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Nanotechnology for photodynamic therapy: a perspective from the Laboratory of Dr. Michael R. Hamblin in the Wellman Center for Photomedicine at Massachusetts General Hospital and Harvard Medical School

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Abstract

The research interests of the Hamblin Laboratory are broadly centered on the use of different kinds of light to treat many different diseases. Photodynamic therapy (PDT) uses the combination of dyes with visible light to produce reactive oxygen species and kill bacteria, cancer cells and destroy unwanted tissue. Likewise, UV light is also good at killing especially pathogens. By contrast, red or near-infrared light can have the opposite effect, to act to preserve tissue from dying and can stimulate healing and regeneration. In all these applications, nanotechnology is having an ever-growing impact. In PDT, self-assembled nano-drug carriers (micelles, liposomes,

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etc.) play a great role in solubilizing the photosensitizers, metal nanoparticles can carry out plasmon resonance enhancement, and fullerenes can act as photosensitizers, themselves. In the realm of healing, single-walled carbon nanotubes can be electrofocused to produce nano-electronic biomedical devices, and nanomaterials will play a great role in restorative dentistry.

Keywords

Hamblin Laboratory; nanotechnology; photodynamic therapy

1 Introduction to PDT

Photodynamic therapy (PDT) is an emerging modality for the treatment of a variety of diseases that require the killing of pathological cells (e.g. cancer cells or infectious micro-organisms) or the removal of unwanted tissue (e.g. neovascularization in the choroid or atherosclerotic plaques in the arteries). It is based on the excitation of nontoxic photosensitizers (PSs) by harmless visible light leading to the production of highly toxic reactive oxygen species (ROS) that kill cells [1]. Suitable PSs possess a high extinction coefficient in the far red or near-infrared spectral region and a high yield of the long-lived triplet electronic state (formed from the excited singlet state by intersystem crossing). The triplet state PSs has a sufficiently long lifetime to be able to react with the surrounding molecular oxygen by one of two distinct pathways. The type 1 pathway involves electron transfer to or from the triplet PSs that can lead to a variety of oxygen free radicals such as superoxide, hydroxyl radicals, and hydroperoxides. The type 2 pathway relies on the fact that molecular oxygen is a triplet in its ground state and, therefore, has a spin allowed interaction with the PS triplet, leading to both species changing to the singlet state. However, the excited state singlet oxygen ($^1\text{O}_2$) is a potent oxidizing agent. Figure 1 shows a Jablonski diagram and the resulting type 1 and type 2 pathways.

1.1 Nanocarriers in PDT

In order for PDT to be both effective and safe, it is crucial that the PS should be delivered in therapeutic concentrations to the target cells (such as tumor cells), while simultaneously being absorbed in only small quantities by non-target cells, thus, minimizing undesirable side effects in healthy tissues. There are two main obstacles to achieving this aim. First, most PSs have extended π -conjugation systems making the molecules highly planar and in addition the molecules tend to be highly hydrophobic, and therefore, most PSs stack up to form aggregates in an aqueous environment [2]. This aggregation process lowers the efficiency of the PSs, which must be in monomeric form to be highly photoactive. Second, the PSs that have been studied so far do not generally have a high specificity for tumor cells or a pronounced tumor-localizing effect, making it difficult to target only the diseased tissue when applying PDT [3]. Many efforts have, therefore, been directed at designing delivery systems that can incorporate PS in monomeric form without diminishing its activity and without causing any harmful effects *in vivo*. The ability of nano-carriers to target tumors due to the enhanced permeability and retention (EPR) effect is also of great importance in PDT using nanoparticles [4]. Figure 2 shows how encapsulation of PS in nanoparticles can potentiate the PDT effects.

These nano-delivery systems include many different lipid and detergent nanostructures (liposomes and micelles). In fact, these nanocarriers were routinely used in PDT before nanotechnology became a separate and rapidly growing area of specialization [5]. Several questions need to be answered in the design of nanoparticle delivery agents for PSs. First, should the PS be noncovalently encapsulated in the nanoparticle or covalently attached to it? If the PS is only noncovalently associated, it is likely to be released more easily and, therefore, better taken up into cells. On the other hand, the PS may be prematurely released in the serum before the nanoparticles has had a chance to accumulate in the tumor as is hypothesized to occur via the enhanced permeability and retention effect. Second, should the nanoparticles be biodegradable or not? If they are biodegradable, the material composition will be limited to lipids or certain polymers, while nondegradable nanoparticles may remain in the body for long periods of time, and this may lead to concerns of toxicity caused by the delivery vehicle and not the drug.

1.2 Fullerenes in PDT

Fullerenes are another allotropic form of carbon nanomaterial comprising a family of closed-cage carbon molecules, C_n , where $n=60, 70, 72, 76, 84$, and even up to 100. The molecules characteristically contain 12 pentagons and a variable number of hexagons arranged in a soccer ball structure. Many applications of fullerenes have been investigated including several in biomedicine [6]. The fullerenes are seen as potential PDT agents as they possess some favorable characteristics, which render them well suited as a photosensitizer [7]. Pristine C_{60} is highly insoluble in water and biological media and, thus, forms nanoaggregates, which makes it poorly photoactive [8]. However, when fullerenes are functionalized with hydrophilic or amphiphilic functional groups, they become much more water soluble and can behave as PS [9]. Functionalization imparts a higher ability to photogenerate 1O_2 , hydroxyl radicals, and superoxide anion, thus, making them potent sources of light-mediated ROS.

Fullerenes have several potential advantages as PSs compared to other structures such as porphyrins. Fullerenes are comparatively more photostable and are less easily photobleached compared to tetrapyrroles. Fullerenes can undergo photochemistry classified into both type I and type II pathways. Fullerenes can easily be chemically modified to tuning the partition coefficient and the distribution in a biological system. The main disadvantage of fullerenes is the fact that the absorption spectrum is in the UV and blue regions, rather than the red and near-infrared regions where tissue transmission of light is maximized.

1.3 Metal nanoparticles in PDT

Gold nanoparticles have been used in two ways in PDT [10]: first, as drug-delivery platforms in a similar manner to other inorganic nanoparticles, which can be enhanced by an additional photothermal effect [11], second as surface plasmon-enhanced agents taking account of the nonlinear optical fields associated with very close distances to metal nanoparticles [12].

1.4 Single-walled carbon nanotubes (SWCNT) in bioelectronic devices

An intriguing new use of single-walled carbon nanotubes (SWCNT) has emerged in the fabrication of nano-electronics that are needed for many implantable biomedical devices. Because (for obvious reasons) it is absolutely necessary to minimize the size of implantable bioelectronic devices, and also on account of the excellent electronic properties of SWCNT (conducting or semiconducting depending on the chirality), it was considered whether SWCNT had a useful role to play in nano-bioelectronics. Therefore, it became necessary to be able to deposit vertically oriented SWCNT in an ordered array on a semiconductor substrate. This challenging goal could be elegantly accomplished by a technique known as “nanoscopic lens electrofocusing” [13]. Using this novel approach, blood glucose sensors, implantable flow sensors, and nano-biofuel cells could all be demonstrated in proof-of-principle form.

1.5 Dental nanomaterials

During the last decade, nanotechnology has become an extremely active field of research in dentistry [14]. In dental materials, for example, the main purpose of nanotechnology is achieving better mechanical properties, higher abrasion resistance, less shrinkage, improved optical and esthetic properties and to provide antimicrobial properties, a very important attribute for nanomaterials that will be used in the mouth. There has been a long-standing discussion about the absence of antimicrobial action in resin-based restorative materials, and the desires have been expressed that the ability to kill oral microorganisms could be a key to extremely long-lived restorations. Moreover, nanotechnology is now being used in the production of a wider range of dental materials such as light-cured composites and their bonding systems, impression materials, ceramics, dental implant coatings, and bioceramics [15]. Although the realization is almost certainly far off in the future, discussion has already commenced on the design of “dental nanorobots” that could be used in a mouthwash and would circulate in the mouth repairing damage and protecting the teeth [16].

2 Dr. Michael R. Hamblin and his team's contribution

2.1 Early research career

Michael R. Hamblin received his PhD in synthetic organic chemistry from Trent University in UK under Dr. Ian G.C. Coutts in 1977. During his thesis research, he synthesized a range of novel bisheterocyclic spirodienones by oxidative phenolic coupling [17, 18]. Furthermore, he developed an interest in the field known as biosynthesis, or unraveling how plants and fungi synthesize secondary metabolites such as alkaloids and other natural products that have a potential as biopharmaceuticals [19].

Dr. Hamblin's postdoctoral research was in biosynthesis carried out in two laboratories in the UK: first with Prof. Michael Grundon at the New University of Ulster; he studied the biosynthesis of the fungal metabolite echinulin [20] and then with Prof. Buchanan at Heriot Watt University; he studied the biosynthesis of the antiviral antibiotics, pyrazofurin [21] and showdomycin [22].

After periods of research at the University of Cambridge and the University of Leicester, Dr. Hamblin took up a Cancer Research Campaign Fellowship at Ninewells Hospital and Medical School at the University of Dundee. Here, he first was exposed to photodynamic therapy and other forms of phototherapy that would become his over-riding research interest over the next 20 years. He devised synthetic routes to conjugates between porphyrin photosensitizers and proteins such as albumin, transferrin [23], low-density lipoprotein, and high-density lipoprotein [24] that could target specific receptors on cancer cells and macrophages [3].

In 1994, Dr. Hamblin joined Wellman Laboratories and initially worked with Dr. Tayyaba Hasan on targeted photodynamic therapy using photoimmunoconjugates (PIC) for treatment of ovarian cancer. He devised a novel synthetic method to attach the photosensitizer chlorin(e6) to antibodies via a poly-L-lysine (PLL) linker [25] that had the added advantage of being able to tailor the overall charge on the PIC from cationic through neutral to anionic [26]. These different charges on the PIC had interesting effects on the uptake and effectiveness of photodestruction of ovarian [27], colorectal [28], and prostate cancer [29] depending on the delivery route (intravenous vs. intraperitoneal). As a follow-on project to this work, Dr. Hamblin was the first to test conjugates between PS and PLL as highly active broad-spectrum antimicrobial photosensitizers [30].

In 1997, Dr. Hamblin set up his own laboratory to work on two aspects of targeted PDT. PDT employs the combination of nontoxic dyes (called photosensitizers) and harmless visible light to kill unwanted cells and destroy tumors and other pathological tissues. He proposed that PDT could be applied to different medical problems from its conventional use as a cancer therapy. First, he devised a way to very specifically target scavenge receptors that are overexpressed on certain pathogenic phenotypes of macrophages (tumor-associated macrophages and alternatively activated macrophages in vulnerable atherosclerotic plaque). This tumor macrophage work led to several publications [31-33]. The atherosclerosis work also led to several publications [34, 35].

Dr. Hamblin expanded on his original discovery of polycationic photosensitizer conjugates [34-40] to start a broad-ranging research program in antimicrobial PDT that has led to the Hamblin Laboratory becoming a world leader in this field [41-44]. In 2001, Dr. Hamblin was awarded a NIH grant entitled "Photodynamic Therapy for Localized Infections," which has been continuously funded up to the present day and is now funded until 2018. Dr. Hamblin formed a collaboration, initially with Dr. Christopher Contag from the Stanford University and later with Xenogen Corp. that allowed his laboratory access to a range of genetically engineered bacteria that could be imaged by a bioluminescence camera. This innovation dramatically simplified the monitoring of localized infections in small animal models [45] and allowed the Hamblin Laboratory to produce a range of papers covering the use of PDT to treat infections in wounds [46], abscesses [47], burns [48], and abrasions [49]. Many of these papers covered clinically relevant drug-resistant organisms such as MRSA [49], *Acinetobacter* [50], and *Pseudomonas* [51].

In the last decade, the Hamblin Laboratory (in common with many biomedical research laboratories both in the USA and throughout the world) has become increasingly involved

with nanotechnology and nanomedicine. Partly, this new research direction has been in response to the availability of new funding opportunities, but equally so, it has been due to the real benefits offered by the nanotechnology revolution that can improve so many different aspects of biomedical research.

2.2 Fullerenes

In 2004, the Hamblin Laboratory formed a collaboration with (the late) Dr. Tim Wharton who was a fullerene chemist at Lynntech Inc. in College Station, TX. Wharton prepared a series of cationic substituted C60 fullerenes that were able to photoinactivate various different micro-organisms and were also highly effective at mediating PDT killing of tumor cells depending on the exact structure and particularly on the number of cationic groups attached to each C60 cage (see Figure 3).

For instance, the monocationic BF4 was highly effective at the photoproduction of type I photochemistry-related ROS such as hydroxyl radicals and had high efficacy at killing cancer cells [52]. On the other hand, the tri-cationic BF6 was able to act as a broad-spectrum photoantimicrobial agent, mediating efficient light-induced killing of Gram-positive, Gram-negative bacteria and fungal yeasts [53]. Both of these compounds were also able to mediate PDT of diseases in selected animal models [54]. BF4 was tested in a challenging mouse model of disseminated metastases originating from colon cancer. PDT using BF4 was able to provide a statistically significant increase in survival [55]. BF6 was tested in two mouse models of wounds that had been infected with highly virulent Gram-negative bacteria [56]. In the case of *Proteus mirabilis*, BF6-PDT saved mice from dying of the infection, while in the case of *Pseudomonas aeruginosa*, PDT could synergistically combine with a sub-optimal dose of antibiotics.

Dr. Hamblin then formed a collaboration with Prof. Long Y. Chiang from U Mass Lowell, MA. Long Chiang's laboratory focuses on the design and synthesis of carbon nanostructures. These include the near-infrared absorbing emerald green fullerenes for near-infrared sensing and imaging, near-infrared filter, and broadband photovoltaic devices. Another project involves the design and synthesis of multiphoton absorptive fullerene-fluorene chromophores for nonlinear photonic sensor protection and optical limiting applications: the design and synthesis of starburst conductive conjugate-polymer molecular suprastructures using functionalized C60 as a molecular core for electronic nanodevices.

Chiang also works on the design and synthesis of hydrophilic molecular self-assembly of fullerene-derived nanospheres as biologically active free radical scavenging agents, PDT drugs, and antibacterial agents (see Figure 4). Several water-soluble C60 derivatives have been developed specifically as free radical scavengers in biological systems against ROS-induced diseases. The high efficiency of FC4S as a biophotonic compound in photodynamic therapy (PDT) against tumor and cancer cells, as shown in our recent *in vitro* and *in vivo* studies, established its platform in conjunction with the use of antibodies and peptides for targeting for potential therapeutic uses.

Chiang has devised a synthetic route to a range of fullerene derivatives characterized by a well-defined chain of 10 cationic charges. These compounds have been tested as

photosensitizers to mediate the PDT of pathogenic microorganisms [57], cancer cells [58], and to treat mouse models of infectious disease [59].

2.3 Carbon nanotubes

Shanmugamurthy Lakshmanan (Shan) is a postdoctoral research fellow in the Hamblin Lab. Shan has carried out research on electrophoresis deposition (EPD) of SWCNTs in narrow spatial windows in order to achieve nanoscale devices. A novel technique called the “nanoscopic lens” was employed along with EPD to produce a variety of 3D nano-structures in a controlled manner based on ion-induced focusing [13] (see Figure 5). This research has shifted the current ability to position nanoparticles on surfaces, to a new dimension of spatial control. The nanoscopic lens technique can serve as the foundation of a multifaceted technology for devices. The advantages are relatively easy setup and operation, CMOS compatible, cost effective, uses commercially available machinery, components, and substances.

2.4 Nanotechnology in PDT drug delivery

Most photosensitizers are highly hydrophobic and, therefore, their poor solubility and aggregation in aqueous solutions require the development of drug delivery systems for their administration. Nanotechnology not only may play an important role in the photodynamic activity of the photosensitizer by preserving its monomeric form thus maximizing the photochemistry that occurs upon photon absorption but also may tune the pharmacokinetics and tumor selectivity of the photoactive drug.

Ying-Ying Huang is an Instructor at the Harvard Medical School and has been a researcher in the Hamblin Laboratory for more than 5 years. Among a large number of research interests, she works on nanotechnology and drug delivery in PDT.

One study involved the investigation of a micellar nano-delivery vehicle for new synthetic stable bacteriochlorin photosensitizers prepared in the laboratory of Jonathan Lindsey from North Carolina State University [60]. Micellar delivery markedly improved the subcellular localization and made the compounds much more active in PDT (see Figure 6).

Maria Garcia-Diaz spent a period in the Hamblin Laboratory in 2011–2012 during her PhD in Barcelona, Spain. She investigated the effects of the formulation on the photodynamic activity, localization, and tumor accumulation of the photosensitizer temocene against the P815 tumor both *in vitro* and *in vivo* after vascular or cellular targeting [61]. Temocene was administered in three different formulations: as free drug dissolved in PEG₄₀₀-EtOH mixture or encapsulated into Cremophor EL micelles or into pegylated liposomes. Although aggregation of temocene was evident when delivered in the solvent mixture, the free drug showed the best *in vitro* response because cells were able to internalize the largest amount of photosensitizer. However, this formulation induced an immediate and acute toxic response when administered intravenously. On the other hand, the nano-carrier-based formulations showed high encapsulation of temocene in the monomeric state. Micelles led to a poor cellular internalization and, therefore, no photocytotoxic effect, whereas liposomes exhibited the highest killing activity per uptaken molecule *in vitro*. Interestingly, the micellar

formulation showed the best *in vivo* response when used in a short drug-to-light interval resulting in a disruption of the tumor-associated vasculature. Liposomes showed the highest tumor extravasation after 24 h after injection and the best tumor selectivity; therefore, the liposomal formulation was found to be an efficient drug delivery system for a tumor cell targeting strategy. In this work, we demonstrated that the choice of nano-delivery vehicle could modulate and direct the photosensitizing agents to specific targets.

Mahdi Karimi spent time in the Hamblin Laboratory during 2012–2013, while he was doing his PhD in the Department of Biophysics, Faculty of Biological Sciences, Tarbiat Modares University in Iran. His research interests include the study of different aspects of nanotechnology in the field of drug and gene delivery. Karimi used chitosan tripolyphosphate nanoparticles for stimulation of dendritic cells as the most potent antigen-presenting cells. He demonstrated that nanoparticles could stimulate allogenic T cell proliferation. In another study, a novel albumin-chitosan core-shell nano-carrier for gene delivery was prepared [62]. On his return to Iran, he set up his own laboratory in the Iran University of Medical Science. There, he started investigations into the use of carbon nano-tube (CNTs) in gene and drug delivery systems [63, 64]. Currently, the Karimi Lab is working on graphene and other nanoparticles such as plant proteins and structural proteins as drug and gene carriers in drug carrier design.

2.5 Nanotechnology in dentistry

The Hamblin Laboratory formed a collaboration with Prof. Dr. Alessandra Nara de Souza Rastelli from the Araraquara School of Dentistry, University of Sao Paulo State-UNESP, Brazil. Alessandra spent 6 months in the Hamblin Laboratory in 2014. In her laboratory in Brazil, she has set up a research program that looks at nanoparticles as critical components of nanocomposites, nanosolutions, nano-fillers for drug delivery and antimicrobial nanoparticles, which may or may not be light activated for PDT as described above. Shown below is a preparation of silver/zinc oxide nanoparticles that can be used as a topical antimicrobial preparation for oral and dental applications (Figure 7).

2.6 Photoactive nanoparticles

Rakkiyappan Chandran is a PhD graduate research student in Nanoscience at the Joint School of Nanoscience and Nanoengineering, at the University of North Carolina, Greensboro, NC, USA. He works in the area of self-assembly of nanostructures and nanomaterials for drug delivery, bio-mimicry, and other biomedical application. He worked in the Hamblin Laboratory for 2 years (2012–2013) on novel antimicrobial agents, employing carbon-based nanomaterials like fullerenes and other upconverting nanoparticles (UCNPs) as photosensitizers. His current interested is to work on theranostic nanomaterials composed of fluorescent and photothermal agents, which possess both imaging and diagnostic property that provides a method for point of care treatment in clinical oncology. For *in vivo* implementation, the near-infrared (NIR) window has been the focus for a majority of studies because of their greater light penetration. Therefore, having both fluorescent and photothermal agents with optical properties in the NIR provides the best chance of improved theranostic capabilities by utilizing nanotechnology.

3 Highlights of other important contributors and contributions to this field

There are a number of other world leading laboratories that have made major contributions especially in the area of nanotechnology and PDT. We can only highlight a few of these laboratories.

Gang Zheng is a Senior Scientist at the Princess Margaret Cancer Centre, and the University Health Network in Toronto, Canada. He invented porphysomes, the first-in-class, all organic, and targetable nanoparticles with intrinsic multimodal photonic properties [65]. Porphysomes were highlighted by the Canadian Cancer Society as one of the top 10 breakthroughs in cancer research in 2011. They are self-assembled from porphyrin-lipid building blocks to form liposome-like nanoparticles (~100 nm diameter) that means the photoactive porphyrins are quenched and inactive until they reach their target *in vivo*, where the PDT active agents are liberated [66]. The Zheng Laboratory has also specialized in the preparation of lipoprotein-like nanoparticles: lipoprotein-like nanocarriers are a novel class of biocompatible, biodegradable, and multifunctional nanoparticles based on chemically modified lipoproteins or artificially engineered lipoprotein mimetics that can be used to deliver photosensitizers for PDT [67].

Ravindra K. Pandey is a chemist who is a Principal Investigator at Roswell Park Cancer Center at Buffalo, NY, USA. His research program covers many aspects of PDT and nanotechnology [68]. One of his goals has been to develop “multifunctional theranostic nanoparticles” that can carry out both tumor imaging using PET, MRI, and fluorescence and at the same time can mediate tumor therapy via PDT [69]. He also develops models for photosynthetic reaction centers [70] and deeply explores the chemistry of porphyrin-based compounds related to chlorins and bacteriochlorins [71]. Most of the PSs and tumor-imaging agents developed in his group are derived from naturally occurring chlorophyll-a and bacteriochlorophyll-a [72]. One of his compounds (HPPH) is currently in phase II human clinical trials [73], and several more are in various stages of preclinical and toxicological studies.

Paras N. Prasad is a Distinguished Professor at SUNY at Buffalo who has collaborated with Pandey. His research interests cover the broad area of photonics. Photonics is emerging as a multidisciplinary new frontier of science and technology and is capturing the imagination of scientists and engineers worldwide because of its potential applications to many areas of present and future information and image-processing technologies. Nonlinear optics provide key operational functions needed for the implementation of photonics technology. His specific areas of interest include the preparation, processing, and theoretical modeling of photonic materials, including nonlinear optical effects in organic polymers [74], laser-induced ultrafast processes in the condensed phase [75], microstructures and dynamics within sol-gel-processed organic/inorganic hybrid materials [76], and three-dimensional optical data storage [77]. In the PDT field, he is interested in investigating multifunctional nanostructured materials that can do both therapy and fluorescence imaging [78] and in particular in multiphoton processes and their applications to PDT [79] including upconverting rare earth nanoparticles [80].

Dr. Raoul Kopelman is the Richard Smalley Distinguished University Professor of Chemistry, Physics, and Applied Physics at The University of Michigan, Ann Arbor, MI, USA. With his student, Jeff Anker, he received the Hall of Fame Collegiate Inventors Grand Prize in 2002. His research interests are in nonclassical chemical reaction kinetics and in ultra-small optochemical sensors and actuators for biomedical use. Smart nanoprobes are being developed for the detection and PDT of cancer. Kopelman invented optical nanosensors for single cell chemical and physical imaging [81], as well as a nanoscale photon source [82], a nanoscale voltmeter [83], and a nanoscale viscometer [84]. In 2003, his pioneering study described the encapsulation of meta-tetra(hydroxyphenyl)chlorin or Foscan[®] into silica nanoparticles for anti-tumor PDT [85]. They also published the encapsulation of methylene blue in ORMOSIL [86], and recently, polyacrylamide-based (PAA) nanoparticles were engineered for the intracellular delivery of PDT agents [87]. He is currently working on nanoparticle-enabled intraoperative imaging and therapy.

Celine Frochot is a CNRS Research Director at the Reactions Laboratory and Process Engineering (LRGP) in Nancy, France. Her research interests lie broadly in the area of using nanoparticles to potentiate PDT. In particular, she has focused on targeting PS-loaded nanoparticles by conjugating additional ligands recognized by receptors that are overexpressed on tumor cells or other pathological tissue states. Examples of this approach have been the use of folate to target both free PS [88] and quantum dots [89]. Attaching the carbohydrate residue mannose can also target the mannose receptors, which are overexpressed on several cell types including some cancer cells [90]. Other targeting strategies have included neuropilin targeting [91] and RGD tripeptide targeting that are recognized by integrin $\alpha_V\text{-}\alpha_3$ [92].

4 Conclusions and future perspective

Although the literature is exploding with a plethora of new applications of nanotechnology in biomedicine (the new field of nanomedicine), it behooves us all to take a long and critical look at which applications will have a real and lasting impact on human health and which fall more readily into the class of “gimmicks.”

In the PDT field, the introduction of liposomal benzoporphyrin derivative (also known as verteporfin or “Visudyne”) in 2000 for the treatment of choroidal neovascularization secondary to wet age-related macular degeneration was an amazing success [93]. At its peak of sales, it was selling \$US billions per year and saving the eyesight of countless people around the world. Although many researchers are developing a diversity of nano-carriers for PDT drug delivery, it appears that another success on that scale will be some time off.

For instance, considering the use of fullerenes in PDT, although it is highly interesting from a scientific and mechanistic point of view, it is probably rather unlikely to ever make it into the clinics in a big way.

Nanotoxicology is also still of great concern, especially with public interest groups. There have been many scary reports of unseen dangers stealthily accumulating in the environment due to injudicious use of nano-materials [94].

We believe the use of SWCNT in implantable nanodevices is just in its infancy. In particular, the design of brain machine interfaces, and implantable neuroprosthetic augmentations is poised to take off in a big way [95]. The long-term goal is to be able to “plug in some more memory” just as one would in a personal computer that was running slow. SWCNT will almost certainly have a role to play in the design of these types of devices.

Of all the medical specialties, the one where nanotechnology has arguably made the most impact is in dentistry. This is probably due to the long-standing use of engineered composite materials in restorative dentistry and the relatively easy access of the oral cavity compared to other organ systems [96]. Moreover, PDT is making an increasing impact as an antimicrobial treatment for periodontitis, endodontic sterilization, per-implantitis, and even caries [97]. These applications will almost certainly be potentiated by the correct design of nano-carriers to target the pathogen and spare the host cells.

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References

- [1]. Agostinis P, Berg K, Cengel KA, Foster TH, Girotti AW, Gollnick SO, Hahn SM, Hamblin MR, Juzeniene A, Kessel D, Korbelik M, Moan J, Mroz P, Nowis D, Piette J, Wilson BC, Golab J. Photodynamic therapy of cancer: an update. *CA: Cancer J. Clin.* 2011; 61:250–281. [PubMed: 21617154]
- [2]. Dandler J, Scheer H. Inhibition of aggregation of [Pd]-bacteriochlorophyllides in mesoporous silica. *Langmuir.* 2009; 25:11988–11992. [PubMed: 19772311]
- [3]. Hamblin MR, Newman EL. On the mechanism of the tumour-localising effect in photodynamic therapy. *J. Photochem. Photobiol. B.* 1994; 23:3–8. [PubMed: 8021748]
- [4]. Nakamura H, Jun F, Maeda H. Development of next-generation macromolecular drugs based on the EPR effect: challenges and pitfalls. *Exp. Opin. Drug Deliv.* 2015; 12:53–64.
- [5]. Derycke AS, de Witte PA. Liposomes for photodynamic therapy. *Adv. Drug Deliv. Rev.* 2004; 56:17–30. [PubMed: 14706443]
- [6]. Chawla P, Chawla V, Maheshwari R, Saraf SA, Saraf SK. Fullerenes: from carbon to nanomedicine. *Mini Rev. Med. Chem.* 2010; 10:662–677. [PubMed: 20236059]
- [7]. Duncan LK, Jinschek JR, Vikesland PJ. C60 colloid formation in aqueous systems: effects of preparation method on size, structure, and surface charge. *Environ. Sci. Technol.* 2008; 42:173–178. [PubMed: 18350893]
- [8]. Hotze EM, Labille J, Alvarez P, Wiesner MR. Mechanisms of photochemistry and reactive oxygen production by fullerene suspensions in water. *Environ. Sci. Technol.* 2008; 42:4175–4180. [PubMed: 18589984]
- [9]. Culotta L, Koshland DE Jr. Buckyballs: wide open playing field for chemists. *Science.* 1991; 254:1706–1709. [PubMed: 17829222]
- [10]. Gamaleia NF, Shton IO. Gold mining for PDT: great expectations from tiny nanoparticles. *Photodiagnosis Photodyn. Ther.* 2015; 12:221–231. [PubMed: 25818545]
- [11]. Vankayala R, Lin CC, Kalluru P, Chiang CS, Hwang KC. Gold nanoshells-mediated bimodal photodynamic and photothermal cancer treatment using ultra-low doses of near infra-red light. *Biomaterials.* 2014; 35:5527–5538. [PubMed: 24731706]
- [12]. Li Y, Wen T, Zhao R, Liu X, Ji T, Wang H, Shi X, Shi J, Wei J, Zhao Y, Wu X, Nie G. Localized electric field of plasmonic nano-platform enhanced photodynamic tumor therapy. *ACS Nano.* 2014; 8:11529–11542. [PubMed: 25375193]

- [13]. Lakshmanan, S.; Hamblin, MR. Nanoscopic Electrofocusing for Bio-Nanoelectronic Devices. Yuhasz, W., editor. IOP Science Publishing; Philadelphia, USA: 2015.
- [14]. Abiodun-Solanke IMF, Ajayi DM, Arigbede AO. Nano technology and its application in dentistry. *Ann. Med. Health Sci. Res.* 2014; 4:S171–S177. [PubMed: 25364585]
- [15]. Mitsiadis TA, Woloszyk A, Jimenez-Rojo L. Nanodentistry: combining nanostructured materials and stem cells for dental tissue regeneration. *Nanomedicine (Lond)*. 2012; 7:1743–1753. [PubMed: 23210714]
- [16]. Shetty NJ, Swati P, David K. Nanorobots: future in dentistry. *Saudi Dent. J.* 2013; 25:49–52. [PubMed: 23960556]
- [17]. Coutts IG, Hamblin MR. Synthesis of spiroheterocycles by oxidative coupling of phenolic sulphonamides. *J. Chem. Soc. Chem. Commun.* 1980:949–950.
- [18]. Coutts IG, Hamblin MR. Spirodienones Part 2: the synthesis of some heterocyclic spirodienones by phenolic coupling. *J. Chem. Soc. Perkin I.* 1981:1294–1298.
- [19]. Coutts IG, Hamblin MR, Tinley EJ. The enzymatic oxidation of phenolic tetrahydroisoquinoline-1-carboxylic acids. *J. Chem. Soc. Perkin I.* 1979:2744–2750.
- [20]. Grundon MF, Hamblin MR, Harrison DM. Biosynthesis of aromatic isoprenoids part 5: the preparation of 1-(3,3-dimethylallyl)-L-tryptophan and cyclo-L-alanyl tryptophan and their non-incorporation into echinulin. *J. Chem. Soc. Perkin I.* 1980:1294–1298.
- [21]. Buchanan JG, Hamblin MR, Sood GR, Wightman RH. The biosynthesis of pyrazofurin and formycin. *J. Chem. Soc. Chem. Commun.* 1980:917–918.
- [22]. Buchanan JG, Hamblin MR, Kumar A, Wightman RH. The biosynthesis of showdomycin – studies with stable isotopes and the determination of principal precursors. *J. Chem. Soc. Chem. Commun.* 1984:1515–1517.
- [23]. Hamblin MR, Newman EL. Photosensitizer targeting in photodynamic therapy. I. Conjugates of haematoporphyrin with albumin and transferrin. *J. Photochem. Photobiol. B.* 1994; 26:45–56. [PubMed: 7799139]
- [24]. Hamblin MR, Newman EL. Photosensitizer targeting in photodynamic therapy. II. Conjugates of haematoporphyrin with serum lipoproteins. *J. Photochem. Photobiol. B.* 1994; 26:147–157. [PubMed: 7815189]
- [25]. Soukos NS, Hamblin MR, Hasan T. The effect of charge on cellular uptake and phototoxicity of polylysine chlorin(e6) conjugates. *Photochem. Photobiol.* 1997; 65:723–729. [PubMed: 9114750]
- [26]. Hamblin MR, Miller JL, Hasan T. Effect of charge on the interaction of site-specific photoimmunoconjugates with human ovarian cancer cells. *Cancer Res.* 1996; 56:5205–5210. [PubMed: 8912858]
- [27]. Molpus KL, Hamblin MR, Rizvi I, Hasan T. Intraperitoneal photoimmunotherapy of ovarian carcinoma xenografts in nude mice using charged photoimmunoconjugates. *Gynecol. Oncol.* 2000; 76:397–404. [PubMed: 10684717]
- [28]. Del Governatore M, Hamblin MR, Shea CR, Rizvi I, Molpus KG, Tanabe KK, Hasan T. Experimental photoimmunotherapy of hepatic metastases of colorectal cancer with a 17.1A chlorin(e6) immunoconjugate. *Cancer Res.* 2000; 60:4200–4205. [PubMed: 10945630]
- [29]. Hamblin MR, Rajadhyaksha M, Momma T, Soukos NS, Hasan T. In vivo fluorescence imaging of the transport of charged chlorin e6 conjugates in a rat orthotopic prostate tumour. *Br. J. Cancer.* 1999; 81:261–268. [PubMed: 10496351]
- [30]. Soukos NS, Ximenez-Fyvie LA, Hamblin MR, Socransky SS, Hasan T. Targeted antimicrobial photochemotherapy. *Antimicrob. Agents Chemother.* 1998; 42:2595–2601. [PubMed: 9756761]
- [31]. Demidova TN, Hamblin MR. Macrophage-targeted photodynamic therapy. *Int. J. Immunopathol. Pharmacol.* 2004; 17:117–126. [PubMed: 15171812]
- [32]. Hamblin MR, Miller JL, Ortel B. Scavenger-receptor targeted photodynamic therapy. *Photochem. Photobiol.* 2000; 72:533–540. [PubMed: 11045726]
- [33]. Liu Q, Hamblin MR. Macrophage-targeted photodynamic therapy: scavenger receptor expression and activation state. *Int. J. Immunopathol. Pharmacol.* 2005; 18:391–402. [PubMed: 16164823]
- [34]. Tawakol A, Castano AP, Anatelli F, Bashian G, Stern J, Zahra T, Gad F, Chirico S, Ahmadi A, Fischman AJ, Muller JE, Hamblin MR. Photosensitizer delivery to vulnerable atherosclerotic

plaque: comparison of macrophage-targeted conjugate versus free chlorine(e6). *J. Biomed. Opt.* 2006; 11:21008.

- [35]. Tawakol A, Castano AP, Gad F, Zahra T, Bashian G, Migrino RQ, Ahmadi A, Stern J, Anatelli F, Chirico S, Shirazi A, Syed S, Fischman AJ, Muller JE, Hamblin MR. Intravascular detection of inflamed atherosclerotic plaques using a fluorescent photosensitizer targeted to the scavenger receptor. *Photochem. Photobiol. Sci.* 2008; 7:33–39. [PubMed: 18167594]
- [36]. Gad F, Zahra T, Hasan T, Hamblin MR. Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria. *Antimicrob. Agents Chemother.* 2004; 48:2173–2178. [PubMed: 15155218]
- [37]. Hamblin MR, Miller JL, Rizvi I, Loew HG, Hasan T. Pegylation of charged polymer-photosensitizer conjugates: effects on photodynamic efficacy. *Br. J. Cancer.* 2003; 89:937–943. [PubMed: 12942129]
- [38]. Hamblin MR, Miller JL, Rizvi I, Ortel B. Degree of substitution of chlorin(e6) conjugated to charged poly-L-lysine chains affects their cellular uptake, localization and phototoxicity towards macrophages and cancer cells. *J. X-ray Sci. Technol.* 2002; 10:139–152.
- [39]. Hamblin MR, Miller JL, Rizvi I, Ortel B, Maytin EV, Hasan T. Pegylation of a chlorin(e6) polymer conjugate increases tumor targeting of photosensitizer. *Cancer Res.* 2001; 61:7155–7162. [PubMed: 11585749]
- [40]. Hamblin MR, O'Donnell DA, Murthy N, Rajagopalan K, Michaud N, Sherwood ME, Hasan T. Polycationic photosensitizer conjugates: effects of chain length and Gram classification on the photodynamic inactivation of bacteria. *J. Antimicrob. Chemother.* 2002; 49:941–951. [PubMed: 12039886]
- [41]. Hamblin MR. Antimicrobial photodynamic therapy and photodynamic inactivation, or killing bugs with dyes and light – a symposium-in-print. *Photochem. Photobiol.* 2012; 88:496–498. [PubMed: 22497420]
- [42]. Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem. Photobiol. Sci.* 2004; 3:436–450. [PubMed: 15122361]
- [43]. Hamblin, MR.; Jori, G. *Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications.* Hader, GP.; Jori, G., editors. RSC Publishing; Cambridge, UK: 2011.
- [44]. Huang L, Dai T, Hamblin MR. Antimicrobial photodynamic inactivation and photodynamic therapy for infections. *Methods Mol. Biol.* 2010; 635:155–173. [PubMed: 20552347]
- [45]. Demidova TN, Gad F, Zahra T, Francis KP, Hamblin MR. Monitoring photodynamic therapy of localized infections by bioluminescence imaging of genetically engineered bacteria. *J. Photochem. Photobiol. B.* 2005; 81:15–25. [PubMed: 16040251]
- [46]. Hamblin MR, O'Donnell DA, Murthy N, Contag CH, Hasan T. Rapid control of wound infections by targeted photodynamic therapy monitored by in vivo bioluminescence imaging. *Photochem Photobiol.* 2002; 75:51–57. [PubMed: 11837327]
- [47]. Gad F, Zahra T, Francis KP, Hasan T, Hamblin MR. Targeted photodynamic therapy of established soft-tissue infections in mice. *Photochem Photobiol Sci.* 2004; 3:451–458. [PubMed: 15122362]
- [48]. Lambrechts SA, Demidova TN, Aalders MC, Hasan T, Hamblin MR. Photodynamic therapy for *Staphylococcus aureus* infected burn wounds in mice. *Photochem. Photobiol. Sci.* 2005; 4:503–509. [PubMed: 15986057]
- [49]. Dai T, Tegos GP, Zhiyentayev T, Mylonakis E, Hamblin MR. Photodynamic therapy for methicillin-resistant *Staphylococcus aureus* infection in a mouse skin abrasion model. *Lasers Surg. Med.* 2010; 42:38–44. [PubMed: 20077489]
- [50]. Dai T, Tegos GP, Lu Z, Huang L, Zhiyentayev T, Franklin MJ, Baer DG, Hamblin MR. Photodynamic therapy for *Acinetobacter baumannii* burn infections in mice. *Antimicrob. Agents Chemother.* 2009; 53:3929–3934. [PubMed: 19564369]
- [51]. Hamblin MR, Zahra T, Contag CH, McManus AT, Hasan T. Optical monitoring and treatment of potentially lethal wound infections in vivo. *J. Infect. Dis.* 2003; 187:1717–1726. [PubMed: 12751029]

- [52]. Mroz P, Pawlak A, Satti M, Lee H, Wharton T, Gali H, Sarna T, Hamblin MR. Functionalized fullerenes mediate photodynamic killing of cancer cells: type I versus type II photochemical mechanism. *Free Radic. Biol. Med.* 2007; 43:711–719. [PubMed: 17664135]
- [53]. Tegos GP, Demidova TN, Arcila-Lopez D, Lee H, Wharton T, Gali H, Hamblin MR. Cationic fullerenes are effective and selective antimicrobial photosensitizers. *Chem. Biol.* 2005; 12:1127–1135. [PubMed: 16242655]
- [54]. Sharma SK, Chiang LY, Hamblin MR. Photodynamic therapy with fullerenes in vivo: reality or a dream? *Nanomedicine (UK)*. 2011; 6:1813–1825.
- [55]. Mroz P, Xia Y, Asanuma D, Konopko A, Zhiyentayev T, Huang YY, Sharma SK, Dai T, Khan UJ, Wharton T, Hamblin MR. Intraperitoneal photodynamic therapy mediated by a fullerene in a mouse model of abdominal dissemination of colon adenocarcinoma. *Nanomedicine*. 2011; 7:965–974. [PubMed: 21645643]
- [56]. Lu Z, Dai T, Huang L, Kurup DB, Tegos GP, Jahnke A, Wharton T, Hamblin MR. Photodynamic therapy with a cationic functionalized fullerene rescues mice from fatal wound infections. *Nanomedicine (UK)*. 2010; 5:1525–1533.
- [57]. Yin R, Wang M, Huang YY, Landi G, Vecchio D, Chiang LY, Hamblin MR. Antimicrobial photodynamic inactivation with decacationic functionalized fullerenes: oxygen independent photokilling in presence of azide and new mechanistic insights. *Free Radic. Biol. Med.* 2015; 79:14–27. [PubMed: 25451642]
- [58]. Yin R, Wang M, Huang YY, Huang HC, Avci P, Chiang LY, Hamblin MR. Photodynamic therapy with decacationic [60] fullerene monoadducts: effect of a light absorbing electron-donor antenna and micellar formulation. *Nanomedicine*. 2014; 10:795–808. [PubMed: 24333585]
- [59]. Zhang Y, Dai T, Wang M, Vecchio D, Chiang LY, Hamblin MR. Potentiation of antimicrobial photodynamic inactivation mediated by a cationic fullerene by added iodide: in vitro and in vivo studies. *Nanomedicine (Lond)*. 2015; 10:603–614. [PubMed: 25723093]
- [60]. Huang YY, Balasubramanian T, Yang E, Luo D, Diers JR, Bocian DF, Lindsey JS, Holten D, Hamblin MR. Stable synthetic bacteriochlorins for photodynamic therapy: role of dicyano peripheral groups, central metal substitution (2H, Zn, Pd), and Cremophor EL delivery. *ChemMedChem*. 2012; 7:2155–2167. [PubMed: 23065820]
- [61]. Garcia-Diaz M, Kawakubo M, Mroz P, Sagrista ML, Mora M, Nonell S, Hamblin MR. Cellular and vascular effects of the photodynamic agent temocene are modulated by the delivery vehicle. *J. Control Release*. 2012; 162:355–363. [PubMed: 22841794]
- [62]. Karimi M, Avci P, Mobasser R, Hamblin MR, Naderi-Manesh H. The novel albumin-chitosan core-shell nanoparticles for gene delivery: preparation, optimization and cell uptake investigation. *J. Nanopart. Res.* 2013; 15:1651. [PubMed: 24363607]
- [63]. Karimi M, Solati N, Amiri M, Mirshekari H, Mohamed E, Taheri M, Hashemkhani M, Saeidi A, Estiar MA, Kiani P, Ghasemi A, Basri SM, Aref AR, Hamblin MR. Carbon nanotubes part I: preparation of a novel and versatile drug-delivery vehicle. *Exp. Opin. Drug Deliv.* 2015; 12:1071–1087.
- [64]. Karimi M, Solati N, Ghasemi A, Estiar MA, Hashemkhani M, Kiani P, Mohamed E, Saeidi A, Taheri M, Avci P, Aref AR, Amiri M, Baniasadi F, Hamblin MR. Carbon nanotubes part II: a remarkable carrier for drug and gene delivery. *Exp. Opin. Drug Deliv.* 2015; 12:1089–1105.
- [65]. Lovell JF, Jin CS, Huynh E, Jin H, Kim C, Rubinstein JL, Chan WC, Cao W, Wang LV, Zheng G. Porphysome nanovesicles generated by porphyrin bilayers for use as multimodal biophotonic contrast agents. *Nat. Mater.* 2011; 10:324–332. [PubMed: 21423187]
- [66]. Jin CS, Cui L, Wang F, Chen J, Zheng G. Targeting-triggered porphysome nanostructure disruption for activatable photodynamic therapy. *Adv. Health. Mater.* 2014; 3:1240–1249.
- [67]. Marotta DE, Cao W, Wileyto EP, Li H, Corbin I, Rickter E, Glickson JD, Chance B, Zheng G, Busch TM. Evaluation of bacteriochlorophyll-reconstituted low-density lipoprotein nanoparticles for photodynamic therapy efficacy in vivo. *Nanomedicine (Lond)*. 2011; 6:475–487. [PubMed: 21542686]
- [68]. Wang S, Fan W, Kim G, Hah HJ, Lee YE, Kopelman R, Ethirajan M, Gupta A, Goswami LN, Pera P, Morgan J, Pandey RK. Novel methods to incorporate photosensitizers into nanocarriers

for cancer treatment by photodynamic therapy. *Lasers Surg. Med.* 2011; 43:686–695. [PubMed: 22057496]

- [69]. Gupta A, Wang S, Pera P, Rao KV, Patel N, Ohulchanskyy TY, Missert J, Morgan J, Koo-Lee YE, Kopelman R, Pandey RK. Multifunctional nanoplateforms for fluorescence imaging and photodynamic therapy developed by post-loading photosensitizer and fluorophore to polyacrylamide nanoparticles. *Nanomedicine.* 2012; 8:941–950. [PubMed: 22115602]
- [70]. Zheng G, Shibata M, Dougherty TJ, Pandey RK. Wittig reactions on photoprotoporphyrin IX: new synthetic models for the special pair of the photosynthetic reaction center. *J. Org. Chem.* 2000; 65:543–557. [PubMed: 10813970]
- [71]. Kozyrev A, Ethirajan M, Chen P, Ohkubo K, Robinson BC, Barkigia KM, Fukuzumi S, Kadish KM, Pandey RK. Synthesis, photophysical and electrochemistry of near-IR absorbing bacteriochlorins related to bacteriochlorophyll a. *J. Org. Chem.* 2012; 77:10260–10271. [PubMed: 23082726]
- [72]. Kozyrev AN, Chen Y, Goswami LN, Tabaczynski WA, Pandey RK. Characterization of porphyrins, chlorins, and bacteriochlorins formed via allomerization of bacteriochlorophyll a. Synthesis of highly stable bacteriopurpurinimides and their metal complexes. *J. Org. Chem.* 2006; 71:1949–1960. [PubMed: 16496980]
- [73]. Bellnier DA, Henderson BW, Pandey RK, Potter WR, Dougherty TJ. Murine pharmacokinetics and antitumor efficacy of the photodynamic sensitizer 2-[1-hexyloxyethyl]-2-devinyl pyropheophorbide-a. *J. Photochem. Photobiol. B.* 1993; 20:55–61. [PubMed: 8229470]
- [74]. Pliss A, Kuzmin AN, Kachynski AV, Prasad PN. Nonlinear optical imaging and Raman microspectrometry of the cell nucleus throughout the cell cycle. *Biophys. J.* 2010; 99:3483–3491. [PubMed: 21081098]
- [75]. Wang Y, He GS, Prasad PN, Goodson T 3rd. Ultrafast dynamics in multibranched structures with enhanced two-photon absorption. *J. Am. Chem. Soc.* 2005; 127:10128–10129. [PubMed: 16028898]
- [76]. Yoshida M, Prasad PN. Fabrication of channel waveguides from sol-gel-processed polyvinylpyrrolidone/SiO(2) composite materials. *Appl. Opt.* 1996; 35:1500–1506. [PubMed: 21085265]
- [77]. He GS, Markowicz PP, Lin TC, Prasad PN. Observation of stimulated emission by direct three-photon excitation. *Nature.* 2002; 415:767–770. [PubMed: 11845202]
- [78]. Ohulchanskyy TY, Roy I, Goswami LN, Chen Y, Bergey EJ, Pandey RK, Oseroff AR, Prasad PN. Organically modified silica nanoparticles with covalently incorporated photosensitizer for photodynamic therapy of cancer. *Nano Lett.* 2007; 7:2835–2842. [PubMed: 17718587]
- [79]. Bhawalkar JD, Kumar ND, Zhao CF, Prasad PN. Two-photon photodynamic therapy. *J. Clin. Laser Med. Surg.* 1997; 15:201–204. [PubMed: 9612170]
- [80]. Chen G, Yang C, Prasad PN. Nanophotonics and nanochemistry: controlling the excitation dynamics for frequency up- and down-conversion in lanthanide-doped nanoparticles. *Acc. Chem. Res.* 2013; 46:1474–1486. [PubMed: 23339661]
- [81]. Clark HA, Kopelman R, Tjalkens R, Philbert MA. Optical nanosensors for chemical analysis inside single living cells. 2. Sensors for pH and calcium and the intracellular application of PEBBLE sensors. *Anal. Chem.* 1999; 71:4837–4843. [PubMed: 10565275]
- [82]. Lieberman K, Harush S, Lewis A, Kopelman R. A light source smaller than the optical wavelength. *Science.* 1990; 247:59–61. [PubMed: 17749492]
- [83]. Tyner KM, Kopelman R, Philbert MA. Nanosized voltmeter enables cellular-wide electric field mapping. *Biophys. J.* 2007; 93:1163–1174. [PubMed: 17513359]
- [84]. Li Y, Burke DT, Kopelman R, Burns MA. Asynchronous magnetic bead rotation (AMBR) microviscometer for label-free DNA analysis. *Biosensors (Basel).* 2014; 4:76–89. [PubMed: 25587411]
- [85]. Yan F, Kopelman R. The embedding of meta-tetra(hydroxyphenyl)-chlorin into silica nanoparticle platforms for photodynamic therapy and their singlet oxygen production and pH-dependent optical properties. *Photochem. Photobiol.* 2003; 78:587–591. [PubMed: 14743867]

- [86]. Tang W, Xu H, Kopelman R, Philbert MA. Photodynamic characterization and in vitro application of methylene blue-containing nanoparticle platforms. *Photochem. Photobiol.* 2005; 81:242–249. [PubMed: 15595888]
- [87]. Qin M, Hah HJ, Kim G, Nie G, Lee YE, Kopelman R. Methylene blue covalently loaded polyacrylamide nanoparticles for enhanced tumor-targeted photodynamic therapy. *Photochem. Photobiol. Sci.* 2011; 10:832–841. [PubMed: 21479315]
- [88]. Schneider R, Schmitt F, Frochot C, Fort Y, Lourette N, Guillemin F, Muller JF, Barberi-Heyob M. Design, synthesis, and biological evaluation of folic acid targeted tetraphenylporphyrin as novel photosensitizers for selective photodynamic therapy. *Bioorg. Med. Chem.* 2005; 13:2799–2808. [PubMed: 15781391]
- [89]. Morosini V, Bastogne T, Frochot C, Schneider R, Francois A, Guillemin F, Barberi-Heyob M. Quantum dot-folic acid conjugates as potential photosensitizers in photodynamic therapy of cancer. *Photochem. Photobiol. Sci.* 2011; 10:842–851. [PubMed: 21479314]
- [90]. Brevet D, Gary-Bobo M, Raehm L, Richeter S, Hocine O, Amro K, Loock B, Couleaud P, Frochot C, Morere A, Maillard P, Garcia M, Durand JO. Mannose-targeted mesoporous silica nanoparticles for photodynamic therapy. *Chem. Commun. (Camb)*. 2009:1475–1477. [PubMed: 19277361]
- [91]. Thomas N, Pernot M, Vanderesse R, Becuwe P, Kamarulzaman E, Da Silva D, Francois A, Frochot C, Guillemin F, Barberi-Heyob M. Photodynamic therapy targeting neuropilin-1: interest of pseudopeptides with improved stability properties. *Biochem. Pharmacol.* 2010; 80:226–235. [PubMed: 20380812]
- [92]. Frochot C, Di Stasio B, Vanderesse R, Belgij MJ, Dodeller M, Guillemin F, Viriot ML, Barberi-Heyob M. Interest of RGD-containing linear or cyclic peptide targeted tetraphenylchlorin as novel photosensitizers for selective photodynamic activity. *Bioorg. Chem.* 2007; 35:205–220. [PubMed: 17223161]
- [93]. Fenton C, Perry CM. Verteporfin: a review of its use in the management of subfoveal choroidal neovascularisation. *Drugs Aging.* 2006; 23:421–445. [PubMed: 16823995]
- [94]. Zhang M, Jin J, Chang YN, Chang X, Xing G. Toxicological properties of nanomaterials. *J. Nanosci. Nanotechnol.* 2014; 14:717–729. [PubMed: 24730292]
- [95]. Borton D, Micera S, Millan Jdel R, Courtine G. Personalized neuroprosthetics. *Sci. Transl. Med.* 2013; 5:210rv2. DOI: 10.1126/scitranslmed.3005968.
- [96]. Melo MA, Guedes SF, Xu HH, Rodrigues LK. Nanotechnology-based restorative materials for dental caries management. *Trends Biotechnol.* 2013; 31:459–467. [PubMed: 23810638]
- [97]. Gursay H, Ozcaker-Tomruk C, Tanalp J, Yilmaz S. Photodynamic therapy in dentistry: a literature review. *Clin. Oral Invest.* 2013; 17:1113–1125.

Figure 1. Jablonski diagram showing how nanoparticle-encapsulated PS can generate different ROS upon illumination that are able to kill pathogens and cancer cells and can also destroy tumors.

Figure 2. Nanotechnology could hasten the progress of PDT research from *in vitro* experiments, moving on to *in vivo* studies, and finally to clinical applications.

Figure 3.
Structures of cationic fullerenes BF4 and BF6.

Figure 4.
Examples of functionalized fullerenes with decacationic chains.

Figure 5.
The nanoscopic lens techniques for electrofocusing SWCNT.

Figure 6.
Confocal images of a bacteriochlorin photosensitizer delivered by micelles.

Figure 7.
SEM images of Ag/ZnO nanoparticles synthesized by co-precipitation at room temperature in Sao Carlos Physics Institute – IFSC – USP/Araraquara School of Dentistry-UNESP.