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Chapter 9

Photodynamic Nanomedicine Strategies in Cancer Therapy and Drug Delivery

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Additional information is available at the end of the chapter

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1. Introduction

Cancer is a group of diseases which cause an abnormal and uncontrolled cell division coupled with malignant behavior such as invasion and metastasis [1]. World Health Organization (WHO) estimates that in 2012, the total number of cancer deaths in the European Union (EU) was 1283101 [2]. For the treatment of cancer various methods have already been discovered and many others are in the process of discovery e.g. chemotherapy (alkylating agents, antimetabolites and natural products - as plant products and micro-organisms), hormonal therapy (with steroids, hormones, anti-estrogens, anti-androgens, gonadotropin releasing hormones analogues, non-steroidal aromatase inhibitors), immunotherapy (interferon, growth factor inhibitors, vaccines, interleukin-2) and different therapies: radiation therapy, photodynamic therapy, surgery, chemotherapy and some traditional therapies [3]. But the anticancer drugs can fail to kill cancer cells for various reasons, the transport of the anticancer drug being governed by physiological and physicochemical properties of the target cell and of the drug itself [4]. These properties include pressure, charge, size, configuration, electro-chemical properties, hydrophilicity, etc. For the therapeutic agents delivery to the tumor cells, the following problems can be addressed, as follows:

a. Drug resistance at the tumor levels (non cellular based mechanisms);

b. Drug resistance at cellular level (cellular based mechanisms);

c. Pharmacokinetic properties of the anticancer agent in the body [5].

The concept of the nanoparticles which permits higher absorption of the drugs in a specific tissue, and this concept has been applied for hyperthermia, radiation therapy, photodynamic therapy, etc. [6]. Meanwhile, the nanoparticles opened new horizons for drug delivery and bringing the term nanomedicines. Nanomedicine is the medical application for diagnosis and
treatment of different human diseases by means of small particles, known as nanoparticles with sizes of 2-100 nm.

The nanoparticles are known by their large surface area, high reactivity, high solubility, reduced side effects and low toxicity [7-9]. The main nanoparticles applied in nanomedicine are: polymeric nanoparticles, liposomes and lipid nanoparticles, micelles, microcapsules, magnetic particles, and carbon nanoparticles (fullerenes, carbon nanotubes, carbon nanofibers, etc) and the nanoassemblies [10-12].

Photodynamic therapy (PDT) as a part of photochemotherapy, is a concerted method where, in addition to light and an administered drug, oxygen is required. PDT represents a concerted action of light, with a sensitizers and an oxygen active specie (singlet oxygen) which preferentially actions on tumor cells and not on healthy cells. The administered drug is generally a substance which can efficiently photosensitize the formation of singlet oxygen (or other reactive species derived from oxygen), and such species react with different biological targets, and cause cellular damage and finally, the cellular death. Activation of the photosensitizers by light is an essential condition for a successful PDT. Doses of light energy applied in PDT are commonly within 60-200 J/cm², though doses may vary from 25 to 500 W/cm² depending on indications, tissues and light sources [13].

Under such circumstances, this chapter offers the most up–to–date coverage of photodynamic therapy including information on how nanosensitizers, have evolved within the field of cancer therapy and more recently for drugs controlled release in this field, by using personal data correlated with literature reports.

2. Short history

Photodynamic therapy is dating from ancient time, the Indian civilizations reported from the first time the combined action of psoralens with sunlight to treat vitiligo [14].

Niels Fienisen used UV light to treat small pox, pustular infections eruptions, cutaneous tuberculosis, and for its results he obtained the Nobel Prize in Medicine in 1903. Similar results obtained Niels Raab in 1905, by using eosin as sensitizer and combining his results with Jesioneck and J.Prime results for skin tumors and epilepsy generated by light induced dermatitis [17]. Meyer-Betz was the only experimentalist who tested this method on himself, by injecting haematoporphyrin, reporting the observed effects: oedema, erythema and light sensitivity [18]. Later, Campbell and Hill studied the PDT effects on microcirculation, reporting the thrombosis and vascular shutdown [19].

Lipson in 1966 went on to treat a patient with a large cancer of the breast following an injection of a derivative of haematoporphyrin (HpD). The modern era of photodynamic therapy was established by Dr. T.J.Dougherty, at the Division of Radiation Biology at Roswell Park Memorial Institute, Buffalo, USA, who reported that a systematically injected porphyrin on activation with red light caused complete eradication of transplanted experimental tumors [20].
3. Mechanism of photodynamic effect

The photodynamic effect mainly results from energy and/or electron transfer of the lowest excited triplet state $T_1$ of the photosensitizer to an organic substrate or molecular oxygen. In the photodynamic therapy occur three types of mechanisms:

- **type I mechanism** – *electron transfer* ($eT$) where the photosensitizer excited state generates a radical species, for example by electron transfer from (or to) a substrate, or by hydrogen atom abstraction from a substrate. The radical species then reacts with ground state oxygen so that the overall reaction is a photochemically initiated autoxidation:

\[
\begin{align*}
\text{Sens} \overset{hv}{\rightarrow} \text{Sens}^* \\
\text{Sens}^* + A \overset{eT}{\rightarrow} \text{Sens}^{**} + A^{**} \\
\text{Sens}^* + AH_2 \overset{H\text{ atom transfer}}{\rightarrow} \text{SensH}^* + AH^* \\
AH^* + ^3O_2 \rightarrow AH + OO^* \rightarrow \text{products}
\end{align*}
\]

(Sens = sensitizer; A = biomolecule; $^3O_2$ = triplet excited state of oxygen)

*Scheme 1.* The type I mechanism of PDT

- **In type II mechanism** - *energy transfer* ($ET$) an energy transfer occurs from the excited photosensitizer to molecular oxygen, to give the sensitizer in its ground state and singlet oxygen. In this mechanism electronic excitation energy is transferred from the excited triplet $T_1$ of the sensitizer (generated by intersystem crossing isc from the excited singlet $S_1$) to triplet molecular oxygen, to give the sensitizer in its ground state $S_0$ and singlet oxygen $^1O_2$.

\[
\begin{align*}
\text{Sens} (S_0) \overset{hv}{\rightarrow} \text{Sens} (S_1) \overset{\text{isc}}{\rightarrow} \text{Sens} (T_1) \\
\text{Sens} (T_1) + ^3O_2 \rightarrow \text{Sens} (S_0) + ^1O_2 \\
A + ^1O_2 \rightarrow \text{products}
\end{align*}
\]

*Scheme 2.* The type II mechanism of PDT

Major biological targets are membranes that undergo rupture and the cells are destroyed through the membranes around the mitochondria and the lysosomes. These organelles induce subsequent cellular destruction by necrosis or apoptosis [21-24].

Except these two types of mechanisms, there is another one: **type III mechanism**, which take place when the oxygen is absent in the system.
Scheme 3. The type III mechanism of PDT (a)AH = porphyrin; B = quinone (b)Don = donor (cysteine); A = porphyrin; Acc = acceptor (methyl viologen).

4. Photosensitizers

4.1. Conventional photosensitizers

All the sensitizers could be natural or synthetic compounds, with proper absorption properties from a light source. They should be pure compounds, soluble in body fluids, with high capacity to be incorporated in malignant cells. Also, they should be fluorescent and able to generate singlet oxygen, which is the excited state of oxygen efficient on malignant cells [25]. Taking into account all these criteria and knowing the compatibility with human body, the porphyrins are known as ideal sensitizers for photodynamic therapy.

The general chemical structure for some porphyrins and phthalocyanines as PDT agents are represented in Figure 1.

**First Generation Photosensitizers,** includes Photofrin® and HpD and exist as complex mixtures of monomeric, dimeric, and oligomeric structures. At 630 nm, their effective tissue penetration of light is small, 2–3 mm, limiting treatment to surface tumors. Although Photofrin® has a low \( \varepsilon_{\text{max}} \) (at 630 nm~3000 M\(^{-1}\) cm\(^{-1}\)), generate singlet oxygen with high quantum yield, \( \Phi_{\Delta} = 0.89 \). In spite of its safe applications in bladder, esophageal and lung cancers, Photofrin tends to be applied to head human part and thoracic part affected by cancer [26].

**Second Generation Photosensitizers,** includes porphyrins and related compounds (porphycenes, chlorins, phthalocyanines, so on), many of them being under clinical tests. From the **porphyrins family,** meta-tetra(hydroxyphenyl)porphyrin \( (m\)-THPP) and 5,10,15,20-tetrakis(4-sulfanatophenyl)-21H,23H-porphyrin (TPPS\(_4\)) are the most used second generation PDT sensitizers (Figure 2). \( m\)-THPP, however, caused skin phototoxicity, and was 25 to 30 times more potent than HpD in tumor photonecrosis when irradiated at 648 nm [27]. TPPS\(_4\) exhibited lower photochemical efficiency than meso-substituted porphyrins containing fewer sulphonated groups [28].

Except the free-bases, the porphyrins can be chelated with a variety of metals, the diamagnetic ones enhancing the phototoxicity. Paramagnetic metals are shortening the lifetime of the triplet state and as result can make the dyes photoinactive [21]. The presence of axial ligands to the centrally coordinated metal ion is often advantageous, since it generates some degree of steric...
hindrance to intermolecular aggregation, without impairing the photophysical properties of the dye [21].

Phthalocyanines (Pc) are currently recognized as one of the best sensitizers used in PDT, have a long-wavelength band with a large extinction coefficient (~ $10^5$ M$^{-1}$ cm$^{-1}$) and generally a low dark toxicity [29-32].

Their absorption maxima are in the region 670-700 nm, with very high molar coefficients. A representative compound is aluminium phthalocyanine tetrasulphonated AlPcS$_4$, commercially known as Photosens, in spite of its skin sensitivity, proper absorption maxima at 676 nm, it is well applied in Russian clinics for stomach, skin, oral and breast cancers [33].

Another clinical phthalocyanine is silicon phthalocyanine 4 (Pc4) which was successful tested in different skin cancers (pre-malignant - actinic keratosis, Bowen disease) or even in malignant forms of cutaneous cancers [34,35,36].

The central metal ions play an important role in the photophysical properties of phthalocyanines. In metallophthalocyanines the central metal (M) has one or two axial ligands or one or
more ring substituents or both. When a diamagnetic ion is in the center of the ring (e.g., Zn, Al, Ga), the phthalocyanine generally possesses a high triplet state yield ($\varphi_T > 0.4$) with a long lifetime ($\tau_T > 200 \mu s$) and enough energy (110-126 kJ/mol$^{-1}$) to generate $^1$O$_2$ (94.5 kJ/mol$^{-1}$ is required) [37-40]. Silicon phthalocyanine allows two appropriate axial ligands, which forbid the ring staking which decrease the clinical efficiency [41-44]. The triplet-state lifetimes of an axially substituted silicon phthalocyanine typically vary from 100 to 200 μs and the yields from 0.2 to 0.5 [43]. Some synthetic silicon phthalocyanin and naphthalocyanine (Figure 2) have been used in some laboratory experiments on K562 culture cellk with excellent results [45, 46].

**Third generation photosensitizers** contains available drugs that are modified them with antibody conjugates, biologic conjugates, etc.[47,48]. These terms are still being used although not accepted unanimously and dividing photosensitizing drugs into such generations may be very confusing. The nanostructures are increasingly being used as carriers for the development of 3rd generation PS, as the most important drug delivery systems used as carriers for PS in the field of anticancer PDT.

![Figure 2. The chemical structures of Cl$_2$SiPc (left) and Cl$_2$SiNc (right)](image)

### 5. Nanoparticles in PDT

The nanoparticles can be classified into:

- ‘Hard nanoparticles’ - inorganic materials that keep unchanged their original shape and size,
- ‘Soft nanoparticles’ - organic materials that could be functionalized capacity, with versatile size and shape under different conditions; pH, T, pressure. Nanoparticles have unusual properties that can improve the drug delivery.

#### 5.1. Hard nanoparticles

**Inorganic Nanoparticles** is the generic term for several nanoparticles including for example metal oxide- and non-oxide ceramics, metals, gold and magnetic nanoparticles.

**Ceramic nanoparticles:** Ceramic-based nanoparticles have some advantages over organic carriers: particle size, shape, porosity, and mono-dispersibility. They are water-soluble,
extremely stable, and known for their compatibility in biological systems without being subjected to microbial attack. For conventional drug delivery, the carrier vehicle should release the encapsulated drug at the target tissue. The works done by Prasad’s group is one of the few examples for the application of ceramic nanoparticles to PDT [49]. Their silica-based nanoparticles (diameter ca. 30 nm) have been entrapped with the hydrophobic photosensitizing anticancer drug 2-devinyl-2-(1-hexyl-oxyethyl) pyropheophorbide via a controlled hydrolysis of triethoxyvinylsilane in micellar media. The resulting silica-based nanoparticles were monodispersed with uniform particle size. By irradiation with suitable wavelengths: 532 or 650 nm, silica nanoparticles with porphyrin embedded, could be efficiently taken up by tumor cells and lead to cells death.

Silica nanoparticles (SiO$_2$), with the following advantages:

1. chemically inert, avoiding interactions with other molecules in the body.
2. available for their synthesis, allowing precise control their particles size, shape, porosity and polydispersity during the preparation.
3. allow to incorporate small molecules encapsulated within the own particle or covalently attached to the surface.
4. These interesting properties have made silica nanoparticles the most studied nanoparticle-based PDT systems.

The delivery of photosensitisers embedded in porous silica nanoparticles has many advantages:

- almost any type of photosensitiser can be used. Second, the concentration of photosensitiser can be modulated as needed (increasing or decreasing it).
- the silica surface offers the possibility for further functionalisation.
- When the photosensitisers are incorporated on to silica nanoparticles trough covalent bonds, it is possible to avoid the eventual release of the compounds in the media, and the consequent lost of efficacy or the appearance of side effects.

Gold nanoparticles: Gold nanoparticles have been targeted to breast cancer cells by incorporating a primary antibody to the ir surface in addition to a zinc phthalocyanine photosensitiser and a bioavailability and solubility enhancer, with promising results [50,51]. Gold particles with various diameters and uniform size distribution have been demonstrated to have novel and fascinating properties. The goal of the synthesis methods is to produce size controllable gold nanoparticles. Many methods are based on the reduction of tetrachloric acid (H AuCl$_4$) to form gold nanoparticles. The formation and stabilization of nanosized colloidal metal particles demands careful attention to the preparation conditions and to the presence of stabilizers. Nanoparticles of silver, gold, platinum, and copper have been prepared by various methods, but most of their shapes have been limited to spheres [52,53].

Magnetic nanoparticles: The magnetic nanoparticles offer the possibility of being directed towards a specific target in the human body and remaining eventually localised, by means of
an applied magnetic field. Iron coated nanoparticles are therefore appropriate to be used as magnetic carriers of medical drugs, magnetic resonance imaging contrasts, biological labels etc, adsorbed into the carbon surface. As one of the most important materials, magnetite (Fe₃O₄) nanoparticles have attracted a lot of attentions for their interesting magnetic properties and potential applications in the fields of biology, pharmacy and diagnostics [54]. The magnetite Fe₃O₄ with oleic acid nanoparticles analyzed by TEM showed a spherical shape with a narrow size distribution. Figure 3 shows Fe₃O₄ nanoparticle surrounded by TPPS₄, AFM for TPPS₄ and Fe₃O₄.

Figure 3. Fe₃O₄ nanoparticle surrounded by TPPS₄ (left), AFM for TPPS₄ (right, up) and for Fe₃O₄ (right, down)

Organic Nanoparticles is the generic term for several nanoparticles including for example porphyrins, phthalocyanines and related sensitizer nanoparticles. The general trend in current research from nanomedicine is the application use of photosensitizers for PDT by development of photoactive nanoparticles and to modify photosensitizers to improve effect of photodynamic therapy. PS can be modified by encapsulated them in delivery agents such as liposomes [93], micelles [81], ceramic based nanoparticles [49], and polymer nanoparticles [57, 67]. Some examplification will be shown bellow.

5.2. Soft nanoparticle

5.2.1. Polymeric carriers for drug delivery

The polymeric carrier are divided into three groups:

1. Biodegradable polymers. These degrade under biological conditions to nontoxic products that are eliminated from the body.

2. Drug-conjugated polymers (Natural polymers). The used polymers are dextran, polyacrylamides and albumins, and offer a targeted drug controlled releasing by drug-polymer cleavage at the proper site.

3. Nondegradable polymers. These are stable in biological systems, and used as components of implantable devices for drug delivery.
Macromolecular complexes of various polymers can be divided into the following categories according to the nature of molecular interactions: complexes formed by interaction of oppositely charged polyelectrolytes, charge transfer complexes, hydrogen-bonding complexes and stereocomplexes. Both synthetic and natural polymers could be used for the production of nanosystems. These polymers may be used alone or in combination to develop nanoparticles. Several fabrication techniques are developed and can generally be subdivided into two categories. The first category includes solvent evaporation or diffusion, ionotropic gelation, etc. The second one includes emulsion, interfacial polymerization and polycondensation [66].

5.2.2. Biodegradable polymers

**Polymer nanoparticles** involve natural or biocompatible synthetic polymers as: polysaccharides, poly lactic acid, poly lactides, poly acrylates, poly alkyl cyano acrylates, poly alkyl vinyl pyrrolidones or acryl polymers. The most important seems to be Polylactic-co-glycolic acid (PLGA) which has shown several advantages over other biodegradable polymers that are routinely used for photosensitiser delivery [49] and has become the most popular polymer for PDT. The size of PLGA 50:50 nanoparticles with m-THPP as photosensitiser influences their photodynamic activity (bigger size, lower activity), but it also affects their interaction with the biological environment (protein absorption, cellular uptake or tissue distribution) [56]. Another important polymer - poly(vinyl alcohol) (PVA) - seems to have certain affinity for the p-THPP photosensitiser, inducing the adsorption of PVA on to the surface of the nanoparticle and leading to higher clearance of the complex [57, 76]. Many sensitizers from the second generation have been encapsulated into polymer nanoparticles, for example PLGA, the final size of the new system being 285 nm, with a polydispersity index of 0.12 and a relatively reduced toxicity. A specific example is bacteriochlorophyll encapsulated into PLGA prepared by solvent evaporation method. This yielded to spherical particles of about 660 nm size with an encapsulation efficiency of 69% and higher singlet oxygen production (ϕ = 0.26) [58]. Another porphyrin sensitizer, a synthetic one, 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (TMPP) has been tested on chick embryo chloroallantoic membrane model, showing a longer retention time when is encapsulated into nanoparticles and an improvement of the vascular effects after light irradiation [59], due to the fact that the pathological tumoral vasculature is “leaky”, most probably due to the pore size 100-780 nm and to the accumulation in the interstitial tumor tissue [60,61]. Also, phophorbide a and chlorin e6 have been encapsulated in PLGA nanoparticles [63,64]. Similar results have been registered in choroidal neovascularization associated with AMD [62], where the lipophilic porphyrins show phototherombic effect and leakage from blood vessels.

5.2.3. Natural polymers

The naturally-occurring polymers of particular interest for delivery of some drugs could be the polysaccharides that include chitosan, hyaluronan, dextran, cellulose, pullulan, chondroitin sulphate and alginate, and polymers as casein and gelatin. They are nontoxic, biocompatible, biodegradable and hydrophilic.

Examples of the natural polymers used to prepare drugs-loaded nanoparticles are:
Dextran sulphate is a polysaccharide that consist from linear 1,6-linked D-glucopyranose units with 2.3 sulphate groups per glucosyl residue, is non-toxic, water-soluble and biodegradable. Because it wears negatively charges, it is used for nanoparticle insulin delivery system based on complexation with oppositely charged polymers [65].

Alginate/chitosan nanoparticles may form complexes with cationic β-cyclodextrin polymers [66]. Some polyphenols have been entrapped in calcium alginate beads and to investigate their encapsulation efficiency and in vitro release [67]. Addition of 0.25-1% CS in coagulation fluid determined an improvement of encapsulation efficiency. This is probably due to increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan during gelation. In the presence of more chitosan, the process will go faster [68]. In vitro polyphenols released of prepared beads was carried out both in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The total polyphenols release rate in SGF was between 40.7% - 93.6% and in SIF was between 3.7 - 15.4%, and the highest content of polyphenols was released in SGF. The release rate (RR) of polyphenols from microcapsules is influenced by the concentration of alginate, this phenomenon is in agreement with the previous study where it is reported that the release rate was quicker for beads prepared in low concentration of alginate but slower for beads prepared in high concentrations [69]. For microcapsules prepared by adding chitosan in coagulation fluid the best encapsulation efficiency (85.2%) was obtained with 0.5% CS (w / v). Weak interactions between polyphenols and calcium alginate have allowed most of the polyphenols to be released in SGF. With the addition of CS in the coagulation fluid is observed a slight increase of polyphenols released in SIF.

Alginate is a linear copolymer composed of β-D-mannuronic acid and α-L-guluronic acid joined by a 1-4 glycosidic bond. The composition is highly dependent on the used polysaccharide. The most common source of alginate is the cell wall of brown algae. Alginate is biocompatible, biodegradable and non-toxic polymer and has many biomedical applications due to the reactivity of its carboxylate side groups and its capacity to form spontaneous gelation when exposed to divalent cations such as calcium [70] and specific drug delivery [71]. Some nanoparticles were prepared by the ionotropic pre-gelation of alginate with calcium chloride followed by complexation between alginate and chitosan [69].

Chitosan (CS) is a copolymer consisting of β (1 → 4)-linked 2-acetoamido-2-deoxy-β-D-glucopyranose (Glc-NAc; A-unit) and 2-amino-2-deoxy-β-D-glucopyranose (GlcN; D-unit) [70]. The primary amine groups make chitosan very useful in pharmaceutical applications [72]. CS nanoparticles proved cytotoxic properties on various tumor cell lines [73]. Moreover, this polysaccharide had been used as immunoadjuvant in laser immunotherapy with positive effects in the treatment of experimental tumors [74]. CS proved antioxidant properties.

5.2.4. Nanovectors

Polymeric micelles have many advantages such as small size (10 to 200 nm) for passive accumulation in solid tumors by enhanced permeation and retention (EPR), improved stability, biodegradability and high flexibility for structural and chemical modifications [75,76]. Micelles polymers are usually formed into core shell structures by spontaneous
assembly when its concentration is above critical micelle concentration (CMC). They have a
number of unique features, including nano size, easy modification of the surface chemistry,
core functionalities, and also, the and also, they serve as carriers and delivery systems [77].
They have been tested for solubilizing some anti-cancer drugs as: docetaxel (DOC), paclitaxel
(PTX), camptothecin [78 -80]. Due to their hydrophilicity, the polymer micelles play an
important part in RES recognition and in the blood circulation of drugs.

Porphyrs could be encapsulated into micelles as Triton X-100 at a critical concentration
2.7x10^{-4} M. Above this concentration, porphyrins and micelles coexist in a dynamic
equilibrium.

Also, from geometrical considerations, two possibilities can occur, i.e. the case of an oblate ellipsoid or that of a prolate ellipsoid. The model of an oblate ellipsoid is supported by energetically considerations, although the second model can’t be neglected as beeing that of a host molecule for the porphyrin. The fluorescence lifetimes of porphyrins are in the range 13-17 ns in micelles and 9-12 ns in organic solvents, which means that the electron transfer should be available for about 10-9s before the excited molecule decays spontaneously back to its ground state. The lifetimes of all the porphyrins in Triton X-100 micelles could be attributed to the monomeric forms and are larger than the values obtained in pure solvents. The increasing of the porphyrins lifetime in micelles can be ascribed to the reduction in the diffusion-limited fluorescence quenching by oxygen in micellar samples (Figure 4). This could be an explanation for the low rate photodegradation of all these porphyrins [81].

Figure 4. The fluorescence quenching of TPP-Mg in benzene (A) and Triton X-100 (B)

Liposomes are known as vesicles with clinical applications, formed by hydration of phospholipids at higher temperature than their transition temperature. They have sizes of 100 nm and allow some drugs to be contained in the lipid space between bilayers. Liposomes are stable microscopic vesicles formed by phospholipids and similar amphipathic lipids. Liposome properties vary substantially with the composition, size, surface charge, and the preparation method. Liposomes are nanoconstructs (approximately 100 nm in diameter) with bilayered membrane structures composed of phospholipids with hydrophilic heads and hydrophobic anionic or cationic long chain tails [82]. Moreover, the hydrophobic membrane can encapsulate hydrophobic drug molecules and prevent leakage of hydrophilic agents from within the core.
Based on their size and number of bilayers, the liposomes are divided into three classes.

1. Small unilamellar vesicles are surrounded by a single lipid layer and are 25–50 nm in diameter.

2. Large unilamellar vesicles as heterogeneous group of vesicles surrounded by a single lipid layer.

3. Multilamellar vesicles formed by several lipid layers separated from each other by a layer of aqueous solution.

Liposomes are used as pharmaceutical carriers due to their unique abilities to efficiently encapsulate both hydrophilic and hydrophobic therapeutic agents, to offer protection to the encapsulated drugs from undesired effects of external conditions, because they can be functionalized with specific ligands that can target specific cells, tissues, and organs of interest, and because they could be coated with inert and biocompatible polymers such as polyethylene glycol (PEG), in turn prolonging the liposome circulation half-life in vivo. They can form desired formulations with needed composition, size, surface charge, and other properties [83]. Liposome vesicles are interesting and useful drug carriers because they can carry both hydrophilic molecules in their aqueous core and hydrophobic drugs among the fatty acid chains in the phospholipid bilayers [84]. Liposomes are vesicles which consist of one to several, chemically-active lipid bilayers. Some drug molecules can be encapsulated and/or solubilised within the bilayers according to their hydrophilic/lipophilic balance. Due to their hydrophobic properties, photosensitizers being poorly soluble in aqueous phases and due to their aggregation property have limited delivery in active form to the desired target [85,86]. Additionally, many photosensitizers have a low affinity to tumor sites inducing some damages of normal tissue following PDT in patients. Nanotechnology based formulations of photosensitizers are attractive systems for improved delivery of photosensitizers [87]. Liposomes are artificial vesicles composed of a lipid bilayer usually used for the formulation and delivery of all kinds of drugs. The benzoporphyrin derivative monoacid ring A (BPD-MA) has been used for antiangiogenic PDT encapsulated in polycationic liposomes modified with cetyl-polyethyleneimine. The encapsulated photosensitiser was better internalised by human umbilical vein endothelial cells and was found inside the nucleus and associated with mitochondria [88]. The commercial liposomal preparation of the same photosensitiser (Visudyne; Novartis) is active against tumours in sarcoma-bearing mice [89]. Photofrin loaded into PEG modified liposomes presents enhanced phototoxicity compared to the free drug or when embedded in the same non-PEGylated liposomes [90]. Although the presence of the PEG inhibited the uptake of the nanoparticles by the tumour cells, it decreased the release of the photosensitiser from the liposome. Another porphyrin derivative (2,3-dihydro-5,15-di(3,5-dihydroxyphenyl)porphyrin (SIM01)) in dimyristoyl-phosphatidylcholine liposomes (DPPC) also yields better results in PDT than the photosensitizer alone, mainly due to a major accumulation in the tumour cells (human adenocarcinoma in nude mice) [91]. Liposomal TPP is effective in PDT of human a melanotic melanoma in nude mice; after being intravenously administered, authors demonstrated that their use can totally disintegrate tumours [92]. Also, TPP, TNP and ChL could be used in E.Coli destroying by PDT treatment (Figure 5), [93].
5.3. Hydrogels and their applications in drug delivery

Hydrogels are a desired material for biomedical and pharmaceutical applications due to their unique swelling properties and hydrated structure. Gels are macromolecular material with an intermediate material between a solid and liquid material. These gels are made up of a combination of local cross-linked polymer chains, noting that the junction zones are of size reduced. The structure of these gels is their property associated with swelling by incorporating a solvent. When the solvent comprises water in a proportion higher to 20 %, the gel will be called hydrogel. Hydrogels are elastic in nature due to the presence of the reference configurations, stored in the hydrogel, which will in turn even after the been distorted over a period of time. The natural polymers chitosan and alginate has been the most studied, for the manufacture of the hydrogel nanoparticles. Among synthetic polymers based nanoparticles, remember poly (vinyl alcohol) PVA, poly (ethylene oxide) PEO, poly (ethylene imide) (PEI), polyvinylpyrrolidone (PVP), poly (N-isopropyl acrylamide), which were used in order to release molecules incorporated.

Hydrogels are crosslinked polymeric networks that are insoluble in water but swell to an equilibrium size in the presence of excess water or biological fluids [94]. Research on hydrogels started in the 1960s with a landmark paper on poly(hydroxyethyl methacrylate) (PMMA) [95]. Due to the unique swelling properties and biocompatible structure, these materials have been extensively studied for biomedical and pharmaceutical applications, such as contact lenses, biosensors, artificial hearts, artificial skin and drug delivery devices [96]. Among them, hydrogels from poly (vinyl alcohol) are especially important due to their advantages of being water soluble, non-toxic, non-carcinogenic and biodegradable. Hydrogels based on poly (vinyl alcohol) (PVA) is characterized by transparency, in a three-dimensional polymeric structure and a water absorption capacity greater volume, without dissolving therein. Poly (vinyl alcohol) has a semi-crystalline nature with applications for encapsulation and controlled release of the porphyrins, especially in cancer therapy. Hydrogel loading procedure with 5,10,15,20-tetra-sulfonato-phenyl porphyrin (TPPS\textsubscript{4}), sorption experiments, the retention efficiency of porphyrins on the PVA hydrogel, and controlled release of TPPS\textsubscript{4} from the PVA hydrogel have been achieved and already reported [97].

PVA becomes more porous and its pores become more larger after porphyrin retention. This observation is more stronger for TPPS\textsubscript{4} than for the other porphyrins with smaller sizes [98,
SEM analysis showed a porous structure of the hydrogel, evidencing interconnected pores with a size distribution in the range of 80-950 nm, Figure 6. The retention efficiency (normalized to the swollen hydrogel mass) has been calculated according to the formula:

\[
RE(\%) = \frac{m_{\text{porphyrin retained}}}{m_{\text{porphyrin initial}} \cdot m_{\text{hydrogel}}} \times 100
\]

where: \(m_{\text{porphyrin initial}}\) is the initial amount of porphyrin to be found in the solution, and \(m_{\text{porphyrin retained}}\) is determined from the difference between the initial and the remaining amounts of porphyrin after retention. Release experiments were carried out by using a TPPS\(_4\)-loaded PVA hydrogel, rinsed thoroughly after loading with distilled water, and then placed in the appropriate quantity of medium (distilled water). The sorption mechanism of the porphyrins onto the PVA hydrogel can be interpreted as having two components: physisorption and chemisorption. In physisorption, the porphyrin is encapsulated in the pores of the nanostructured hydrogels. This mechanism is mainly controlled by diffusion. The diffusion mechanism is not ideal (Fickian), but rather Stephan diffusion, because of the anisotropic porous structure of the gel [101]. The chemisorption mechanism consists of the hydrogen bonding between the \(-\text{OH}\) groups of the poly(vinyl alcohol) and the pyrrolic nitrogen of the porphyrin molecule. The nature and the intensity of the chemisorption depends largely on the conformation of the porphyrin molecule and the solvent used.

![Figure 6. Aspect of PVA hydrogel with (left) and without TPPS\(_4\) hydrogel (right)(up), SEM images of PVA 90 hydrogel (TPPS\(_4\)) before (left) and with TPPS\(_4\) (right) (middle) and kinetics of TPPS\(_4\) loading in PVA hydrogel (left) and kinetic of TPPS\(_4\) release from the PVA hydrogel (normalized to encapsulated TPPS\(_4\) amount)(right) (down)](image)
Endothelial cell line, HUVEC, as adherent cells line and photodynamic therapy model, were grown on the surface of hydrogels mentioned, and monitorized by microscopic techniques, following the cellular membrane integrity. Also, the influence of TPPS₄ forms on hydrogel properties are analyzed. For this purpose HUVEC cells pre-incubated with TPPS₄ were illuminated with red light. PDT led to a dramatic change in the morphology of these endothelial cells. The photosensitizer accumulated in mitochondria and its fluorescence emission is detected in red region (~590 nm), before (left) and after PDT protocol. A deformation of the cells, as a sign of the cellular death, is observed after PDT (right) (Figure 7).

Figure 7. Laser scanning confocal microscopy of HUVEC before (left) and after (right) the PDT protocol with TPPS₄ hydrogels

5.4. Non–biodegradable nanoparticles for photodynamic therapy

Compared to biodegradable polymeric carrier systems, non-biodegradable nanoparticles have several advantages: they are very stable to fluctuations in temperature and pH [102], taking into account that the particle size, shape, porosity and mono-dispersibility can be controlled during their preparation [103]; they are not subject to microbial attack [104]; and the tiny pores in the ceramic particle, which are 0.50 – 1.00 nm in diameter, are too small to allow the drug to escape the matrix but are large enough to enable efficient oxygen diffusion to and from the particle [105]. Biodegradable polymer nanoparticles degrade readily to release the photosensitizers, whereas the shells in non-biodegradable particles are difficult to collapse. However, the efficiency of PDT is only attributable to the production of \( ^1\text{O}_2 \); it is unnecessary to release the loaded photosensitizers, but it is essential that the oxygen can diffuse in and out of the nanoparticles. The lifetime of singlet oxygen is able to induce PDT-induced oxidative damage around few miliseconds in aqueous media [106]. The nanoparticles size is under 100 nm and have a negligible effect on the delivery of \( ^1\text{O}_2 \). The use of non-biodegradable nanoparticles has some advantages with respect to their degradable counterparts. As the nanoparticle keeps its integrity, the photosensitizer has a permanent protection from the environment; besides, it is possible to use the nanoparticles as platforms to incorporate additional functionalities and they can be of smaller size. These nanoparticles kept their integrity over several months and were effective with just 5 minutes of irradiation. 5,10,15,20-tetrakis(1–methyl-4-pyridino)porphyrin tetra(p-toluenesulfonate) (TMPyP), has also been encapsulated in polyacrylamide -based nanoparticles. Its phototoxicity with two photon IR radiation was demonstrated in vitro by modulating the time of exposure to light [106]. The main difference between classic PS and nano-PS is their relative size (Figure 8).
However, several strategies have now been developed to encapsulate photosensitizer into nanoparticles and also improve delivery to the required area and many formulations have been described whereby the nanomaterials have an additional active intermediary role in the photodynamic process [107]. Recent trends in the use of fullerene derivatives in medicine are related to development of nanoplatforms that contain drugs with different composition able to carry out selective delivery of them to some human organs. The main medicinal targets are cancer cells of different types. It is believed that in this aspect the fullerenes are of great interest because of their opportunity to participate in the composition of such nanoplatforms in several roles: cytotoxic agent as well as, conversely, an antioxidant (these roles may change depending on accumulation in different organs and tissues) ones; as transporter of drugs; as photo- or radiosensitizer (or protector). Recently, the conjugates of C60 with meso-aryl-porphyrins with long chain substituents were obtained for using in PDT, Figure 9, [108]. Apoptosis without participation of caspase-3 was observed when the human lymphoblast cell line (K562) was treated by TPP/PVP/C60 [109]. TPP generates singlet oxygen with high quantum yield (0.63) [110]. Three types of interactions were registered in this dyad: electrostatic, hydrogen bonds and the donor acceptor bonds between fullerene and other components [111]. Here the high ability of these compounds to the formation of photo-induced state with divided (isolated) charges was first noted. Cell survival was dependent on illumination rate and the phototoxic effect persisted even in an atmosphere of argon. Depending on the microenvironment of the sensitizer site localization, the tissue is damaged either through the mechanism of \( \cdot{O}_2 \) mediated photoreaction process or through ROS attack at a low concentration of oxygen. Apoptosis by caspase-3-dependent pathway (58% of apoptotic cells) has been replaced by predominant necrotic phenomena in anaerobic conditions. Starting from the characteristics of fullerene compounds, we tried to study in vitro C60 fullerene and some functionalized derivatives (C60 complexes with PVP (poly-vinyl-pyrrolidone) and with the oxo-dimer (TPP-Fe)\(_2\)O experimental models in vitro with normal and tumor cells and investigation of their toxicological profile, in order to identify novel anti-neoplastic therapeutic devices.

However, the complexity of the problem is that until now there is no predictive model of action of fullerene derivatives under concrete conditions for a specific cell type. Moreover, the set of
possible mechanisms of the effect of fullerenes on the signaling pathways of apoptosis varied and depends on many factors that are difficult to administrate. However, the success of some fullerene derivatives against HIV, the selectivity to certain lines of cancer cells without damaging normal tissue, the possibility of using in theranostics suggest promising perspectives of fullerenes in the field of nanomedicine.

6. Study of the photodynamic effects of selected photosensitizers on human biological samples

6.1. Incorporation of nanoparticles in human blood

Significant nowadays research efforts are focused on finding new photosensitizers with antineoplastic activity and an acceptable toxicological profile. Although consistent information exists regarding PDT in solid tumors, relatively few data are available for PDT of blood cancers. Therefore, we carried out a comparative study on lymphoblastic K562 cells and human normal peripheral blood mononuclear cells (PBMC) treated at a density of 2 x 10^5 cells/mL with 5,10,15,20-tetra-sulphophenyl-porphyrin (TPPS_4) and then irradiated with He-Ne laser light (λ = 632.8 nm). The following cell functions were investigated: viability, multiplication, RNA synthesis, total RNA levels and apoptosis. Human normal PBMC subjected to TPPS_4 loading and laser-irradiation develop a different cellular response, their viability and proliferative capacity not being altered by experimental PDT. Accordingly, it appears that TPPS_4 is a non-aggressive compound for cellular physiology and becomes cytotoxic only by irradiation; moreover laser-activated TPPS_4 affects only cells that have a tumoral pattern [112].

There are several differences between lymphocytes obtained from healthy donors undergoing artificial activation in vitro and genuinely leukemic cells. The cells membrane structure is different in healthy and malignant cells, resting and stimulated cells can be compared using fluorescence spectroscopy [27]. In the concentration range 10–100 μg/mL, TPPS_4 loaded for 24h in normal PBMC does not significantly alter cell viability (Figure 10).
Irradiation of K562 cells, either loaded or not loaded with TPPS$_4$, drops off cellular viability (assessed by the Trypan Blue exclusion test 4hrs after irradiation) for both tumor cells and normal PBMC (Figure 11). TPPS$_4$-loaded K562 cells are almost similarly affected by irradiation as the corresponding control. TPPS$_4$ alone has no significant effect on cell viability.

Human normal PBMC react differently at the PDT procedure than tumor cells. Their viability and capacity to incorporate uridine are not altered by laser-activating of TPPS$_4$ loaded into cells (Figure 12).

4-5 ml of human peripheral blood were fast collected. Blood mononuclear cells (PBMC) were separated by gradient dental equipment screened using Histopaque 1077 (Sigma), washed twice with RPMI 1640 culture medium (Sigma) and then normalized to $10^5$ cells / ml RPMI 1640 culture medium. Samples containing cell lines loaded with various concentrations of TPPS$_4$ 24h were investigated in flow cytometry fluorescence recorded at wavelengths above 670nm. Increasing the concentration of TPPS$_4$ fluorescence intensity increases cell suspension directly proportional both total fluorescence and fluorescence maximum. In flow cytometry, the regions of lymphocytes, granulocytes and monocytes are clearly distinguished. The fluorescence of sample can be measured by using properly chosen filter. Fluorescence is
analyzed separately for each type of cells. A flow cytogram represents the graphical representation of light scattering vs. right angle scattering and help to determine lymphocytes and granulocytes. The aspect of flow cytometry (mean fluorescence intensities) could offer data about dye aggregation or dye interaction with cellular membrane [41, 113, 114]. The number of stained cells decrease from 91.02% (for control cells), to 89.27% for 10 μg/ml TPPS4, 87.80% for 20 μg/ml TPPS4 and 86.93% for 40 μg/ml TPPS4 (Figure 13.)

Figure 13. The flow cytometry results for the PMBC cells incubated with TPPS4 at different concentrations

Ion [115] and Frackowiak [27] evaluated the incorporation of sulphonated porphyrin TPPS4, which is better incorporated in cells by comparison with the non-sulphonated ones, most probably due to the spatial forms of J-aggregated forms (helical forms) able to penetrate the membrane and recovering to the monomeric forms after penetration. It is shown that during irradiation cells are actively destroyed (Figures 14-17), [116].
For a longer photodynamic activity it is important to correlate the photophysical activity (the lifetimes of the excited states) with photochemical activity (singlet oxygen efficiency and the photodegradation rate. The aggregated forms (J-aggregates) of porphyrins favor the penetration of the membranes. The porphyrins incorporation in cells is well correlated with singlet oxygen generation capacity. In monomeric forms the non-sulfonated porphyrins are better incorporated than the sulfonated ones which are better incorporated in leukocytes than in granulocytes (Figures 18, 19), [113].

The non-sulphonated porphyrins (TNP and TPP) in DMSO-water mixture (0.05% DMSO) exist as monomeric and J-aggregated (dicationic - aggregated forms), the last ones with comparable fluorescence properties with the monomers [117].
Figure 16. The flow cytometry of different forms of TPPS₄

Figure 17. The flow cytometry of leukocytes and granulocytes incorporated with TPP and TNP
6.2. Application of B2 Vitamin in liposomes for ophthalmologic diseases

After PDT treatment was possible to see by angrography a rapid and complete vasooclusion (Figure 20), because the vessels were filled with erythrocytes and due to platelet aggregates. Another observed effects are: vacuolization of mitochondria and endoplasmatic reticulum (ESR), clumping of nuclear chromatin and finally, a subconjunctival hemorrhage, chemosis and cyanotic color of the neovascular areas (Figures 21 and 22).

![Figure 18](image18.png)

**Figure 18.** Emission properties of TPP and TNP in DMSO:water and in lymphocytes

![Figure 19](image19.png)

**Figure 19.** The correlation graphic of absorption and emission for TPP and TNP

![Figure 20](image20.png)

**Figure 20.** Fluorescein angiography after PDT treatment with Riboflavin on rabbit. Dark area delimitates the irradiated sites where hypofluorescence indicates vascular occlusion
From Figure 21 could be observed the disappearance of peritumoral neovascularization and complete disappearance of tumoral neovascularization and tumoral atrophy (6 months). Angiography showed an immediate and complete vasooclusion (Figure 22).

6.3. Dermatological applications

PDT produces cytotoxic effects through photodamage of cellular organelles and biomolecules. It is known that PDT mediates tumor destruction by three mechanisms: direct cell killing, tumor vasculature damage and immune response activation. The combination of the three mechanisms is required to obtain long-term tumor control [122].

Actinic keratosis (AK) is the most common skin lesion with malignant potential, with a prevalence ranging from 11% to 25% in the Northern Hemisphere and from 40% to 50% in Australia [123]. There are some factors responsible for skin lesions (squamous cell carcinoma (SCC)) as follows: UV light, heat and pollutants resulted from carbon processing. The most sensitive persons are Fitzpatrick I and II phototype and men by comparison with women [124]. In time, these lesions could remain unchanged, could spontaneously regress or could progress to SCC and further developing on the support of pre-existing actinic keratosis.
In our experimental approach, we have obtained from untreated skin biopsies a mean of $2.8 \times 10^6$ keratinocytes/cm$^2$ skin with a mean of 65% viability. After therapy from the same skin region and from the same surface isolated keratinocytes were less than half compared to the control skin, displaying as well a lower viability (Figure 23).

![Graph showing viability and proliferative capacity of keratinocytes before and after PDT with TPPS$_4$](image)

**Figure 23.** Primary keratinocytes isolated from normal human skin before (control) and after PDT with TPPS$_4$ (viability and proliferative capacity)

Primary keratinocytes were further cultivated until the culture could not be maintained. The proliferation capacity of primary keratinocytes extracted from PDT skin biopsies was significantly lower compared to control skin (Figure 23). Annexin-V and propidium iodide labelling of isolated keratinocytes after in vivo PDT compared to control keratinocytes yielded to the following values: control = 100 %; An-PI- = 10 %; An+PI- = 18 %; An+PI+ = 65 %.

The tested TPPS$_4$ showed an effective *in vivo* destructive effect on keratinocytes in the patient with actinic keratosis doubled by a good clinical response (Figure 24).

![Image showing skin with AK before and after PDT](image)

**Figure 24.** The aspect of the skin with AK before PDT (left) and after PDT (right) with TPPS$_4$

### 7. Conclusions

Photodynamic therapy is a recognized alternative used in successful cancer therapy, in a society when the clinicians are seeking new and efficient methods for saving people. There is
no doubt that the long scientific efforts of photodynamic therapy will allow useful patient
treatments in the future.

The main benefits of PDT are the following:

a. The patients can avoid surgery.
b. PDT patients usually don’t even need to check into the hospital.
c. PDT can be repeated, unlike radiation and chemotherapy.
d. PDT can work in places where surgery would not be feasible, such as the trachea, the
   major airway leading from the voice box to the lungs.
e. The photosensitizing agent will selectively accumulate in cancer cells and not in sur‐
   rounding normal tissues. Hence, cancer cells can be selectively destroyed while most
   normal cells are spared.
f. The treatment occurs only in the presence of light.
g. PDT might be applied in many cases where surgery, chemotherapy and X ray radiation
   are contra-indicated.

Side effects, limitations could be registered, especially for photosensitivity or sensitivity to light,
the pain of PDT, which is usually mild to moderate, easily controlled with some drugs.

However, there are many problems for the clinical application of existing photosensitzers.

a. Most PS molecules are hydrophobic and can aggregate easily in aqueous media, decreas‐
   ing its quantum yield. Moreover, the aggregated PS cannot be simply injected intrave‐
   nously.
b. Selective accumulation of the PS molecules in deceased tissues is required to avoid
   collateral damage to healthy cells. Although third generation PS based on nanomaterials
   have been prepared for selective targeting, their selectivity is not high enough for clinical
   application.
c. Nanomaterials (organic or inorganic) are more promising because they are hydrophilic,
   possess enormous surface areas, and their surface can be modified with functional groups
   possessing a diverse chemical or biochemical properties;
d. Owing to their sub-cellular and sub-micron size, nanoparticles can penetrate deep into
   tissues through fine capillaries, and are generally taken up efficiently by cells.

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References


[40] K.A. Bello, I.A. Bello, Some observations on the visible absorption spectra and stability properties of the silicon phthalocyanine system, Dyes and Pigments 35 (1997) 261-266.


Photodynamic therapy using tetrapyrrolic macrocycles, Brazilian Journal of Medical and Biological Research 44(1) (2011) 53-61.


