



## Nanophotosensitive drugs for light-based cancer therapy: what does the future hold?

“Nanotechnology offers unique advantages over small-molecule drugs by delivering a high payload of photosensitizers to the target tissue, providing flexibility in the excitation wavelengths, enabling longer retention in the target tissue, possessing regenerative reactive oxygen species-producing capability and stimulating the immune system.”

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Light-based therapies are as old as the advent of human medicine. From bathing in sunlight and killing harmful microbial organisms to the use of highly focused lasers to ablate disease tissue, understanding of the interaction of light with tissue has enabled major advances in modern medicine. Photomedicine, as the ensemble of light-mediated therapies and imaging is called, relies on high-intensity light to destroy disease tissue or sufficiently alter the composition of a target tissue such that it atrophies over time. Although direct laser ablation has found its mark in medicine, the realization that light can augment the therapeutic effects of certain natural products and synthetic drugs (photosensitizer [PS]) launched the field of photodynamic therapy (PDT). Beginning from the 1970s, when PDT found its way into modern clinical practice, it is now widely used clinically against many types of diseases, especially cancer [1]. Typically, the PS is not toxic until exposed to light of the appropriate wavelength. Upon activation by light, PS produces reactive oxygen species (ROS) or other cytotoxic radicals that can directly kill cancer cells, disrupt blood vessels and stimulate immune cells. The multidimensional cell killing mechanisms are responsible for the downstream thera-

peutic effects observed in cells that were not directly exposed to light. Additionally, the spatiotemporal control of light and PS during PDT minimizes off-target toxicity, which is responsible for many side effects of chemotherapies.

Conventional PDT employs a variety of illumination sources, including halogen and arcing lamps, lasers and light-emitting diodes to activate a PS. However, the high tissue absorption and scattering of visible light confine most PDT to superficial disease or those that can be reached through the use of an endoscope or optical fibers. Utilizing light in the near-infrared (NIR) range (700–1100 nm) minimizes the attenuation in tissue, and when combined with an NIR-sensitive PS, can maximize the depth of treatment. Even under these optimal conditions, the transmission into tissue is limited to only 5–10 mm [2].

The nature and spectral properties of a PS play a major role in the efficacy of PDT. Small organic molecules are the primary PSs used in the clinics today for historical reasons and the ease of synthesis, reproducibility and biocompatibility. However, the poor solubility, rapid depletion of photoactive molecules upon excitation, narrow excitation window and occasionally transient retention in the

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target tissue have ushered new nanoplatforms for PDT. Nanotechnology offers unique advantages over small-molecule drugs by delivering a high payload of PSs to the target tissue, providing flexibility in the excitation wavelengths, enabling longer retention in the target tissue, possessing regenerative ROS-producing capability and stimulating the immune system. As such, researchers have developed diverse types of nanophotosensitive drugs for PDT. These photosensitive nanoparticles (nanophotosensitizers) are designed to be biocompatible, retain in tumors by enhanced permeation and retention effect, target tumors by active transport mechanisms or remain in circulation for prolonged period. Current nanoplatforms can be grouped into vehicular, intrinsic and dynamic nanophotosensitizers.

### Vehicular nanophotosensitizers

Vehicular nanophotosensitizers are formed by encapsulating or conjugating known PSs with nanomaterials. Nanoparticles, such as, liposomes, polymers, or hollow particulates are not themselves a source of ROS. Instead, they are used to increase PS payload, improve solubility, modulate pharmacokinetics, enhance bioavailability and minimize the inherent toxicity of the drugs to vital organs. This strategy has been successfully used to improve the therapeutic effect of drugs, such as, doxorubicin [3]. Compared with the traditional single-molecule or PS carriers (e.g., aqueous suspension, matrix cream and thermosensitive gel), vehicular nanophotosensitizers can promote the effective targeting and cellular internalization of PS molecules through a combination of multivalent cancer-targeting strategies and enhanced permeation and retention effect [4]. These dual targeting mechanisms ensure prolonged retention and release of the PS in cancer. The local increase in the effective concentration of PS in tumors improves PDT outcomes.

Solubilization of hydrophobic PSs in lipid-based nanoparticles, such as, liposomes and nanoemulsions is a standard method to formulate these PSs without altering their structures [5]. Visudyne, a liposomal formulation of benzoporphyrin derivative, is widely used to treat ophthalmologic diseases, such as, age-related macular degeneration [6]. Similarly, a liposomal formulation of zinc(II) phthalocyanine allowed the use of this hydrophobic PS for the treatment of squamous cell carcinomas in human patients [7]. A drawback of liposomes is the short plasma half-life due to the rapid lipid exchange between the liposomal constituents and the lipoproteins as well as the rapid uptake by cells of the mononuclear phagocyte system [8].

Polymeric nanoparticles have also emerged as promising vehicular nanophotosensitizers. Polymer-based PSs offer multiple advantages over small organic PSs,

including the ability to deliver a high payload of PS to the infected area, versatile surface modification for improved targeting and delivery efficiency, capacity to prevent rapid degradation in biological systems, and the potential to load different PSs for combination therapy and multimodal imaging capability. Examples of these nanoparticles include polylactide–polyglycolide copolymers [9], N-(2-hydroxypropyl)-methacrylamide copolymers [10] and polyacrylamide [11]. These synthetic polymers are either loaded or conjugated with the conventional small organic PSs, such as, porphyrins, chlorins, hypericin or phthalocyanines. Natural polymers composed of polysaccharides, such as, chitosan and alginate as well as proteins, such as, albumin and collagen have also been used to produce vehicular nanophotosensitizers [12].

### Intrinsic nanophotosensitizers

In addition to the advantageous properties of nanomaterials for PDT, some materials, such as, nanofullerenes, titanium dioxide ( $\text{TiO}_2$ ) and zinc oxide ( $\text{ZnO}$ ) nanoparticles exhibit intrinsic photosensitizing properties [1]. The fullerenes have extended conjugated  $\pi$  bond, which can generate long-lived triplet state by absorbing blue–violet light and promote the production of ROS. Compared with small-molecule PSs, the fullerenes have much better photostability and *in vivo* stability. These nanomaterials serve as both type I and type II PDT agents because of their ability to produce ROS in an oxygen-dependent and -independent manner [13].

$\text{TiO}_2$  is an excellent intrinsic nanophotosensitizer because of its capacity to produce ROS from the reduction potential of the photo-generated holes (2.53 eV) and electron oxidation potential (-0.52 eV) [14]. The primary advantages of  $\text{TiO}_2$  are its catalytic ROS generation, ideal band gap, high photo-cross-section and stability in aqueous systems. The regenerative production of hydroxyl and superoxide radicals from water and molecular oxygen makes  $\text{TiO}_2$  a versatile nanophotosensitizer for killing cells under both hypoxic and normoxic conditions. However, the nonbiodegradability of the nanomaterial has the potential effect of building up in nontarget tissues. As such, the long-term biological effects of these particles are still unknown [15].

$\text{ZnO}$  is another important nanophotosensitizer. Unlike  $\text{TiO}_2$ , the relatively lower stability of  $\text{ZnO}$  nanoparticles allows slow biological breakdown. Additionally, the  $\text{ZnO}$  band gap of 3.3 eV corresponds to 370 nm absorption, a lesser damaging light with higher tissue penetrating capability than the 150 nm (3.8 eV) for  $\text{TiO}_2$ . Unfortunately,  $\text{ZnO}$  has a low electron oxidation potential (-0.1 eV), which is not capable of forming superoxide radicals from water. Moreover, the

absorption of light in the UV wavelengths preclude the use of these materials for treating lesions deeper than a few microns from the incident light. Recent efforts to overcome these impediments include the discovery of generation of singlet oxygen species from gold nanoparticles irradiated with an NIR laser. The PDT effect of nanogold particles presumably arises from the surface plasmon resonance [16].

### Dynamic nanophotosensitizers

Many PSs absorb light in the UV and visible wavelengths, which confines PDT to shallow or accessible tissue. As a result, alternative strategies to optimize PDT outcomes are under development. This can be accomplished by either converting light from one form into another for PS activation or spectrally shifting light to improve PS activation in deep tissue. Because ROS is generated through a secondary process after excitation, we refer to these particles as dynamic nanophotosensitizers. Notable examples include upconversion nanoparticles, quantum dots (QDs) and scintillating nanoparticles (ScNPs).

Upconversion nanoparticles exhibit nonlinear optical properties where multiple absorption events in a particle result in an emission at a higher energy than the exciting photons. This process is mediated by long-lived excited, metastable species that can either transfer energy to other excited species (energy transfer upconversion), undergo consecutive absorption events (excited-state absorption), or by photon avalanche effect to reach a highly excited state. These processes are distinct from multiphoton excitation where photons must be coincident on an absorber. Idris *et al.* designed a multicomponent system consisting of upconverting rare earth materials encapsulated by mesoporous silica shells loaded with two different PSs, merocyanine 540 and zinc (II) phthalocyanine [17]. Upon excitation at 980 nm, the rare earth materials emit light that was able to excite the two PSs in the visible wavelengths. The NIR excitation and the simultaneous production of multiple radicals enhanced the treatment of tumors in deep tissue [18].

The broad absorption spectra of QDs provide flexibility in the excitation wavelengths and allow the efficient harvesting of energy from a broadband light source. Conversely, the narrow emission spectra of QDs can be fine-tuned to selectively activate diverse PSs at the optimal excitation peak. Hsu *et al.* demonstrated the potential of intracellular bioluminescence energy transferring from coelenterazine-treated luciferase immobilized QDs to meta-tetra-hydroxyphenylchlorin PS. By using the light from within cells, the approach overcomes the difficulty in delivering light beyond a few millimeters from external sources, pro-

ducing ample ROS to efficiently kill tumors [19].

ScNPs can convert ionizing radiation in the kilovoltage or megavoltage range into visible light that can activate photosensitizing molecules. As the radiation traverses an ScNP, it generates electron–hole pairs, which can transfer energy to luminescent centers in the particle. This form of activation overcomes the depth limitation of most PDT procedures, allowing the treatment of tumors anywhere in the body. Chen and colleagues used ScNPs to deliver PSs to the treatment site *in vivo*. The ScNPs transduced the absorbed x-ray light to activate the PSs, resulting in the production of singlet oxygen to kill the cancer cells [20].

### What does the future hold?

Despite the unique attributes of PDT, it remains an underutilized cancer treatment paradigm in the clinics due to several factors. The adoption of different nanoconstructs could overcome some impediments to progress, broaden PDT appeal and uncover new applications. One major obstacle is the shallow penetration of light in tissue, which confines PDT to only superficial or endoscope-accessible tissues. Multiple tissue depth-independent methods for PDT are under investigation. One approach is to employ ionizing radiation from x-rays to stimulate ROS production from nanophotosensitizers (see above). The potential hazards caused by ionizing radiation lowers enthusiasm for this approach. An alternative approach is to stimulate ROS production from Cerenkov radiation (CR) and the interaction of radionuclides with nanophotosensitizers. By activating PSs from within cells, this method expands the use of PDT for the treatment of localized and disseminated diseases. CR can be generated by both  $\beta$ -emitting radionuclides as well as external beam radiation. CR-producing external beam radiation combined with PSs can synergistically inhibit cancer cell growth *in vitro*, while CR-producing radionuclides can destroy cancer both *in vitro* and *in vivo* [21]. New methods that avoid the use of ionizing radionuclides are attractive. The emerging ultrasonic and microwave stimulation of nanophotosensitizers to produce ROS are also promising treatment methods.

Another major limitation of type II PDT is the reliance on molecular oxygen to generate reactive singlet oxygen species. This premise suggests that PDT will be less efficient under hypoxic conditions and that prolonged exposure of PS-containing tissue to light will deplete oxygen, which diminishes the PDT effect. To overcome this challenge, PSs can be incorporated into perfluorocarbon nanoparticles, which are known to carry a high concentration of molecular oxygen. Under hypoxic conditions, the nanoparticles will release the oxygen for ROS generation and boost PDT. Alterna-

tively, the multidimensional ROS-generating capability of nanophotosensitizers can be used to construct materials that produce diverse ROS types under different conditions.

Except for liposomes and some organic polymers, the clinical translation of the new nanophotosensitizers is lagging behind. Comprehensive validation studies are needed to establish the impact of nanophotosensitizers on PDT outcomes. There is no consensus on the therapeutic index of the various multifunctional nanophotosensitizers reported to date. Instead, differences in the experimental conditions, synthetic methods and sources of materials confound results, which are difficult to reproduce. As a result, it would be important to establish criteria for evaluating the safety and effectiveness of nanophototherapeutics before clinical translation. For many of the nonorganic nanomaterials, long-term toxicity remains a concern. One approach is to incorporate features in the nanoconstructs to make them biodegradable, resulting in byproducts that are nontoxic and readily removable from the body. Understanding the biological effects of nanophotosensitizer–cell interactions will drive the development of biocompatible nanomaterials for PDT.

In summary, PDT uses a combination of nontoxic organic molecules and light to produce toxic ROS for the selective treatment of diverse diseases, including cancer. Although small organic molecules are widely used as PSs in the clinics, their poor solubility, rapid depletion of photoactive molecules upon excitation and occasional rapid clearance from the target tissue have ushered new nanoplatforms for PDT. Either through increased delivery of PSs or enhanced intrinsic ROS-

generating capability, the emerging nanophotosensitizers promise to overcome some of the impediments to a broad adoption of PDT in clinical practice. Developing innovative methods to overcome the rapid opsonization and predict the long-term toxicity of these nanomaterials will accelerate the clinical translation of this treatment paradigm. Despite the ongoing challenges in nanophotosensitizer-based PDT, the innovative approaches under development will not only solve critical problems with small molecule based PDT but also will open a new chapter for the personalized treatment of various diseases with high efficiency and low toxicity to healthy tissues.

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