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REVIEW ARTICLE

Use of nanoparticles (NP) in photodynamic therapy (PDT) against cancer

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Abstract

Nanotechnology is an interdisciplinary field that holds promise for the development of better diagnostic methods and treatments for different diseases, including cancer. Given the optical, magnetic and structural properties of nanoparticles (NP), their use has been proposed in the development of non-conventional treatments against cancer, such as photodynamic therapy (PDT). In PDT, a photo-sensitizing (PS) agent, which accumulates in tumor cells and causes the death of malignant cells after irradiation with light at a certain wavelength, is used. However, the use of PDT has different problems due to hydrophobic characteristics of the PS that hinder treatment administration and efficiency. Therefore, the use of NP as carriers is proposed and their coupling to PS to optimize treatment administration. In this review, the use of NP in PDT for the treatment against cancer is described, as well as their characteristics and molecular mechanism of action when coupled to a PS.

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Application of nanotechnology in biomedicine

Nanotechnology development started early in the past decade. Conducted studies propose an explanation for the interactions between nanoparticles (NP) and biological systems, in order to verify their application in biomedicine[1]. There are different works that suggest that NPs can be used as drug delivery systems to increase the response to anti-cancer compounds[2,3]. A definition of nanotechnology could be the creation and use of materials or systems whose dimensions in the nanometer scale are in the range of 0.1-100 nm[4], although there are some exceptions, such as the liposomes.

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The NP synthesis offers the possibility to manipulate some of their physical and chemical properties that are necessary to achieve the goal of designing molecules that provide highly specific biological interactions. These have a variety of formulations for several uses, with advantages over other drug molecules in *in vivo* studies[1]. Currently, NPs have been used as carriers to deliver compounds directly into cancerous tissues because they are able to cross endothelial, vascular and tumorcell-membranes[5].

Nanotechnology allows for a more efficacious administration of drugs against cancer, but regulatory approval of nanodrugs is slow. Up to date, there are different types of drugs approved by the Food and Drug Administration (FDA), such as Dixil®, a formulation of liposomes with doxorubicin approved in 1995 for the treatment of breast and ovarian cancer[6], or Abraxane®, a conjugate of albumin bound to paclitaxel, approved

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Table 1. Drugs using nanocarriers approved against cancer

Commercial name/compound	Manufacturer	Nanocarrier	Indication
Abraxane/paclitaxel	Abraxis Biosciences	Albumin bound to paclitaxel	Metastatic breast cancer[6]
DaunoXome/daunorubicin	Diatos (France)	Liposome	Kaposi Sarcoma[9]
Doxil/Caelix/doxorubicin	Ortho Biotech	Liposome	Kaposi Sarcoma, recurrent breast cancer, ovarian cancer[10]
Myoset/doxorubicin	Cephalon (Europe)	Non-pegilated liposome	Combined therapy against recurrent breast cancer and ovarian cancer[11]

Adapted from Jain[7] and Yu et al.[8].

in 2005 for the treatment of metastatic breast cancer[7]. Table 1 presents some manufacturers that produce NP-coupled drugs that are approved in treatments against cancer up to the year of 2010. To date, nanotechnology integration to molecular biology and medicine has translated into an active development of a new research area known as nanobiotechnology[2].

Formulations of a nanocarrier are designed to reduce drug clearance time and to provide protection against enzymatic degradation agents or against the environment[4,12]. Nanobiotechnology is beginning to change the drug delivery scale and method, and is highly valuable for investigation of treatments against cancer[5], which in the past few years have focused on the development of molecular vehicles that serve as anti-cancer agents[13].

The topology of a NP comprises a nucleus, the lining and the surface with functional groups. The use of NPs in cancer therapy shows unique pharmacokinetic characteristics; NPs are rapidly internalized and stabilized[14]. Due to the different application that the NPs have demostrate, there are formulations coupled with drugs approved for clinical use that are utilized as nanocarriers[15] (Table 1).

The purpose of this review is to analyze those works that, using NPs coupled to photo-sensitizing agents, report improvements in photodynamic therapy (PDT) in studies conducted *in vivo* and *in vitro* against cancer.

NPs in PDT

PDT is a FDA-approved technology that uses lasers to activate photosensitive drugs to treat cancer and other conditions by non-surgical, minimally-invasive means. The conditions that can be treated must be in places accessible to light, such as the esophagus, the skin, the stomach, the vagina, the cervix, etc.[16]. However, recently, owing to the development of optic fibers, its use has extended to internal organs such as the brain, the ovary and the liver, amongst others.

PDT requires three components: a photosensitive chemical substance known as photo-sensitizer (PS), a light source (lamp, laser, light emmiting diode [LED]) and intracellular molecular oxygen (O_2); the energetic interaction of these three essential elements in the PDT is detailed in the Jablonsky diagram[17] (Fig. 1). PS, in its electronic baseline status, which is in the singlet status (PS_0), when absorbing light with an appropriate wavelength (a), reaches a first short-lived excited singlet status (PS_1*). The excited PS can return to its baseline status (PS_0) by emmiting the absorbed energy as fluorescence (b) or by internal conversion (c). Alternatively, the excited PS (PS_1*) can change into a first excited triplet status (T_1*) by means of a process known as intersystem crossing (d). This is a forbidden transition (spin forbidden); however, a good PS achieves a high yield of these states at the so-called quantum capacity. The T_1* status has a half-life sufficiently long to take part in the following three chemical reactions (f) and, therefore, most of the photodynamic action is mediated by PS in this energetic state:

- Type I reaction: T_1* can transfer an electron to a substrate (water or biomolecule) or substract a hydrogen atom from an AH_2 substrate to generate peroxyde and superoxyde free-radicals.
- Type II reaction: electronic excitation energy is transferred to molecular oxygen present in the tissue in normal triplet status ($^3O_2$), giving rise to singlet oxygen ($^1O_2$), which is highly reactive and cytotoxic.
- Type III reaction: The Ps in triplet status reacts directly with with biomolecules through an oxygen-independent pathway.

Type II reaction seems to play a central role in photodynamic cytotoxicity due to high-efficiency interaction of $^1O_2$ with different biological molecules. All three reactions can occur simultaneously and in competence to generate cell death, but the relationship between them depends on the type of PS and on the nature of the substrate molecules the reaction products interact with[16-19].

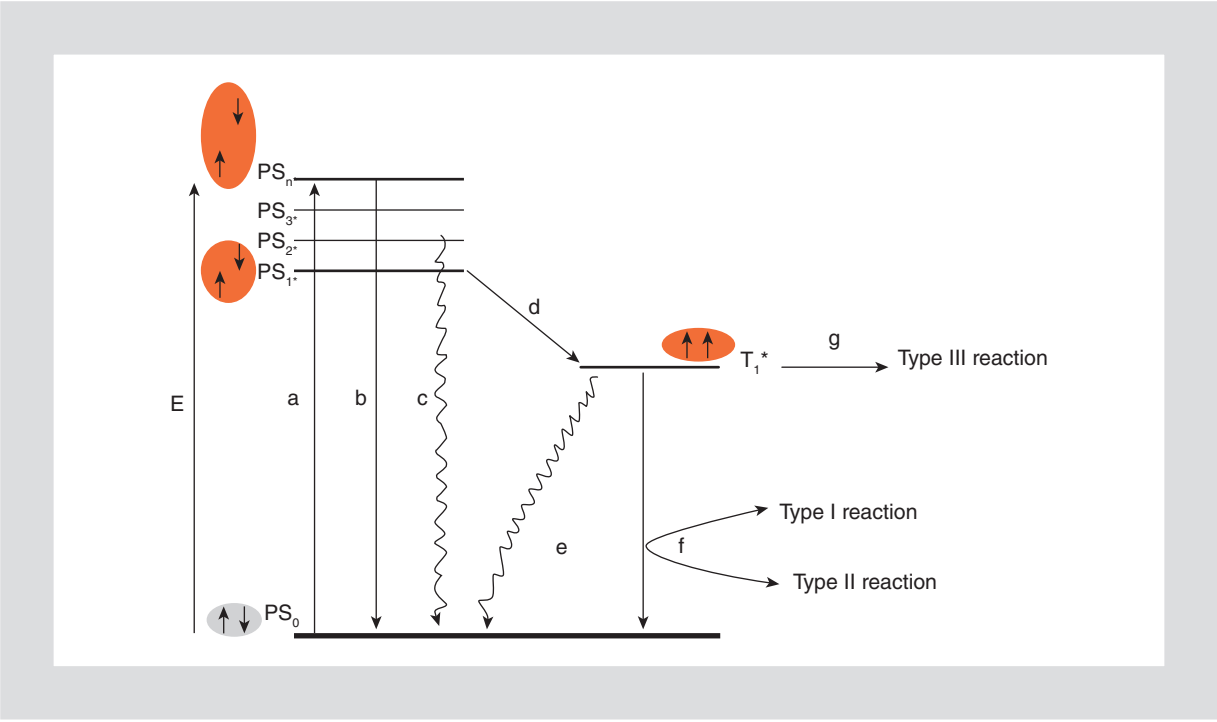

Figure 1. *Jablonsky diagram, modified to explain cell biological response mechanisms induced by PS after irradiation with light at a specific wavelength. Photophysical processes: absorption (a), fluorescence (b), internal conversion (c), intersystem crossing (d), phosphorescence (e), reactive oxygen species formation (f), PS reaction with biomolecules and oxygen-independent in the triplet state (T1*) (g).*

In its triplet status (T_1*), PS can also return to its basline status (PS_0) by emmiting a photon (phosphorescence) (e). Finally, it is degraded by the light; this process is known as photobleaching[19].

PDT clinical application is carried out as follows:

- The photosensitizing drug is administered to the patient topically or systemically.
- Some time is waited in order for the photosensitising drug to selectively accumulate in tumor cells or in cells affected by other condition. Here, fluorescence can also be measured.
- An optic fiber is introduced into the cavity of the patient in order to carry light to the tumor.
- The tumor is localizedly irradiated with a laser system or other source of light[20].

PDT has a relatively low cost, it is non-invasive, it can be locally applied and no severe side-effects are observed. Limitations of the method are associated with PS distribution and local and deep tissue irradiation, since wavelenghts do not sufficiently penetrate into the tissue[12,21]. To date, market-available PSs have specific characteristics that can benefit the patients, but no one is completely satisfactory.

In general, the main disadvantages of PSs are: short half-life within the tissue, partial retention in normal tissue, they are difficult to synthesize and reltively unstable; however, they are not mutagenic and are minimally allergenic, easy to reconstitute and cost-effective[22]. In spite of these downfalls, clinical success is possible. Nanotechnology offers many advantages to optimize PS administration, thus improving PDT[23], where NPs can be employed as: PS, photosensitizing molecule carriers, skeletons to photosensitize molecules and multifunctional carriers[24,25].

NPs have the potential to improve PDT beyond its current limitations[21]. There are many ways to modify PSs: for example, coupling them with delivery agents within liposomes[26], micelles[27-30], ceramic NPs[31], gold nanoparticles (AuNP)[32] and polymer NPs[33]. In table 2, PSs that have been conjugated to different types of NPs are presented, as well as the maximal absorption wavelength for each PS, showing that it falls within the visible light spectrum, thus improving the excitation efficiency of each PS. The advantage of using a NP is that the PS is delivered to the tumor site in a more selective form and with low toxicity, which causes very little damage to normal tissues[15,34].

The first studies within the area of NPs coupled to PSs started in 1991; for this review, publications made on the subject in different Latin American databases such as BIREME[51], LILAC[52] and SCIELO[53] have been examined, as well as European databases including SCOPUS[54] and SCIENCEDIRECT[55] and, finally, PubMed[56]. A total of

Table 2. PS conjugated to NPs

PS	Types of conjugated NPs	Abs (nm)
PpIX	Mesoporous silica NP[35] Silica NP[36]	630
Hematoporphyrin	Polyacrylamide NP[37]	630
5-aminolevulinic acid	Conjugated with AuNP[38] Alginate and chitosan NP-modified folic acid[39]	630
mTHPC	Silica NP[40], micelles[4]	652
BChl-a	PLGA NP[41]	740
Ce6	Glycol-chitosan NP[42]	690
Phthalocyanines	AuNP[43], liposomes[44] PEG-PCL micelle NP[45], silica NP[46] PLGA NP[47,48]	680
Zinc phthalocyanine	CdTe QD[49,50]	

BChl-a: Bacteriochlorophyll-a; Ca6: chlorin e6; PEG-PCL: polyethlene glycol-poly-e-caprolactone.

514 published original articles have been found up to the first quarter of 2014; performing a search for the number of patents in the USPTO database with the key terms "nanoparticles" and "photodynamic therapy", 196 registered patents were found up to date[57]. The graph on the number of publications per year is presented in figure 2.

The first works were published by two groups. One of them, the Brasseur et al. group, in 1991, used the PS hematoporphyrin coupled to an organic polyalkylcyanoacrylate NP; the conjugate demonstrated easy PS release from the formulation[37].

The number of publications, however, did not increase significantly until the year 2003, when PS-coupled NP patents started simultaneously; the synthesis mechanism and some *in vitro* tests had been described. These works include those by Konan et al. (2003), using the PS meso-tetra (4-hydroxyphenyl) porphyrin bound to NP with poly(D,L-lactic co-glycolic acid) (PLGA) employing the emulsification-diffusion technique[58]. Phototoxicity was assessed in the breast cancer EMT-6 cell-line and higher citotoxic effect was found at low PS concentrations than when it was administered only in the cell-line[59].

Other type of NPs patented in 2003 was developed by the Prasad group, with ceramic NPs that would serve as PS carriers[60]. Other works followed, such as the one by Ricci-Junior et al., who in 2006 proposed the syntesis of zinc-containing, phthalocyanine-coupled nanocapsules covered with PLGA, with a 265 nm diameter, which generated 60% of cell death after the PDT in the P388D1 cell-line, murine macrophages[33,48]. Other types of PSs coupled to NPs are shown in table 2.

NPs developed to be used when applying PDT

Since PDT efficacy is attributed to the production of the singlet oxygen ($^1O_2$), two strategies can be followed when NPs are used to achieve this goal: with biodegradable PS-releasing NPs, or with non-biodegradable NPs, in which case there is no need to release them[12]. In a review by Konan et al., the PS delivery processes are divided into passive and active base according to the presence or absence of the target molecule on the surface of target cell[61]. However, this definition does not consider the role played by NPs in the PDT process.

The use of NPs as carriers plays an important role as an active intermediary in the photodynamic activation process. Currently, a new classification has emerged according to their function. NPs can be divided in two classes: as passive or active carriers for PS excitation. As passive carriers, they can be subclassified according to the composition of the material into biodegradable and non-biodegradable (for example, metallic and ceramic NPs); and are termed active by the activation mechanism in therapy that favors the PS excitation process[62]. Classifications of NPs that have been used in PDT are shown in table 3.

NPs coupled to PS currently in preclinical phases include, for example, the liposome-covered meta-tetra(hydroxyphenyl)chlorin (mTHPC) formulation of Foslip®.

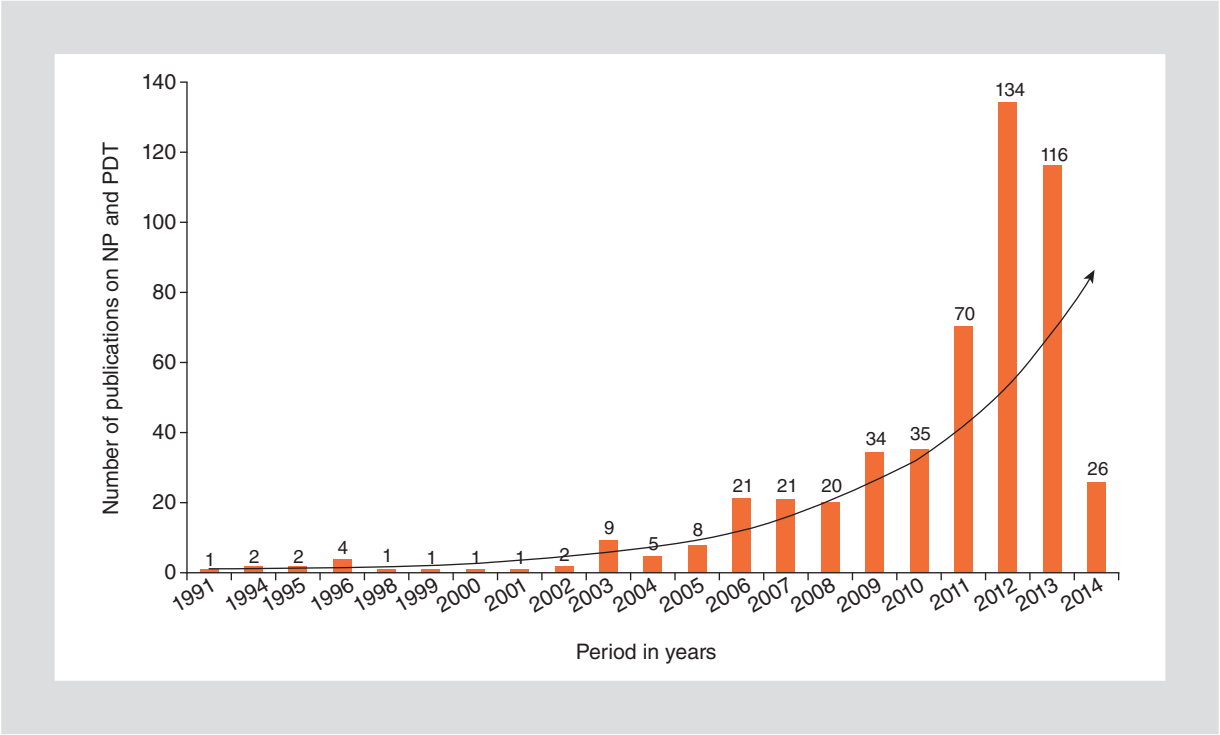

Figure 2. *Number of publications made in the area of NP and PDT.*

However, several studies have reported secondary effects with Foslip®, such as tachypnea, salivation and agitation[63], reduced damage in healthy tissues[64,65], tumor selectivity[66] and high absorption into the skin[67]. The potential use of a Foslip® liposomal formulation has been assessed in a murine model of breast cancer local recurrence[68]. The data show that Foslip® is a very satisfactory PS for PDT, with promising efficacy, improved selectivity and reduced side effects. Further studies are necessary for its development and optimization of the formulation of liposomes for PDT.

Silica phthalocyanine 4 (Pc-4) has been clinically tested[46]. It is one of the most efficient phthalocyanine-based PSs and has demonstrated high photodynamic activity. However, it is an insoluble, hydrophobic agent and has a tendency to aggregate in aqueous solutions that reduce its photodynamic activity[69]. It has been incorporated in porous silica NPs (Pc-4SNP) enabling for its solubility, stability and PS delivery to melanoma A375 cells to be improved and showing increased photodynamic activity compared to free phthalocyanine[46].

A different type of PS carriers are mesoporous silica-covered lipidic NPs, encapsulated to improve PS focalization and biocompability in the PDT. The results show better *in vitro* absorption in MCF-7 human breast carcinoma cells compared with the non-covered agent[70]. Recently, preclinical studies have been conducted with a new type of liquid ionic PS (cholinium-purpurin-18 [Chol-Pu-18]) and AuNP. They were prepared using the soluble PS based on a purpurin and choline hydroxide[71], showing better response with the conjugate when the PDT was applied on cell-lines.

These results suggest that the use of NPs as PS delivery vehicles increases the response to treatment with PDT. In the following sections we approach the administration of different types of NPs coupled to PS that have been tested in *in vitro* and *in vivo* studies and have demonstrated better response than with standard PDT.

Polymer-based biodegradable NPs

Biodegradable NPs are made out of polymers that degradate, thus releasing the PS[12]. They comprise a mixture of lactic acid and glycolic acid polymers. The increase in the biodegradation rate is achieved by the increase of the glycolic acid molar proportion in the copolimer. In 2003, Konan et al. synthesized NPs with a 150 nm diameter with a combination (50:50 PLGA:poly DL-lactic acid) loaded with a meso-tetra (p-hydroxyphenyl) porphyrin (p-THPP) second-generation PS. In this study, the effects of PDT using the NP conjugate with p-THPP were found to generate 95% of cell death[21]. However, there are not yet reports of *in vivo* studies on the use of biodegradable NPs.

Table 3. Classification of NPs used in PDT

Passive NPs		
Biodegradable	Mainly PLA and PLGA	Solid matrix/capsules containing controlled-release PS through biodegradation
Non-biodegradable	Ceramic (silica)	PS is adsorbed/covalently bound to a porous surface; in addition, it is used to co-encapsulate two photons
	Gold	5-mm NPs acting exclusively as carriers
	Iron oxide	Carries drugs directly or co-encapsulated in micelles
	Polyacrylamide	Encapsules two photons of a colorant by microemulsion
Active NPs		
PS	QD	NPs transfer incident light energy directly to the surrounding oxygen
Self-illuminated	BaFBr:Eu^{+}, Mn^{+}	Scintillation (with luminous persistence), X-ray excitation activates the bound PS
	$NaYF_4$:Yb,Er/Tm	Transduces low-energy light with the emissions of energy, activates the associated PS

It should be considered that *in vitro* photoactivity depends mainly on the photochemical and penetrating properties of the NPs loaded with PS in cells, whereas *in vivo* activity is driven by different factors, such as pharmacokinetics and tissue distribution of the NPs, which are affected by tissue components[12].

Polymer-based non-biodegradable NPs for PDT

The use of non-biodegradable NPs presents many advantages. The most important is that no time is required for biodegradation of the NPs; the PS is protected from the environment by the NPs, which serve as multifunctional platforms and are smaller in size[62]. Polyacrylamide polymers can be used for the synthesis of non-biodegradable NPs, but most are ceramic (silica) or metallic-based[72].

Ceramic NPs

These are porous-surface inorganic systems that have emerged as drug carriers with a huge potential; they can be made out of silica, titanium and aluminum materials[73,74].

The first PS-bound silica NP systems were synthesized and tested by Kopelman et al. in 2003 and by Gary-Bobo et al. in 2011[75,76]. They used mesoporous silica NPs (MSN) coupled to the PS 5-p-aminophenyl-10,15,20-sulfonatophenyl-porphyrin; this conjugate was coupled to the galactose ligand and the drug camptothecin (CPT). The photodynamic effect was assessed in colon cancer HCT-116, breast cancer MDA-MB-231 and pancreas cancer Capan-1 cell-lines; a percentage of cell death of 73 and 79% was found in colon and breast cancer cells, respectively, and in pancreatic cancer cells, 100% cell death was observed after the PDT[77]. Location of the conjugate in cell lysosomes was assesed by confocal microscopy[76,77]. Prasad et al. encapsulated, in a silica matrix, the PS 2-de-vinyl-2-(1-hexyloxyethyl) pyropheophorbide (HPPH), a PS currently in phase I and II clinical trials. *In vitro* experiments showed that the conjugate induced a percentage of cell death of 60% after the PDT[60].

Quantum dots (QD)

QDs were identified in the decade of the 80's and their synthesis was achieved in the early 90's. Currently, many uses have been found for them as solar cells, LED and imaging and diagnostic agents. Furthermore, due to their characteristics, Bakalova et al. suggested in 2004 that they could be used as possible PSs in PDT[78].

Since they show wide absorption spectra, it has been suggested that their conjugation to PSs might provide more flexibility to use different excitation wavelengths in order to activate the PS. However, most of these complexes are not water-soluble and the biocompatibility and biodisponibility properties of these compounds have not yet been demonstrated and for

these reasons they might not be optimal in biological settings[79].

Magnetic NPs

These MPs are made out from iron oxide or other superparamagnetic compounds. There are two types of iron oxide that have been investigated mainly for use in the formulation of magnetic NPs: maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4), both biocompatible, although magnetite is the most promising candidate. Generally, they are covered with dextran, phospholipids or other compounds to inhibit aggregation and to increase their stability. Their magnetic characteristics confer them usefulness in techniques to obtain images with magnetic resonance[23,73.74].

AuNP

AuNPs can be easily prepared from tetrachloroauric acid ($HAuCl_4$), which yields stable monodisperse coloidal systems with a size ranging from 1 to 150 nm[80]. AuNPs have unique chemical properties that make them suitable for promising applications in gene therapy and drug delivery to specific cells. Gold nanocapsules have been tested in several cancer models, both *in vitro* and *in vivo*[73,74].

Among other nanostructures, AuNPs play an important role in cancer therapy since they can improve radiation-induced damage; they produce heat during the exposure to UV rays and near-to-infrared radiation; therefore, they offer the possibility of cancer cells destruction by thermal ablation; they improve the administration of anti-cancer drugs that are highly insoluble in water or unstable in the biological environment; they increase the half-life time of drugs and imaging agents by modifying the NPs surfaces to avoid the loss of drug caused by rapid clearance and metabolism[81].

The use of PS-coupled AuNPs was first proposed by Russel et al. in 2002, showing a clear quantum increase of the $^1O_2$, attributed to the metal due to higher fluorescence[25]. Other PSs have been employed, such as porphyrins, chlorins, protoporphyrin IX (PpIX)-ALA. An attractive characteristic of this approach is that AuNPs are nor toxic and are already used in therapy. Therefore, approval and clinical application can be ultimately expected to be easier to achieve[21].

In 2008, Cheng et al. developed a complex consisting of AuNP, polyethylene glycol (PEG) and phthalocyanine-4 (Pc-4), for *in vivo* administration of drugs in PDT[82]. When the PS Pc-4 is injected *in vivo* in PDT it takes one or two days until it accumulates in the tumor site. Using the NP conjugate, accumulation time in the tumor was reduced to 2 h[34,82,83].

In 2011, Cheng et al. assessed the efficiency of AuNP conjugated with phthalocyanines in mice and demonstrated a system that provided rapid release and tumor penetration in a matter of hours. Pharmacokinetics of the conjugates, in a seven-day test period, demonstrated rapid excretion of the drug, verified by its fluorescence in urine. This study suggests that non-covalent delivery through an AuNP offers an attractive approach for drugs against cancer to penetrate deeply into the center of tumors[84].

This work shows the diagnostic potential of PDT using AuNP with phtalocyanines in mice. The system showed a unique versatility, since it enabled drug administration, quantitative control of the delivery process and cancer therapy. However, in the study, the fluorescence images of tumors in the mice showed the presence of the conjugate not only in the tumor, but also in other areas. These types of delivery systems can be improved, for example, with monoclonal antibodies with specificity for ligand-receptors at the tumor site. Using this system of NPs coupled to PSs, the drug-delivery process in the future might be easily controlled and quantified[84].

Table 4 presents different models of studies on PDT applied with PSs coupled to NPs. The main results of studies conducted in different cancer cell-lines *(in vitro)* are presented. Most of these studies suggest that there is a percentage of cell death equal or higher than 90% after having received PDT in comparison with controls. The results of *in vivo* studies conducted in nude mice with grafted tumors are presented: surprising effects have been found, with PDT achieving a reduction in tumor size or even tumor ablation in thermal therapy and PDT combined treatments. Finally, the start of a clinical trial in lymphoma patients using silica NP-encapsulated Pc-4 as PS is reported.

Development of NPs coupled to PS in Mexico

In Mexico, there are many institutions assigned to different research centers that work with nanotechnology and nanoscience. Different groups are developing NPs coupled to PSs for PDT. The first work published in Mexico on NPs combined with PDT was performed by our working group: we demonstrated that the use of PDT combined with AuNP increased the percentage of cell death in cervix carcinoma C33-A cells from 50%

Table 4. Models of sudies on PDT application with PS coupled to NPs

PS	NP	Study model	Cell-death percentage	Doses of light
		In vitro		
Photofrin[85]	Micelle complexes encapsulated in phthalocyanine dendrimers	A-549 lung cells	88%	80 J/cm^2
HPPH[86]	Silica NP	Colon-26 colon cells	95%	3.2 mW/cm^2
Pc-4[46]	Pc-4 encapsulated in silica NP	A375 B16F10 melanoma cells	92%	25 mW/cm^2
ZnPc[45]	PCL	A-549 lung cells	92%	100 J/cm^2
PHPP[87]	Magnetite (Fe_3O_4) NP	SW480 colon cells	40%	4.35 J/cm^2
Meso-tetraphenyl porpholactol[88]	Encapsulated with PLGA	Glioblastoma cells	95%	42 mW/cm^2
Ce6[89]	Albumin NP	HT29 colorectal cancer cells	90%	6 J/cm^2
	Magnetic NPs[90]	MGC803 gastric cancer cells	80%	30 mW/cm^2
PpIX[91,92]	AuNP	HeLa cervix cancer cells	92%	64.23 J/cm^2
		In vivo		
PS	**NP**	***In vivo* study model**	***In vivo* study model**	
Zinc Phthalocyanine[35]	Encapsulated with PLGA	Nude mice with grafted tumors	Decrease in tumor size in comparison with controls after the PDT	
Ce[69]	Albumin NP (Ce6-HAS-NP)	Nude mice with grafted tumors with HT-29 cells	Significant decrease in tumor size; there was high accumulation of the conjugate in the tumor site. Side-effects with damage to the liver	
Ce6[93]	Gold nanorods	Nude mice with grafted tumors	Tumor reduction after PDT followed by PTT. Synergistic treatment	
Pc-4[82,84]	AuNP	Nude mice with grafted tumors with glioma cells (9L)	No side effects were observed with the conjugate. Reduction of tumor and efficient penetration of drug in the tumor was found	
		Clinical trials		
PS	**NP**	**Patients**	**Trial phase**	**Observations**
Pc-4[94]	Pc-4 encapsulated in silica NP	Patients with stage IA-IIA non-Hodgkin Lymphoma	Phase I	Patient entollment

PCL: poly-ε-caprolactone; PHPP: 2,7,12,18-tetramethyl-3,8-di(1-propoxyethyl)-13,17-bis(3-hydroxypropyl)porphyrin; Ce6: chlorin e6; PTT: photothermal therapy.

with conventional PDT to 70% with PDT using the NP conjugate[95,96].

These NPs were characterized and thermal diffusivity of the PS, PpIX, was determined by thermal lens spectroscopy (TLS) in a solution mixed with an AuNP at different concentrations. The results showed that this value increased proportionally with the NPs concentration due to the strong electrostatic interaction between PpIX and the NPs, favoring an increase of the light absorption peak intensity, as well as heat transference between the PS and the NPs[97]. This was demonstrated in culture media that contained AuNP, where thermal diffusivity increased compared to the control[98].

On the other hand, the ***in vitro*** assesment of the non-radioactive relaxation time (NRRT) of PpIX in combination with AuNP was performed with photoacoustic spectroscopy (PAS), and the value of the PpIX NRRT signal with AuNP was found to be higher than the mean value of standard PpIX. Relaxation time was shown to be consistent with the useful life of the triplet status of

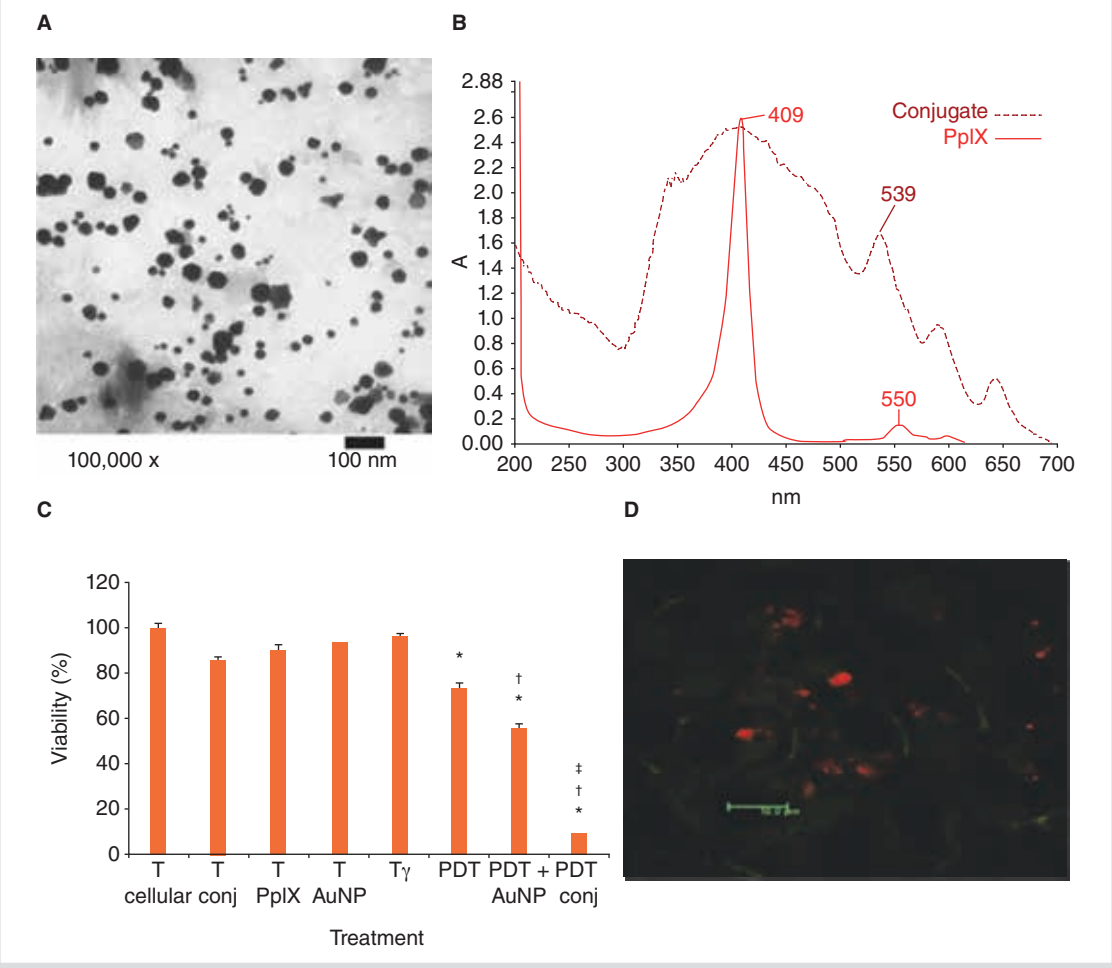

Figure 3. *Development of AuNPs coupled to the PS PpIX in Mexico.* ***A:*** *micrograph of the conjugate obtained by TEM.* ***B:*** *absorption spectrum of PpIX in the red line and absorption spectrum of the conjugate in the blue line,* ***C:*** *treatment with PDT in HeLa cells with PpIX and with the conjugate.* ***D:*** *co-localization of the conjugate in HeLa cells (images used with authorization of the authors)*[101].

porphyrins used in PDT[98]. Additionally, a maximal absorption peak of 404 nm was observed in a frequency range of 17-80 Hz[99]. This led to propose the synthesis of AuNPs coupled to PpIX, a conjugate known as AuNP-PpIX, using the monodisperse coloid method used by Maldonado et al. In 2010[91], NP with a 25 nm diameter were obtained, characterized with transmission electronic microscopy (TEM)[91,100] (Fig. 3 A). PpIX was found to have an absorption peak at 409 nm and a secondary one at 550 nm, whereas the conjugate showed a main broad peak at 409 nm and a secondary one at 620 nm[100] (Fig. 3 B), a very desirable characteristic in all nanosystems designed for future use in cancer diagnosis.

When PDT was applied to cervical cancer cells (HeLa), a significant effectivity increase was found when the conjugate was used as PS, in comparison with the effectivity obtained when the classical PDT (with PpIX) was applied (Fig. 3 C). In the HeLa cells, when PDT was applied with PpIX + NP, greater effectivity was obtained (45% mortality) with regard to the treatment wlth the classical PDT (27% mortality), but when the conjugate was employed as PS, 91% cell death was achieved[91].

In 2012, Roblero-Bartolón et al. demonstrated that AuNP-PpIX conjugates accumulate in the nucleus and cytoplasm of HeLa cells, with no affinity for the mitochondria[101]. Figure 3 D shows fluorescence indicating the intracellular localization of the conjugate in HeLa cells by confocal microscopy. Our group has been interdisciplinary integrated for the design, synthesis and characterization of AuNps coupled to PpIX. On the

other hand, Eshghi et al. published in 2001 on the synthesis of a conjugate similar to ours, AuNP coupled to PpIX (GNP), but with a 7 nm diameter. They found the conjugate to have a 630 nm peak of absorption and high efficiency in the production of ROS. GNP demonstrated to be an efficient PS for PDT in the HeLa cell-line. The effect of toxicity induced by the conjugate was compared with the control experiments, and a percentage of cell death of 92% was found in the treated lines, which suggests that the PpIX-GNP conjugate is an excellent cadidate for PDT[92]. This indicates the potential of gold and PS nanoconjugates in PDT optimization.

Another working group in Mexico is based in the Universidad Autónoma Metropolitana (UAM), and is directed by Dr. Tessy López. This group has published the synthesis of titanium dioxide NPs coupled with the PS zinc-phthalocyanine (ZnPc). The conjugate was stable at temperatures as high as 250 °C, and the photodynamic effect was tested in four cell lines: monkey epithelial cells (Vero), human hepatocellular carcinoma cells (HepG2), acute monocytic leukemia cells (THP-1), and cells of a human-derived fibroblast primary culture (HDF). They demonstrated that the conjugate was located preferably in organelles such as mitochondria and lysosomes, which might suggest a cell death mechanism by apoptosis after the PDT. In addition, in this work, the HepG2 cell-line was found to be sensitive to PDT, with an up to 90% cell death percentage being induced[102].

Conclusions and perspectives

It is important to observe that in the last 35 years thousands of patients have been treated with PDT in the world. The use of this therapy has been increasing but, although PDT is well established and approved by the FDA for conditions such as macular degeneration, skin and Barret esophagus cancer, at an international level, its use is still marginal. This may partially be due to the following PS-related factors: light absorption capacity in visible spectral regions is below 600 nm, which hinders penetration into tissues; the preparation of formulations that allow for parenteral administration is complex because most PS are hydrophobic, and selectivity for accumulation in diseased tissues is often not enough for clinical use. This makes acceptance of PDT difficult, since it is a personalized therapy, i.e., depending on the type of tumor, variables such as PS type and dosage, wavelength to be irradiated, type of tissue (more or less irrigated), time interval between PS administration and irradiation (drug-light interval) and oxygen concentration in the tissue to be treated. In view of all this, NPs offer solutions to improve the use PDT, they favor the PS properties by conferring them hydrophilic properties and an appropriate size to target tumor tissues, by increasing permeability, the retention effect and specificity, the latter with the use of biomarkers such as antibodies and peptides. Furthermore, they allow for low-energy light activation, wich enables penetration into tissues.

Physical and chemical properties of the NPs turn them into a useful tool in therapies against cancer. Several researchers have focused on the search for strategies to increase PDT efficiency, and NPs offer this possibility, since an energetic transference between the NPs and the PS has already been observed, providing they maintain a distance equal or below 10 nm. To this end, ZnO, Au and Fe_3O_4, and PS, mainly porphyrins and phthalocyanines, have been employed. Some advantages offered by the use of NPs in PDT is that dispersion of the remaining PS to other parts of the body is prevented, thus reducing photosensitivity; other advantage is that it increases the quantum capacity of the used PS, which increases therapy efficiency.

Applying the PDT using NP conjugates with PS helps to significantly improve the efficiency of such therapy. These results are encouraging and drive us to the search for those aspects that make possible to further increase the efectiveness of this therapy until 100% elimination of cancer cells is achieved, as well as to improve the PDT as a diagnostic method, so that it allows for cancer to be detected at early stages of the disease in order to improve patient's expectations. For this, further studies have to be conducted to ensure the absence of side-effects that have not been reported to date in the *in vivo* models.

PDT has been adopted as an emerging therapy for some conditions in the USA, the European Community, England, Canada, Russia, Japan, South Korea and Latin American countries such as Brazil, Argentina, Chile, Mexico, Venezuela, San Jose, Costa Rica, Guatemala, Honduras, Nicaragua, Panama, the Dominican Republic, El Salvador and Trinidad and Tobago. We consider that PDT combined with nanotechnology will soon be accessible for everybody as an established rather than experimental therapy.

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