MMP2 and then irradiated with 670 nm light, PPa was efficiently liberated from the nanorods due to the degradation of MMP2P. As a result, the released PPa recovered its phototoxicity and fluorescence. This light-activatable MMP2P-AuNRs achieved MMP2-mediated, cancer-specific fluorescence imaging and subsequent PDT.

A multi-therapy based on gene silencing with PPT was attempted using lipid-coated gold nanoechinus (Au NEs) (Fig. 4). siRNA that silences superoxide dismutase 1 (SOD1), one of the effective anti-apoptotic and self-defending genes that can destroy the free radicals or reactive oxygen species in the body, was complexed with the cationic lipid-coated AuNEs (Fig. 4). When HeLa cells were treated with siRNA-lipid–coated Au NEs, ultra-high gene silencing efficiency (~86%) of SOD1 was achieved. In addition, significantly higher phototoxicity was observed with the cells treated with the siRNA–lipid-coated Au NEs under NIR (1064 nm) irradiation, as compared to the cells treated with lipid-coated Au NEs alone. In vivo studies also demonstrated that the combination of gene silencing and Au NEs–mediated PDT was able to destroy the deep tissue-buried melanoma tumors more efficiently than the Au NEs–mediated PDT alone under ultra-low doses of NIR light irradiation. This study demonstrated for the first time that complete destruction of deep tissue-buried tumors can be accomplished by combination of Au NEs–mediated PDT and gene silencing under exposure to ultra-low doses of NIR light.

Trimodality fluorescence/thermal/photoacoustic (PA) imaging–guided synergistic PTT/PDT cancer treatment was achieved by using multifunctional photosensitizer Ce6-loaded plasmonic gold
vesicles (GVs) (Fig. 5). The GVs consisting of mono-layered, assembled gold nanoparticles exhibit a strong absorbance in the NIR region. The NIR irradiation (671 nm) simultaneously excited both GVs and Ce6 to produce heat and singlet oxygen for combined PTT and PDT, destroying cancer cells (Fig. 5). The GV–Ce6 showed high loading efficiency of Ce6 in the hollow interior of GVs. The heating effect upon NIR irradiation dissociated the GVs and thus released the encapsulated Ce6, enhancing the delivery of Ce6 into cells. A feasibility of using GV–Ce6 for trimodality fluorescence/thermal/PA imaging-guided synergistic PTT/PDT was evaluated in vivo. Tumor tissues were clearly visualized by the fluorescence, thermal, and PA signals simultaneously. Taken together, synergistic PTT/PDT treatment with improved efficacy was observed under NIR irradiation, as compared to either individual PTT or PDT alone.

3.2.2. Silica nanoparticles

Organically modified silica (ORMOSIL) nanoparticles have recently emerged as an attractive candidate for delivery of PS drugs due to efficient loading of hydrophobic PS drugs68. ORMOSIL nanoparticles can be loaded with either hydrophilic or hydrophobic drugs, protecting them against extracellular barriers. ORMOSIL nanoparticles covalently linked to PS drugs were developed for effective PDT while preventing the premature release of PS drugs during blood circulation, which is a critical obstacle in physical encapsulation of PS drugs69. The ORMOSIL nanoparticles were synthesized by alkaline hydrolysis and polycondensation of the iodobenzyl-pyro-silane, a precursor for ORMOSIL incorporating photosensitizer iodobenzylpyropheophorbide. Size of the prepared nanoparticles was ultralow (~20 nm), and they were greatly monodispersed and stable in aqueous solution. In vitro study confirmed that the prepared nanoparticles were efficiently taken

Figure 4  Schematic illustration of combined PDT and gene silencing for effective destruction of deep tissue buried tumors using SOD1-silencing siRNA/lipid-coated Au NEs. Reproduced with permission from Ref. 65. Copyright 2015, Elsevier Ltd.

Figure 5  Schematic illustration of trimodality fluorescence/thermal/photoacoustic imaging-guided synergistic photothermal/photodynamic cancer therapy using photosensitizer (Ce6)-encapsulated plasmonic gold vesicles (GVs). Reproduced with permission from Ref. 67. Copyright 2013, American Chemical Society.
up by cancer cells and showed phototoxicity to colon carcinoma cells under exposure to 514 nm light.

PDT activity and two-photon fluorescence imaging using ORMOSIL nanoparticles encapsulating PpIX and NIR fluorophores IR-820 has been demonstrated [Fig. 6]. PpIX-doped ORMOSIL nanoparticles were efficiently internalized by cancer cells, confirmed by two-photon fluorescence imaging. Additionally, two-photon-excited PpIX after internalization induced photocytotoxicity against cancer cells. In vivo NIR fluorescence imaging documented microinjected IR-820-doped ORMOSIL nanoparticles localized beneath 4-mm depth in the brain of a mouse after microinjection. Moreover, sentinel lymph node (SLN) mapping of mice was achieved using intradermally injected IR-820-doped ORMOSIL nanoparticles. NIR fluorescence imaging also confirmed that IR-820-doped ORMOSIL nanoparticles were also selectively accumulated in tumors after intravenous injection into mice bearing xenografted tumors. This study clearly demonstrated the high potential of NIR fluorophore-doped, PS-loaded ORMOSIL nanoparticles for effective PDT and bioimaging in the clinic.

Mesoporous silica nanoparticles (MSNs) have been widely employed as a promising carrier for PDT due to large surface area, easily tunable pore size and volume, and chemical stability. Utilization of PEG- and PEI-functionalized, ZnPc-loaded MSNs (PEG-PEI-MSNs/ZnPc) for PDT has been reported. PEI played a role in facilitating endosomal escape of MSNs after endocytosis through a “proton sponge effect.” As a result, phototoxicity of the nanoparticles against cancer cells was highly enhanced compared with MSNs/ZnPc. In vivo study demonstrated that PEG-PEI-MSNs/ZnPc could be selectively accumulated in the tumor due to the EPR effect. Moreover, effective tumor destruction was achieved in tumor-bearing mice upon intravenous injection of PEG-PEI-MSNs/ZnPc and the subsequent exposure to 680 nm light.

FA-conjugated, ALA-loaded hollow MSNs (FA-ALA-hMSNs) were employed to transfer ALA into skin cancer cells for PDT. FA-ALA-hMSNs showed efficient accumulation of ALA and PpIX in skin cancer cells through folate receptor–mediated endocytosis. Upon irradiation with light at 635 nm, FA-ALA-hMSNs efficiently killed the skin cancer cells due to generation of phototoxic PpIX.

A feasibility of using multifunctionalized MSNs for combined drug delivery and PDT was investigated. The MSNs were equipped with PS drugs (i.e., porphyrin) and chemotherapy agents (i.e., camptothecin) for synergistic anticancer effects. The MSNs were also anchored with galactose to achieve enhanced cellular uptake in colorectal cancer cells via galactose receptor–mediated endocytosis. The multifunctionalized MSNs indicated a significant synergistic effect for killing cancer cells under light at 650 nm due to combined anticancer drug delivery and PDT.

MSNs with phosphorescent reagents were utilized as oxygen sensing PDT agents. Phosphorescent Pd-meso-tetra(4-carboxyphenyl) porphyrin (PdTPP) that can detect oxygen in tissues via oxygen-dependent quenching of phosphorescence was used as a PS. When PdTPP-entrapped MSNs are internalized by MDA-MB 231 breast cancer cells and irradiated with 532 nm light, they induced photocytotoxic cell death. Simultaneously, PdTPP-loaded MSNs could act as imaging probes detecting the generation of singlet oxygen upon photo-irradiation.

### 3.2.3. Quantum dots (QDs)

QDs have been intensively utilized for multifunctional nanocarriers for PDT due to high emission quantum yield, tunable optical properties, and facile surface modification. QDs can also be used as excellent donors in the fluorescence resonance energy transfer (FRET) process. Biocompatible, porphyrin-conjugated CdSe QDs were developed for PDT via two-photon excitation. Efficient energy transfer between the QDs and the porphyrin was confirmed by employing FRET process. A feasibility of using QD–porphyrin conjugate for effective photosensitizing agent under two-photon excitation was clearly demonstrated. Singlet oxygen generation by QD–porphyrin conjugate under two-photon excitation was greatly higher than that by the porphyrin solution alone.

Stable water-soluble complexes of Ce6 and ZnSe/ZnS QDs were prepared for PDT in cancer cells. Efficient photoexcitation energy transfer between Ce6 and ZnSe/ZnS QDs was confirmed. PDT efficacy of Ce6 was significantly enhanced due to increased cellular uptake of the Ce6 using ZnSe/ZnS QDs. Efficient PDT in cancer cells was also achieved by sulfonated aluminum phthalocyanines (AlPcSs)-conjugated QDs. AlPcSs were used as PS drugs. Since AlPcS–siRNA-lipid coated QDs conjugates were positively charged, they were able to efficiently penetrate into human nasopharyngeal carcinoma cells in contrast to negatively charged, free AlPcSs. With 532 nm light irradiation, combination of direct and indirect FRET excitations for AlPcS–siRNA-lipid coated QDs conjugates exhibited remarkable PDT effect to cancer cells.

**Figure 6** Synthetic scheme of (A) PpIX-doped ORMOSIL nanoparticles for two-photon fluorescence imaging and PDT and (B) PEG-coated, IR-820-doped ORMOSIL nanoparticles for in vivo NIR fluorescence imaging. Reproduced with permission from Ref. 70. Copyright 2012, Elsevier Ltd.
Utilization of desirable external photoexcitation to activate PS drugs in deep tissues is crucial for successful clinical application of PDT. Recently, a novel QD conjugate incorporating light-emitting Renilla luciferase 8 (RLuc8) was developed to generate bioluminescence as an external light source for activating PS drugs in PDT85,86. When the QD conjugates are treated with coelenterazine, a substrate of RLuc8, energy is transferred from the substrate to the QD conjugates via bioluminescence resonance energy transfer (BRET), thereby emitting 655 nm photons and activating meta-tetra-hydroxyphenyl-chlorin (m-THPC, Foscan®) PS for ROS generation (Fig. 7)86. A549 cells treated with QDs–siRNA-lipid coatedRLuc8, Foscan®-loaded micelles, and coelenterazine were effectively killed by Foscan®-mediated PDT. BRET-mediated PDT by QD–RLuc8 combined with Foscan-loaded micelles plus coelenterazine significantly delayed the tumor growth in a mouse model while decreasing proliferation factor (i.e., proliferating cell nuclear antigen) and microvessel densities. This study demonstrated that BRET-mediated PDT can overcome light penetration issues of current PDT.

4. Conclusions and perspectives

A variety of organic and inorganic nanomaterials combined with PS drugs have been developed for targeted and effective delivery of PS drugs. It has been shown that nanomaterials can offer solutions to address crucial limitations of conventional PS drugs. Nanomaterials combined with PS drugs increase the water solubility of hydrophobic PS drugs. They also improve the target-specificity of PS drugs via passive targeting to tumor tissues though the EPR effect. Surface modification of PS-loaded nanoparticles with active targeting ligands further enhances the selective accumulation of PS drugs into tumors.

Despite impressive progress in developing nanomaterials for PDT, a number of challenges still remain toward clinical applications of the nanomaterial-mediated PDT. For example, development of PS drugs with strong absorbance at long wavelengths and high chemical- and photo-stability must be accompanied in parallel with the development of nanomaterials. Systemic toxicity, long-term toxicity, and dose-dependent toxicity of nanomaterials are still problems to be addressed. The use of biocompatible nanomaterials (e.g., lipids, polypeptides, and natural polymers) and biocompatible surface coatings is crucial to reduce the long-term toxicity and dose-dependent toxicity of the nanomaterials. In addition, utilization of stimuli-responsive nanomaterials that can achieve controlled release of PS drugs responding to biochemical stimuli in target tissues/cells is indispensable to reduce the systemic toxicity and dose-dependent toxicity of the nanomaterials by achieving target-specificity and biodegradation. Several studies in this review have demonstrated the advantages of using stimuli-responsive nanomaterials for efficient, safe, and cancer-targeted PDT82,83. In addition to the development of biocompatible nanomaterials, their biosafety evaluations such as systemic clearance and biological effects must be conducted prior to their clinical uses for PDT. These efforts will facilitate the successful translation of nanomaterial-mediated PDT into clinical settings.

Acknowledgments

This work was supported by the Incheon National University Research Grant in 2014.

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