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REVIEW

Bio-nanotechnology and photodynamic therapy—State of the art review

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Summary Photodynamic therapy (PDT) and bio-nanotechnology (NT) show striking similarities in clinical design and mechanistics. The PDT paradigm of photosensitizer application, light activation and singlet oxygen generation does in fact occur on the nanoscale level as does the resultant outcomes. NT has the ability to explain as well as modify each of the critical steps of PDT particularly photosensitizer design and delivery, light source miniaturization and optimization, location and intensity of the photodynamic reaction as well as offering a far greater insight into dosimetry and mechanisms of action. This review will explore the current and potential future interactions and modifications NT may have on PDT.

Contents

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Introduction

Just as life on Earth depends upon the successful transfer of sunlight's energy, human life depends upon the successful transfer of molecules throughout our body. While the study of light can be compartmentalized under the broad field of photonics, so too can the study of these cellular and subcellular biological systems be studied under the emerging field of bio-nanotechnology [1,2].

While we may not recognize it, nanotechnology (NT) is already widely at work, naturally [1]. A biological example is evidenced in the transfer of lipids and proteins from, to and between cells occurring at the nanolevel via the low density lipoprotein (LDL) receptor system. Further, neural transmission and likely memory is based on material storage and degradation on the nanoscale particle level. Gene expression is intimately involved in these nanoscaled processes as well. Naturally occurring NT is alive and well and plays a vital basic role for life and health [3].

As the name implies, bio-nanotechnology is the endeavor that analyzes, develops, and implements synthetic and naturally occurring tools measuring in size from approximately 1—1000 nm (though mainly 1—200 nm) and whose goal is to explain and modify biological systems [4]. This less than satisfactory definition is a result of the explosive emerging knowledge concerning the physical, chemical and biological behavior of these materials which remains to be better defined and refined [5]. This study is further complicated by the fact that the behavior of NT may very well change at differing sizes on the nanoscale [6].

It is highly likely that NT will modify and alter both the basic science and clinical applications of photodynamic therapy (PDT). This review will summarize how this emerging technology may change the face of PDT.

PDT—current paradigm and shortcomings

For the last 100 years the treatment paradigm of PDT has changed little [7]. A photosensitizing agent, when appropriately activated by light, creates what is termed the oxygen dependant photodynamic reaction (PDR). This ultimately results in a system change, for example ablation of a lesion without undue morbidity. To better understand the ramifications of NT on PDT, we need to examine its current state of the art.

Photosensitizers

Numerous naturally occurring and synthetic agents act as photosensitizers (PS). Fundamentally these are defined as agents that produce singlet oxygen following light stimulation [8]. However, in the clinical arena, few agents have made it to the commercially available state, which importantly, allows patients to be treated of. Each of the handful photosensitizers that currently are commercially available has specific characteristics that can benefit patients but none are totally satisfactory [9—13] (Table 1). Depending on the criteria used, the drawbacks of all these pharmaceuticals can be summarized as having some or all of the following shortcomings: inappropriate tissue half-lives causing difficulty in optimizing illumination schedules; inappropriate normal (or non-target) tissue retention so morbidity to these bystanders can be significant and of concern both during and for prolonged periods post therapy; activation energies requiring prolonged illumination times; dark toxicity; minimally clinically useful wavelength severely limiting targeting depth and equally important difficulty in synthesis and manufacture of a product that is stable; minimally allergenic; storable; easily reconstituted and cost effective. Still, even with these serious issues and shortcomings, clinical success is routinely possible. Nanotechnology may be a means to refine these molecules for improved PDT.

Table 1 Current PS drawbacks

- Hydrophobicity
- Difficulty in synthesis
- Suboptimal half-life
- Lack of specificity
- Normal tissue uptake
- Dark toxicity
- Suboptimal activation energy
- Suboptimal wavelength for activation

Table 2 Current illumination drawbacks

- Localization difficulties
- Fluence non-optimization
- Size of light source
- Flexibility of light source
- Timing of illumination
- Cost of light source
- Energy needs of light source

Light sources

A myriad of light sources, including the sun (often unintentionally) can reproducibly activate photosensitizers [14,15]. A major PDT advance has been seen through technology with the continuing refinement of commercially available and reliable illumination devices. Filtered fluorescent lights, portable diode lasers and more recently, light emitting diodes (LED) devices specifically designed and adapted for PDT, are available worldwide [16]. With the expanded implementation of fiber optics as well as miniaturized scopes and delivery systems even the most deep seated tumor beds can be theoretically illuminated [17]. However, even with these progressively improved devices, illumination remains relatively inaccurate and poorly monitored (Table 2). Any pigmented particle can block the bulk of lights transmission and current devices have no means to identify this. Further, light dosimetry reveals rapid fall off at most angles of incidence. Crevices, tissue and surface irregularities lead to inhomogeneous light distribution, a likely critical cause of under and/or over treatment. Current devices are a magnitude better than the earlier devices, but certainly nowhere near what is required to optimally achieve homogeneous or intentionally modulated illumination. NT may offer these possibilities.

Photodynamic reaction

The basis of a therapeutic outcome in conventional PDT is the creation of the PDR [18]. Here, a type II oxidative reaction creates various free radicals and most importantly singlet oxygen; all may induce necrosis, apoptosis or both. Many photosensitizers accumulate in vascular membranes, cell membranes, organelle membranes or in a combination [19]. Following light activation and PDR, these membranes are critically damaged or destroyed with release of contents [8]. Potentially, one may see a rapid vascular shut down, release of cytokines with downstream immunologic reaction or even programmed cell death induction. Certainly exploiting the location and intensity of these components could offer significantly different clinical outcomes ranging from the equivalence of a vaccine to an extremely localized treatment [20]. Currently, clinicians appear to be concentrating on lesion ablation without undue morbidity and not attempting to exploit these potential refinements (Table 3).

Table 3 Current PDR drawbacks

- Location non-specificity
- Intensity non-specificity
- Downstream and normal tissue toxicity
- Poor dosimetry

Dosimetry

An underappreciated aspect of any treatment is the control of the photodynamic reaction to allow for maximal lesion ablation with minimal normal tissue toxicity [21]. The key to maximizing outcome is by a reliable dosimetry system that accounts for all relevant variables in a therapy. This dosimetry system would guide and refine treatment. The effects and side effects of radiation therapy are highly predictable due in part to an accurate dosimetry system based on the interaction of radiation and matter. No such reliable system exists for PDT. Rather a crude, yet somewhat successful system involving administered drug dose, DLI (drug infusion to light illumination interval) and light fluence (in a rough calculation) remains the mainstay of dosimetry. No feedback on an individual's PS concentration, normal tissue as well tumor bed concentration, actual illumination fluence, oxygen level, singlet oxygen production or any of perhaps a dozen other variables are routinely employed. Using the same drug, same infusion dose, same light dose and same DLI result in widely disparate outcomes even in the same patient who may have multiple lesions. Some patients respond well, others minimally, due in large part to an archaic, inefficient dosimetry system. Until accurate real time dosimetry, with built in feedback, is developed and brought to commercial status excellent and reliable clinical outcomes for PDT will remain out of reach. There are in the last few years renewed efforts to improve the dosimetry in PDT [22,23]. As we shall see, NT may play a crucial role in this arena.

Nanotechnology

Nanotechnology has the great potential to alter each critical component of PDT to ultimately allow for clinical and scientific advances. While not widely recognized, NT is already in use in PDT and may ultimately help explain the mechanisms of PDT. However, prior to exploring nano-PDT, a brief review of NT as it applies to medicine is required.

Safety

It is established that NT occurs naturally in the body. Similarly, NT exists naturally in the earth's environment [24,25]. Nanoparticles (NPs) are created after lightning strikes and have been found in glacial ice dating back eons. NPs have also been found in meteorites, providing a universal connection. While this may offer some comfort for safety, these naturally occurring levels are far from what may be expected with widespread industrial synthesis and introduction to the environment. As a cautious example: soot, a NP produced from incomplete carbon based combustion, is quite capable of aerosolization, widespread atmospheric transfer across continents and transfer deep into the mammalian pulmonary system generating significant toxicity potential [26]. While

one can argue that limited NP production and use in the biomedical sciences outweigh potential risks, the current state of the art on safety is in its infancy. Further, the majority of NP production has been industrial based and there too, significant safety issues and environmental laws governing release are lacking so far.

Efficacy

As naturally occurring NT in our body is a prime example of the bounty, simplicity and ultimate efficacy of these processes it would seem appropriate to try to mimic and design clinically relevant NT for the diagnosis, treatment and potential prevention of disease. Among the NT bioindustrial uses is the creation of simpler synthetic processes for production of pharmaceuticals [27], controlled systems for degradation and release of chemicals and molecules [28,29], detoxifying agents [30], and biosensors able to detect miniscule amounts of various substances including poisons [31,32].

Nanoparticles types

By definition these are structures between 1 and 100 nm [25,33]. However when these structures combine they may be larger than this definition. Complicating the NT field is that size generally determines physical, chemical and biological activity so when these structures combine, their characteristics likely change [34]. In general, NPs are defined first as being either naturally occurring or synthetic and then subclassified as being organic (carbon containing) or inorganic (Table 4). Subsequent classification is generally based on structure (sphere, tube, etc.) and what they may contain such as oxides, metals or salts that are critical to function. Some examples of naturally occurring, organic NP includes soot, colloids, aerosols, and even viruses. Synthetic NP examples include catalysts used widely in chemistry and probes encapsulated by biologically localized embedding (PEBBLEs) designed specifically to advance the field of photonics [35].

Table 4 Nanoparticle classification

- Natural versus synthetic
- Organic versus inorganic
- Attribute
  - Oxide
  - Metal
  - Salt
  - Polymer
  - Aerosol
  - Other
- Structure
  - Colloid
  - Quantum Dot
  - Fiber
  - Rod
  - Crystal
  - Fullerene
  - Other

Tools

For biomedicine NT tools may be broken into two broad categories: devices for diagnosis (sensors) and devices for therapy. Perhaps, nanorobots may one day accomplish both.

Diagnostics

At this point, tools for diagnosis are various nanosensors and probes that may be used at the cellular and subcellular level to help gain understanding of both the normal and disease process at this fundamental level [31,36,37]. Examples of nanoprobes to help define chemical process and mechanism of molecular response are becoming widespread in the biological, chemical and physical sciences [31]. One common application is NT on a chip. The chip may be designed to indicate or analyze DNA, proteins, immunoassays among many other categories. The NPs can also be designed to tag onto stem cells, bacteria, viruses, normal and malignant cells to act not only as a detector but also as tracking devices. Applications include observation of cell maturation and travel, use as a biomarker for presence or absence of a particular bacteria, virus or specific metabolic pathway activation. This later use can signal a cell's transformation to the malignant state. By blocking this activation perhaps a novel treatment can be defined.

As these are nanoscale tools, one can identify single cells and molecular pathways in great detail leading to important advances in basic science. Bio-Bar-Code assays [38] use various NP to detect multiple different normal and abnormal pathways simultaneously and will likely, as an example, allow for one drop of blood to be used to replace chemistry panels that currently take several vials.

Whereas prior to NT many probes were too large to allow these processes to be directly observed and analyzed without significant disruption, NT may have diminished if not solved this problem. This is emerging on several fronts. One successful pathway has employed gold NPs which can be attached to DNA down to 10 nm in size [37]. The gold is easily identified and allows tracking of millions of different DNA sequences simultaneously. Quantum Dots (QD) are inorganic fluorophores that due to their NP size allow for introduction into and on the cell without disruption of normal processes [37]. By attaching the QD to normal and tumor cells, observation on normal and tumor physiology is possible, allowing the determination of differences between these processes. QD have also been designed to detect viruses and bacteria in real time, certainly of great use to an ill individual whose medication choice and survival may depend on rapid diagnosis [39]. But also it may be of great use in the case of bioterrorism in which tiny amounts of poisons can be detected in air, water or food. By designing paramagnetic, pH sensitive or electrically sensitive NP, one can also direct these sensors to particular anatomical regions. As mentioned, PEBBLEs are NP platforms that can be designed as highly accurate sensors to measure intracellular and extracellular ion levels (such as Na^+, K^+, Cl^-, Ca^+, etc.) as well as oxygen, OH radicals and even singlet oxygen

[40], clearly of great importance to understanding cellular reactions and in particular, to understanding of PDT and PDR. Since PEBBLEs are on the nanoscale size they do not necessarily interfere with the naturally occurring processes of the cell as prior, larger scaled sensors typically had, thus opening the door to a greater understanding of many activities, not just PDT.

Therapy

Multiple options exist to create NP of various sizes, shapes and materials that can impact organic processes [37]. Currently, most NP oncology research is based on creating NP to bind and enter cells at very specific sites and receptors. For the most part these attempts are based on a desire to achieve transfer of a pharmaceutical such as a chemotherapeutic agent into a specific targeted cell, usually one transformed to the malignant state. The goal is to allow a highly targeted, lethal event while sparing surrounding normal cells. On a more sophisticated level the NT agent may also be designed not only to apply a lethal dose but also to prevent the cell from using a detoxifying method. With NT design, so long as the appropriate pathway and entry point is clearly defined, a lethal intervention may be created. Similarly, premalignant cells, expressing, for example, abnormal proteins, again an abnormal pathway that must be defined, may be targeted by appropriately designed NP. Further, NP may be carriers for a pharmaceutical or may be lethal on their own, again depending on NP design. Gold NPs can be heated by light energy to create a tumoricidal hyperthermia effect [41]. An additional aspect is that the NP, usually designed as a QD, can also be measured and correlated with disease progression or response.

PEBBLEs have recently begun to play an important role in intervention [40]. The same platforms that were created to load sensors can be loaded with photosensitizers, imaging dyes, even singlet oxygen along with a myriad of other molecules that may offer therapy. The PEBBLEs platform can also be designed for binding to specific antigens or cell receptors that can direct this molecule precisely to a specific target.

Nanorobots

Ultimately, as NT progresses it would seem likely that a device could be created that monitors and detects abnormalities and then intervenes to attempt restoration. A feedback loop would be needed to ensure complete therapy. While not nanoscale, consider clinical PDT: a drug is introduced; light is brought in for a determined time and lesion destruction accomplished. The paradigm is set. Only the appropriate tools are needed on a nanoscale. Potentially a nanoscale PDT would be possible to destroy malignancy, infection or even allow for a regenerative medical process.

Nano-PDT

As we can see NT may have a great impact on each step of PDT. We will now examine the current state of the art and how it may alter the science and practice of PDT.

Photosensitizers

It should come as no surprise that NT is already directly involved with PS [20]. Any PS that is brought into cellular or subcellular membranes likely has been taken in by NP sized receptor molecules. PSs that are intravenously introduced do not travel far before the body detects and binds these molecules. Generally, binding is by the lipoprotein system of the plasma consisting of albumin or LDL. The immune system macrophages may ingest these foreigners, or the NP—PS complex may continue to travel. A common and routine means exploited by commercial pharmaceuticals is to encapsulate therapeutic substances into lipoproteins, particularly VLDL (very low density lipoprotein). As fats and proteins are required to allow for the excess metabolism and reproduction of malignant cells (and normal cells with high turnover) this encapsulation is a reliable way to target these regions. It is highly likely an identical mechanism brings the current generation of PS to its target as well. The NP—PS complex may then enter the target by various means.

Photofrin® is composed of porphyrin monomers, dimmers, and oligomers. The smaller components appear to be taken into the cell passively by diffusion, ultimately to the mitochondria. The larger often are actively brought into plasma membranes by phagocytosis. All components are needed for clinical success [18]. Mono-*N*-spartyl derivative of chlorin e6 (MACE) is actively brought to the lysosomes by endocytosis [42,43]. The phthalocyanines specifically accumulate in mitochondria [19]. Benzoporphyrin derivative (BPD) accumulates in the Golgi apparatus [20]. When amino levulinic acid (ALA) is introduced in tissue it is transformed to protoporphyrin IX and may localize in cell membranes (monomer) and lysosomes/mitochondria (aggregates). Therefore, it also appears clear that current PS can accumulate preferentially in different subcellular components by naturally occurring processes on the NP scale.

In this naturally occurring NP—PS complex virtually all tissues may accumulate this NP—PS complex. However, as previously mentioned rapidly proliferating tissue see this lipoprotein NP—PS as a form of energy so preferential accumulation occurs in this manner. Additional selective accumulation occurs as tumors have increased lipoprotein receptors. The leaky neovasculature of tumors may also favor accumulation and diminished clearing. What is also important to consider is that once the PS is activated and generates singlet oxygen a rapid destruction of cellular organelles occurs. Therefore, the PS accumulated in the mitochondrial membranes may then be redistributed throughout the cell and cytoplasm. As illumination will still be occurring, the PS may now generate singlet oxygen in new locations. If the PS was in the cell membrane and this resulted in destruction, the PS may now travel to interstitial regions or microvasculature and generate singlet oxygen here. Therefore, in reality one already has achieved a NP—PS complex for very specific delivery; however, ultimately where the PS actually ends up (particularly during illumination) may vary dramatically. This has important ramifications for NP—PS where rational design is employed for specific PS localization.

NP—PSs constructs are well underway and follow several pathways [40,44]. Naturally occurring NP likely assist in the delivery and uptake of current PS into the membranes

and cells organelles. Synthetic, designed NP will have a further reach. Many naturally occurring and synthetic PS are hydrophobic. This means they reconstitute poorly, have difficulty traveling in biological systems and may be rapidly cleared by the reticulo-endothelial system (RES) (lymphatic) of the body. By creating NP to encapsulate or attach to current or innovative PS the NP become the critical structure for tissue delivery. Appropriate NP design therefore can have a very far reach where PSs are concerned. By rational design, specific tissue half-life, targeting, avoidance, immune tolerance, and hydrophilicity, among other characteristics, can be created as the shell for these new PS or as a means to modify current ones. The actual size of the NP appears critical. NP > 200 nm can be taken up by the RES of the immune system. Smaller NPs appear able to travel to their destinations generally undetected by immune surveillance. One should recall though that NP can bunch together, so even those designed at the smallest end of the spectrum may behave far differently in vivo [45,46]. Further, the NP can have specific additions such as monoclonal antibodies for potential greater targeting and even additional imaging agents attached to allow better visualization by CT scan, MRI, or PET. In addition to PDT, paramagnetic attachments (for MRI) could also be spun for local hyperthermia. It should be emphasized that by design of the NP one can circumvent natural barriers to drug delivery such as the skin, pulmonary system, blood—brain barrier—even the nuclear membrane. One can also prevent the uptake of the drug by the immune system and how it clears the body to avoid, for example, the liver or the kidneys [47]. Imagination appears to be the limit but so far clinical evidence is lacking (Table 5).

Encapsulation of PS by NP

The characteristics of the NP will determine the biological and pharmacokinetics of the molecule. Encapsulation is well established in the pharmaceutical industry where, for example, lipids via NP emulsions are commonly employed. This is likely already accomplished when the clinical PS Purlytin ($SnET_2$) is reconstituted in an emulsion. As this PS showed excellent clinical selectivity, intentional encapsulation via emulsion (a NP) shows proof of principle. A number of different techniques for encapsulation exist with PEBBLEs technology [48,49]. These techniques also result in highly concentrated packets of PS within the NP. Using PEBBLEs, Kim et al. [48] embedded Indocyanine Green (ICG) into an organically modified silicate (ormosil) NP. ICG has the advantage of FDA approval and activates at about 800 nm for excellent tissue penetration. This dye may also have two photon PDT characteristics [50] and can readily fluoresce. ICG alone is hydrophobic and clears too rapidly to be an effective PS on its own. ICG-PEBBLEs had excellent characteristics for both imaging and therapy. When encapsulated by poly lactic-*co*-glycolic acid (PLGA) NPs the PS activity remains but half-life characteristics became clinically viable. When using PLGA polymers as NP the NP—PS complex appears to be very selective as a drug delivery system as well [51]. Similarly Kim et al. [49] encapsulated the highly active PS HPPH (2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-alpha or Photochlor) with dye containing ormosil NP. This allowed for conjugations enhancing two photon PDT. NP polyacrylamide (PEG) encapsulated methylene blue as a PS or Photofrin® as a PS showed both excellent imaging capability and PDT on a single encapsulated platform [52]. When iron oxides were incorporated as well, the potential for local toxic heating via magnetic resonance was demonstrated. Encapsulation with PGGA NPs altered the hydrophobic states of the highly efficient PS bacteriochlorophyll and achieved high concentration of the PS in a simple technique [53].

Dyes are a rich source of PS but those used clinically had prolonged tissue retention—potentially in the skin for several months of photosensitivity. By NP encapsulation with PEG-coating, zinc phthalocyanine prolonged tissue half-life could be modified extensively [54,55]. Ricci-Junior and Marchetti [56] reported NP-zinc phthalocyanine had numerous improved characteristics for drug delivery. In a CAM (chick chorioallantoic membrane) model Pegaz et al. [57] reported NP encapsulation of Verteporfin allowed for greater targeting than non-NP encapsulation. NP emulsions of meso-tetra-(4-hydroxyphenyl) porphyrin (p-THPP) allowed for improved tumor uptake in vitro via a simple synthetic process [46,58].

Table 5 NP—PS opportunities

- Synthesis
- Specificity
- Variable half-life
- Coupling

Surface bound PS

In this design, the PS appears to remain on the surface of the NP, but the NP itself still dictates pharmacokinetics [59]. Theoretically, the singlet oxygen would be more available when generated from the surface than from diffusing within a NP [60]. In a study by Wieder et al. [61], gold NP was attached to the PS phtalocyanine via a thiol moiety. Synthesis allowed for the hydrophobic PS to behave hydrophilically with excellent PDT.

NP conjugating PS with additional tools

It should be clear that NP design significantly influences biological, physical and chemical characteristics of the PS. Intentional design and attachments of additional moities to the NP can also influence behavior. As an example iron containing NP allows for both PDT and hyperthermia [62]. Antibodies have also been attached to NP—PS. By selecting an antibody to specific targets seen only in malignant cells further selectivity may be possible [63,64]. NP-Verteporfin conjugated to VEGF (vascular endothelial growth factor) antibodies showed additional selectivity in a CAM model [65]. Additional antibody targets such as PSA (prostate) and HER-2 (breast) have been explored. The placement of magnetic particles within the NP—PS to allow an externally directed means to bring the PS to the target has also shown preliminary success [64,66,67].

NP conjugated with biosensors and PS

This concept would allow for monitoring of the PS reaction and with appropriate telemetry give significant feedback on the treatment. In its simplest form, a change in fluorescence, if possible monitored in real time, would allow for

Table 6 NP illumination opportunities

- Less expensive
- More flexible
- Matched to specific PS
- Uptake with PS
- Lack of external light source
- Optimal timing programmed

extrapolation to treatment success (or failure) and improve therapy dramatically. If the biosensor had a feedback loop it could even stop therapy when the targets were destroyed.

NP alone

It is clear that theoretically NP can be designed to have very great specificity and activity. Perhaps a singlet oxygen generating NP designed to attach to malignant or abnormal cells, will eventually be all that is really needed. Possibly this would not defined be as PDT.

Illumination

Currently various devices supply the light energy required to activate the photosensitizer. Illumination is in fact considered a separate component of the clinical PDT paradigm. NT may alter this significantly (Table 6). First NT may significantly improve current illumination devices. Lasers are precise, expensive and are also relatively large in physical dimension as sources of illumination. NT will decrease the cost of production and the size of many light sources. This could allow for prolonged or metronomic PDT. More likely NT will improve diodes as to allow these illumination devices to become pre-eminent. Current LED's are traditionally based on semiconductor technology using inorganic structures such as silicon. These are less expensive to produce than lasers but not as precise a source of illumination. The actual LED device remains relatively large, and can be far larger and inflexible compared to current fiber optics. With NT organic-light emitting diodes (O-LED's) are possible [68]. Here, organic chemicals can be deposited on various substrates such as a film and when current is introduced very precise illumination of a specific wavelength or a spectrum can be generated depending on design. In this paradigm, custom illumination sheets could be created for a specific patient who needs large cutaneous therapy just as easily as a pinpoint light film placed in a deep seated tumor. Biodegradable OLED's which would harmlessly dissolve upon completion of illumination could be ideal. With very low electricity needs, battery operated LED and O-LED devices are possible, allowing flexible illumination schedules even in third world conditions.

A second alteration in illumination is possible with NT. NP design may also directly enhance illumination, a concept particularly useful for deep seated lesions. Packets of metallic NP can be designed to concentrate in the target tissue, along with the PS. This can create a light cavity in which, by definition, illumination intensity will increase manifold. For surface lesion illumination far less PS and light energy would be required for successful outcome. More important, deep seated lesions could be treated by infrared (IR) light which, while deeply penetrating, is on its own too weak to activate a conventional PS. However, with the NP creating a light cavity a PS with two photon PDT could readily activate. This has significant clinical ramifications. Further, the metallic NP could be heated by the IR. Such studies are underway in Brazil and elsewhere [69]. Another possible effect to be studied would be the plasmonic effect of NP to produce local heating and killing of tissue by hyperthermia.

A third possibility would be to create nanoscale light sources. Interestingly, many materials that have poor luminescence can actually have significant light emission when designed as NPs, thus opening the door to innovative light sources [70]. Potentially, NPs will be employed as the illumination agent, as a means to deploy illumination, as a light squelcher or as part of a larger NP complex containing the PS agent.

Conceivably, the NP itself is devised to fluoresce or luminesce at a particular wavelength matching the PS [71]. As the illumination is in fact within nanometers or micrometers of the PS no great need for deeply penetrating longer light wavelengths would be required. In fact most of the currently available PS activate most effectively at the Soret band (400 nm). Thus the intimate interaction of light with PS at 400 nm would be a means to enhance PDT both via greater efficiency of singlet oxygen production and likely result in less need for PS as well. Alternatively, luminescence could be induced by an external energy source to ultimately activate the PS. Luminescence NPs of both short and long half-life (afterglow) exist that emit light from about 400—700 nm and can also be matched to a particular PS [72,73]. In this paradigm external energy from, for example, radiation is absorbed by the NP leading to luminescence. As long as the NP light emission is matched to the PS a PDR can occur [74]. Luminescence can also be created by transfer from other energy sources such as heat. An optimal design would be via NP that bind to specific sites as needed (membrane, organelle, etc.) and the actual binding would alter the NP structure so that illumination would be initiated. The binding would set off a series of reactions that create the energy for illumination. The binding could set off reactions that generate heat for hyperthermia or radiation as well [62].

NP as illuminating agents

The NP illuminating agent may be administered separately from the PS. This would allow the PS to accumulate in the desired region and clear ''normal'' tissue. The paradigm would then be a NP that generates appropriate illumination/luminescence at a clinically relevant wavelength to activate the PS. Ideally the NP would be designed to have similar targeting specificity as the PS for even greater selectivity of intervention localization. Light emission could be controlled with design of luminescence for shorter or longer intervals as clinically indicated.

NP to deploy illumination

Naturally fluorescing and luminescing agents can be attached to the NPs that are designed for specific tissue targeting. Therefore the NP is a means to transport the illuminating agents. These agents may be self-activating or activated by external energy sources such as radiation, heat,

Table 7 PDR NP opportunities

- Biosensors
- Squelching
- Dosimetry
- Two photon PDT
- Localization

or magnetic fields. As an example, those activated by radiation would travel to their destination, and also, likely travel to other anatomical regions but in lower quantities. However as radiation (by external beam therapy for example) serves as the source of activation energy and would only target the tumor bed in question no PDR would be generated outside this beam. As radiation penetration and dosimetry is well defined this may be a means to provide a more precise photodynamic therapy in combination with radiation therapy.

Squelching agents
Ideally light or free radical squelching agents could be created and delivered by NP to preferentially absorb in critical normal tissues. This photo protector may then enhance the therapeutic ratio by allowing more intense therapy to the abnormal tissue which does not contain much squelching agent.

Combined illumination/PS NP
Here, one NP containing both the illuminating and PS agent would be synthesized. If developed, with accurate targeting, this NP would allow for a rapid one stop treatment. While theoretically attractive, having an activated light source in combination with the PS from the beginning of synthesis of the NP may lead to rapid degradation of the PS, unintentional PDT to all tissue as the NP is traveling, and excess PDT to tissues that clear and eliminate the NP. This may be solved by creating an illumination NP requiring an additional energy source for activation such as radiation, heat or magnetic induction.

Summary
Illumination is a major difficulty in current PDT and NP may well improve this aspect. However unintentional PDT to tissues traveled and tissue eliminating the illuminating NP may be a consequence. Using an outside source of activation energy such as radiation or magnetic field may be possible but this assumes that devices such as linear accelerators and magnetic resonance imaging (MRI) are readily available with appropriate dosimetry and anatomical localization. This is an unrealistic expectation in most parts of the world. On the other hand, ultrasound devices may offer an outstanding potential as the energy source to activate illumination particularly as these sophisticated, precise and powerful tools are cost effective and available worldwide.

Photodynamic reaction

No doubt NP already plays a key role in the PDR (Table 7). Classically, highly reactive singlet oxygen species are created by the interplay of light energy and the PS leading to cellular and subcellular responses mediated by naturally occurring NP. The actual location of PS concentration can be further pushed by design of the NP. Routes favoring specific cell membranes or organelles can be created. This could allow for apoptotic runs if the PDR were pushed entirely intracellularly or for more systemic/vascular outcomes if the PDR were pushed to the cell/vascular membranes exclusively. Of note, currently PDT is not felt to be carcinogenic/mutagenic due to the lack of direct action on DNA. If NP were designed to bring the PS into the cell nucleus, PDT might then become even more efficient as DNA is exquisitely sensitive to free radical damage, but at the potential cost of creating second malignancy.

This direct singlet oxygen reaction may not be the entire photodynamic process. Mounting evidence indicates that two photon absorption (two photon PDT) may be a component in clinically relevant outcomes. In this case the photosensitizer also is activated by fluorescence resonance energy transfer (FRET) generated from the PS, naturally occurring fluorophores or introduced dyes [49]. Additionally, in two photon PDT more penetrating (longer wavelength) light may initiate the PDR allowing for deeper tissue penetration. As previously mentioned, NP-photosensitizers favoring two photon reactions have been created; therefore, the PDR can also be manipulated in another sense via NP. As free radical production remains the key ingredient, conceivably this type II reaction can be created directly without the need for illumination, directly via appropriately designed nano-molecules (not PS) that emit singlet oxygen. Likely this would not be called PDT.

Dosimetry

NP can play a key role. Using PEBBLEs technology nanoplatforms that can advance the field of PDT are now available [48]. One particularly interesting application of PEBBLEs technology was to create a nanolevel singlet oxygen sensor [40]. Traditionally, these constructs were too large to use at the cell level, too cumbersome to offer accurate measurements and unable to routinely be used in both hydrophobic and hydrophilic environments. The dye used to measure singlet oxygen production was also toxic to the cell. Singlet oxygen nanoprobes were created using PEBBLEs technology that overcame each of these serious issues. The toxic dye used to measure singlet oxygen production was successfully embedded within the NP to eliminate toxicity and the construct of the NP was such that it allowed successful accurate singlet oxygen measurement within a variety of cells and environments [75]. Appropriately designed nanosensors could monitor oxygen level, PS level, free radial production, cytokine response, assay for DNA/RNA/protein damage, among a myriad of other relevant variables. Particularly important could be real time monitoring of light transmission, absorption and scatter to give feedback on this critical aspect of therapy. These devices could be used both in vitro and in vivo to allow for a significantly improved understanding of the basic and clinical science involved with PDT and photodiagnosis. Even if some of these variables could be easily monitored in a real time basis no doubt clinical dosimetry could make a quantum leap forward. These nanosensors, which have the great potential to assay without disrupting

the natural biological process, could be one major advance PDT has been waiting for.

Conclusion

Clearly NT is a highly efficient naturally occurring process and just as clearly NT is already a part of PDT. The road ahead that creates a fully operational NT-PDT paradigm will allow for far greater understanding of the critical steps so poorly defined in our current use of PDT. The potential to improve PS, illumination, PDR and dosimetry, appears to be a fundamental possibility through NT. However, just as PDT promised to be revolutionary, the same revolutionary expectation associated with NT will likely calm itself and move forward in an evolutionary fashion. The same difficulties to create a commercially available NT PS exist as for current PS. This is even more apparent in the use of NT illumination concepts. Certainly, though, the emergence of NT biosensors is critical for our understanding of the PDR and development of dosimetry. With NT we may finally bring PDT to the forefront of oncological diagnosis and intervention.

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