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Nanotechnology in Antimicrobial Photodynamic Inactivation

SAMI NAZZAL[1], CHUEH-PIN CHEN[2] AND TSUIMIN TSAI[2]*

[1.] Department of Basic Pharmaceutical Sciences, College of Pharmacy, University of Louisiana at Monroe, Monroe LA, U.S.A.
[2.] Graduate Institute of Biomedical Materials and Tissue Engineering, College of Oral Medicine, Taipei Medical University, Taipei 110, Taiwan, R.O.C.

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ABSTRACT

Photodynamic therapy (PDT) and photodynamic inactivation (PDI) are technologies that utilize visible light and photosensitizers (PS) to inactivate cells. PDT is currently in use for the treatment of several types of tumors. Although cancer has been successfully treated with PS and light, antimicrobial PDI is emerging as a new treatment modality for bacterial infections due to its effectiveness and less likelihood of inducing bacterial resistance. Resistance to therapy is in part due to the ability of the organisms to form a biofilm, which provides a microenvironment that protects the microorganism from antibiotics and attack by the host's immune system. *In vitro*, PDI nonetheless was shown to be effective against Gram-negative and Gram-positive bacteria. When used *in-vivo* however, several factors were shown to influence and diminish the effectiveness of PDI, such as aggregation of PS and plasma protein binding. To circumvent these factors, different nanotechnology platforms were used to enhance the photodynamic inactivation efficacy, such as liposomes, micelles and nanoparticles, by reducing the PS aggregation and albumin binding to the PS. In general, studies have shown that photodynamic inactivation efficacy could be enhanced when suitable nanocarriers are used to deliver the PS.

Key words: photodynamic inactivation, liposome, micelle, nanoparticles

INTRODUCTION

I. *Photodynamic Therapy (PDT)*

The phototoxicity of chemical compounds towards microorganisms was first published at the turn of the 20th century. Oskar Raab observed that the toxicity of acridine hydrochloride against *Paramecia caudatum* was dependent on the amount of light incident on the experimental mixture[(1)]. von Tappeiner and Jadblauer reported that the observed toxic effect in the presence of light was not attributed to heat. In 1904, experiments performed to exclude the direct influence of light led von Tappeiner to coin the term photodynamic reaction[(1)], which later became known as PDT.

PDT represents a well-established therapeutic modality, which was originally developed and recently approved for the treatment of a variety of solid tumors[(2)]. It involves the systemic administration of the photosensitizer (PS), such as phenothiazines[(3)], prophyrins[(4)], chlorins[(5)] and phthalocyanines[(6)], followed by photoactivation of PS at the disease site with light of specific wavelength[(7)]. The three fundamental requirements for PDT are therefore oxygen, light source and PS. Each factor is harmless by itself, but their combination can produce cytotoxic agents, which may be used to kill tumor cells or pathogenic microorganisms.

In order to be effective, the PS ideally should be selective to tumor tissues and should preferentially localize in the rapidly growing tumor cells before they release the highly reactive singlet oxygen species (ROS)[(2)]. Due to its short lifetime, singlet oxygen intracellular diffusion distance is not more than 0.01 - 0.02 μm and therefore its direct action is dependent on and limited to the intracellular structure where the sensitizer is localized[(8)]. For example, PSs localized in the mitochondria induce apoptosis very rapidly[(9)], where cytochrome c release is one of the best-known apoptotic events after photosensitization[(10)]. Furthermore, despite its significant advantages, the biodistribution of the PS[(11)] is limited. In addition, phototoxicity to the skin, due mainly to the hydrophobicity and non-selectivity of PS, is another considerable limitation[(12)].

The mechanism by which PS works is illustrated in Figure 1. PS in a singlet state at the lowest or ground state energy level (S_0) may absorb a photon from light of a specific wavelength to enter an excited state (S_1). When excited to a triplet state, the photosensitizer may undergo two kinds of reactions. In type I reaction, the PS can react directly with

* Author for correspondence. Tel: +886-2-27361661 ext. 5203; Fax: +886-2-27390082; E-mail: tmtsai00@tmu.edu.tw

a substrate, such as the cell membrane or a molecule, and transfer a proton or an electron to form a radical anion or radical cation. In type II reaction, the PS in the excited triplet state would directly form highly reactive singlet oxygen. Both type I and type II reactions can occur simultaneously. The ratio between these reactions depends on the type of PS used and the concentrations of the substrate and oxygen[13].

II. *Photodynamic Inactivation (PDI) of Bacteria*

Since the middle of the last century, antimicrobial PDI was overlooked by the discovery of antibiotics. In the last decade, the worldwide increase in antibiotic resistance has driven research into the development of new antimicrobial strategies[14]. A relatively novel antimicrobial application of PDI has been made possible by the preparation of PS, whose molecule is engineered to promote a very fast interaction with bacterial cells, hence a highly preferential inactivation of pathogenic agents in comparison with the main constituents of host tissues, such as fibroblasts and keratinocytes[15]. These findings paved the way to the use of PDI for the treatment of localized and drug resistant microbial infections[16].

In the 1990s, it was observed that there was a fundamental difference in susceptibility to PDI between Gram-positive and Gram-negative bacteria (Table 1)[17,18]. It was found that, in general, neutral or anionic PS molecules efficiently bind to and photodynamically inactivate Gram-positive bacteria, whereas they bind only to the outer membrane of Gram-negative bacterial cells rendering them less effective[19]. The high susceptibility of Gram-positive species could be explained by their physiology, as their cytoplasmic membrane is surrounded by a relatively porous layer of peptidoglycan and lipoteichoic acid that allows PS to cross[19]. The cell envelope of Gram-negative bacteria, however, consists of an inner cytoplasmic membrane and an outer membrane that are separated by a peptidoglycan-containing periplasm (Figure 2)[19]. The outer membrane forms a physical and functional barrier between the cell and its environment. In the outer membrane, different proteins are present; some of which function as pores to allow passage of nutrients, whereas others have an enzymatic function or are involved in maintaining the structural integrity of the outer membrane and the shape of the bacteria (Figure 2).

Several approaches have been attempted to potentiate the efficacy of PDI against Gram-negative bacteria. Nitzan and coworkers used polycationic peptide, polymyxin B nonapeptide (PMBN) to increase the outer membrane permeability of Gram-negative bacteria and allow the PS, that are normally excluded from the cell, to penetrate into the bacterial cell where illumination-generated reactive oxygen species can result in fatal bacterial damage[20]. PMBN does not release lipopolysaccharide (LPS) from the cells. On the contrary, it 'expands' the outer leaflet of the membrane, allowing PS such as deuteroporphyrin (DP) to penetrate and induce a PDI effect on *E. coli* and *P. aeruginosa*[20]. This method was also used to kill a multi-antibiotic resistant strain of *A. baumannii*. Nitzan *et al*. speculated that the interaction and bonding between PMBN and DP in solution facilitated PS penetration into the microorganism as DP seemed to work much better in concert with PMBN than many other PS, including porphyrins, phthalocyanines and merocyanine 540[21]. It was also observed that the growth medium of the bacteria made a difference to their susceptibility to PDI, with the high protein nutrient broth leading to less killing, compared to the low protein nutrient broth medium. Furthermore, it was noted that the type of protein present in the medium, as well as its concentration, made a difference to the susceptibility of the bacteria.

A second approach involved the use of PS molecules with an intrinsic positive charge. Wilson and coworkers used Toluidine Blue O to study the effect of PDI on a range of both Gram-positive and Gram-negative bacteria[22]. These studies were mostly concerned with oral bacteria[23], though PDI was also tested on *S. aureus* and the Gram-negative

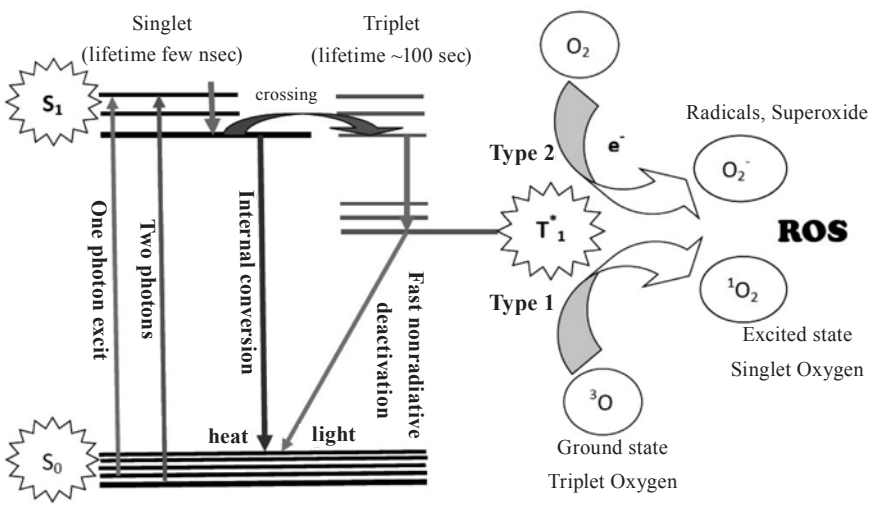

Figure 1. Jablonski diagram showing the energy transferred from photosensitizers to molecular oxygen.

Table 1. List of photosensitizers and Gram-positive and Gram-negative bacteria for which *in vitro* photoinactivation studies were performed

Species (Gram status)	Photosensitizers	References
Staphylococcus aureus (+)	Methylene blue	(78,79)
	Photosens	(80)
	Toluidine blue	(78,81)
	Hematoporphyrin	(82)
	Photofrin	(83)
	m-THPC	(83)
	Hypericin	(83)
	Malachite green isothiocyanate	(84)
	Cationic, neutral and anionic Tetraphenylporphyrins	(85)
	Rose bengal	(86)
Staphylococcus epidermidis (+)	Methylene blue	(79)
	Toluidine blue	(81)
	Photosens	(80)
Streptococcus pyogenes (+)	Methylene blue	(79)
Streptococcus pneumonia (+)	Methylene blue[78,79]	(78,79)
	Toluidine blue	(78)
Enterococcus seriolicida (+)	Zinc pyridiniumphthalocyanine	(87)
Enterococcus hirae (+)	Cationic, neutral and anionic Tetraphenylporphyrins	(85)
	ALA	(88)
	Zinc phthalocyanine tetrasulfonate	(88)
Enterococcus faecalis (+)	Methylene blue	(78)
	Toluidine blue	(78)
Bacillus subtilis (-)	Hematoporphyrin derivative	(89)
Streptococcus faecalis (-)	Hematoporphyrin derivative	(89)
Deinococcus radiodurans (-)	Rose bengal	(90)
*P*seudomonas aeruginosa (-)	Photosens	(80)
	Methylene blue	(78)
	Toluidine blue	(78)
	Zinc pyridiniumphthalocyanine	(87)
	Rose bengal	(86)
Escherichia coli (-)	N-Alkylpyridylporphyrins	(91)
	Cationic hydrophilic porphyrin	(92)
	Cationic, neutral and anionic Tetraphenylporphyrins	(85)
	ALA	(88)
	Photosens	(80)
	Zinc pyridiniumphthalocyanine	(87)
	Thiazines	(93)
	Xanthenes	(93)
	Acridines	(93)
	Phenazines	(93)
	Methylene blue	(78)
	Toluidine blue	(78)
	Zinc phthalocyanine tetrasulfonate	(88)
Acinetobacter baumannii (-)	Cationic hydrophilic porphyrin	(92)
Corynebacterium minutissimum (-)	Methylene blue	(79)
Haemophilus influenza (-)	Methylene blue	(78)
	Toluidine blue	(78)
	ALA	(94)
Propionibacterium acnes (-)	Methylene blue	(79)
Proteus mirabilis (-)	Photosens	(80)
Helicobacter pylori (-)	Hematoporphyrin derivative	(95)
Salmonella typhimurium (-)	Rose bengal	(96)

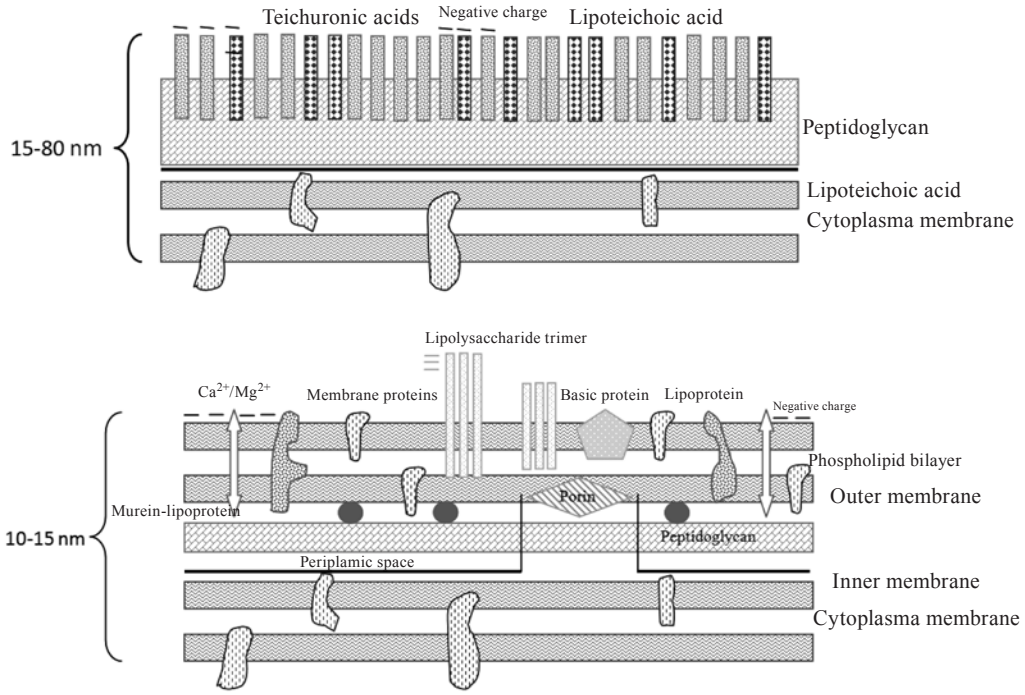

Figure 2. Diagrams illustrating the differences in the membrane structure between Gram-positive (A) and Gram-negative bacteria (B).

H. pylori[23,24]. It was observed that the growth phase of the bacteria did not influence their susceptibility to PDI, whereas the presence of serum in the medium decreased the killing[25]. In another study, washing the loosely bound PS from the cells before illumination was found to decrease the killing. This could be attributed to the limited photodamage caused by the first dose of light, which in turn may not have been sufficient to allow further penetration of bound PS[4].

There are several reports on PDI of Gram-negative bacteria in which it was clear that PS does not have to penetrate the bacterium to be effective or even come into contact with the cells[18]. According to these reports, if singlet oxygen can be generated in sufficient quantities near to the bacterial outer membrane, it will be able to diffuse into the cell to inflict damage on vital structures[26]. In one set of studies, bacteria were separated from the PS by a layer of moist air. Singlet oxygen in the gas phase was generated and allowed to diffuse across the gap before contacting the bacteria[27]. Nonetheless, Gram-negative species remained harder to kill than Gram-positive bacteria, where the intracellular content of carotenoids was found to protect the bacteria from photoinactivation.

III. *Mechanisms of PDI-Induced Damage*

Two mechanisms were proposed to account for the lethal damage caused to bacteria by PDI: (i) DNA damage and (ii) damage to the cytoplasmic membrane, allowing leakage of cellular contents or inactivation of membrane transport systems and enzymes[18]. Evidence suggests that treatment of bacteria with various PS, light and singlet oxygen leads to DNA damage. Cleavage in both single- and double stranded DNA, and the disappearance of the plasmid supercoiled fraction have been detected in both Gram-positive and Gram-negative species after PDI treatment with a wide range of PS structural types[28,29]. The damage may be repaired by various DNA repairing systems[30]. Nonetheless, various authors have also concluded that although DNA damage occurs, it may not be the prime cause of bacterial cell death. Disturbance of cell-wall synthesis and the appearance of a multilamellar structure near the septum of dividing cells, along with loss of potassium ions from the cells were reported by Nitzan *et al.*[20]

LIMITATIONS OF PDT/PDI

The principle of PDT and PDI is based on the combined use of a PS and low-intensity visible light of an appropriate wavelength[31]. After light irradiation, activated PS generates cytotoxic reactive oxygen species that induce a bactericidal effect. Although the possibility to inactivate microbes by PDI has been known for more than 10 decades[32], it is only recently that this modality gained attention as a viable tool to eradicate infectious pathogens[18]. The main advantage of PDI is that bacteria can be eradicated almost instantly while avoiding damage to adjacent host tissues. PDI is effective against antibiotic-resistant and antibiotic-susceptible bacteria, without inducing resistance even after repeated photosensitization.

Despite the frequent clinical use of PDT for the treatment of several malignancies[33], the use of PDI to inactivate microorganisms is still in the research phase[34]. This is in

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part due to the fact that PDI of Gram-negative bacteria with first generation PS, such as the anionic hematoporphyrin, had to be mediated by membrane permeabilizers, such as ethylenediaminetetraacetic acid or polymyxin nonapeptide[19]. Later it was discovered that cationic porphyrins did not require membrane permeabilizers in order to successfully inactivate Gram-negative bacteria[4]. It has been demonstrated that Gram-positive and Gram-negative bacteria, and fungi can be successfully photodynamically inactivated by a single cationic photosensitizer, for instance 5-phenyl-10,15,20-tris(N-methyl-4-pyridyl)porphyrin (TriP[4])[35] or toluidine blue[36]. Furthermore, it has been shown that both antibiotic-sensitive and resistant strains can be successfully photoinactivated[15,37] without inducing resistance to photosensitization.

In general, most intravenously administered PS for PDT or PDI are rapidly cleared from the circulation, although some of the molecules bind to serum proteins, such as albumin and low-density lipoprotein (LDL), and remain in circulation for a longer period[38]. Facilitated uptake of LDL-bound PS by tumor cells expressing a large number of LDL receptors on their surface has been reported to increase the specificity of the PS to the tumor cells[38,39]. However, LDL-bound PS can also be taken up by macrophages, which may localize in the skin, leading to skin hyperphotosensitivity[38,39]. To circumvent this side effect, considerable efforts have been devoted to the development of new PS that would accumulate quickly in tumor tissues and could be rapidly cleared from the skin. One such example is taporfin sodium (Talaporfin), a second generation PS that shows rapid clearance from the skin and has been approved for the treatment of early-stage lung cancers in Japan[40]. Nonetheless, while PDT with Talaporfin can reduce skin phototoxicity; the patient is required to stay in the dark for at least 2 weeks[7].

Other factors, such as the presence of wound fluid, are expected to influence the antimicrobial activity of PDI. For example, in many *in vitro* studies, it was shown that the consistency of the buffer or broth used as a suspending medium strongly influences the efficacy of antimicrobial PDI[21]. The *in vivo* efficacy of PDI is further complicated by the fact that biological membranes seem to be an important target for many antineoplastic photosensitizer agents[34]. Nonetheless, the significant biomedical applications of PDI could not be ignored. For example, commonly used implants that have been developed to assist in the performance and recovery of physiological functions present a constant risk because of bacterial colonization and biofilm formation on their surface. Fortunately, many of the *in vivo* deficiencies of PDI could be circumvented with the use of nanocarriers, which will be discussed in the subsequent sections of this review.

NANOCARRIER-ENCAPSULATED PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY AND INACTIVATION

Alternatively, nanocarriers for PS delivery may be used to enhance the efficacy of PDT and PDI. While the use of long-circulating nanocarriers for PS delivery may seem to contradict the aforementioned trends in the development of the new PS[7], these delivery systems will reduce the likelihood of aggregation of the potential PS[31]. Encapsulation techniques that have been used to prevent the formation of aggregates include liposomes[41], polymeric micelles[42], nanoparticles[43] and nanofilms. A list of nanocarriers and photosensitizers that were used in PDI and tested for their efficacy against Gram-positive, Gram-negative and yeast microorganisms is given in Table 2.

I. *Liposomes*

Liposomes (Figure 3A) have been widely investigated as drug delivery systems due to their structural similarity to the cell membrane[31]. They are versatile and allow for triggered drug release. For example, drug release can be triggered by near-infrared irradiation of hollow gold liposomes containing light-sensitive polymeric materials[44]. Also, thermal- and pH-sensitive, as well as enzymatically triggered and receptor-targeted liposomes were developed[45].

From early studies, it has long been known that liposome lysis (or destruction) can be provoked by irradiation with visible light in the presence of a photosensitizer[46]. These studies aimed at elucidating the mechanisms of photodynamic damage to cells including such significant structural elements as bilayer lipid membranes[46]. Studying the photosensitization and oxidation of both lipid and protein components of biological membranes is important for understanding basic processes underlying photodynamic therapy[44]. Several approaches have been employed to study the mechanism of photomodification in sensitized membranes[47]. Only few studies, however, included observations of membrane permeabilization, though the latter is generally accepted to represent one of the most crucial processes in photodynamic action leading to cell death[48]. In particular, earlier works on liposomes and planar bilayer lipid membranes (BLM), using a hematoporphyrin derivative, have shown photosensitized vesicle disruption[49] and a dramatic increase in planar BLM conductance resulting in membrane breakdown, provided that membrane-forming lipids contain double bonds[50].

Liposome-delivered photosensitizers have been adopted in anti-tumor PDT and proven to yield a more pronounced and selective targeting of the neoplastic lesion[51]. Therefore, liposomes were also investigated for their effect on the affinity of photosensitizing agents to the bacterial cells and the efficiency of their photo-induced bacterial killing[16]. Previous studies have shown that the disruption of the bacterial outer wall can be most efficiently achieved by using positively charged liposomes, analogous to the use of polylysine or polyethyleneimine[16,52]. Methicillin-resistant *staphylococcus aureus* (MRSA) were inactivated by using two noncationic liposome incorporated dyes, hematoporphyrin (HP) and chlorophyll[16].

Overall, present research suggests that the use of suitable liposomal vesicles as delivery systems for antimicrobial

Table 2. List of nanocarriers that were loaded with photosensitizers and tested for their *in vitro* photoactivity against bacteria.

Nanocarriers	Photosensitizers	Species	References
Liposomes	Xanthomonadin	*Xanthomonas oryzae* pv. *Oryzae strains*	(97)
	Hematoporphyrin (Hp) Chlorophylla (Chl)	MRSA	(16)
	Phenothiazine Porphyrin	Gram-positive Gram-negative	(98)
	TDPyP	MRSA	(99)
	m-THPC	MRSA	(100)
	Hp	MRSA *S. epidermidis* *S. pyogenes*	(31)
	Ce6	*S. aureus*	(31)
	Porphyrin	Multiresistant strains	(101)
	Methylene blue (MB)	*Staphylococcus aureus* *Sarcina lutea* *St. epidermidis* *Shigella flexneri*	(102)
	Neutral red (NR)	*Staphylococcus aureus* *Sarcina lutea* *Escherichia coli* *Salmonella paraB*	(102)
	Rose bengal (RB)	*St. epidermidis* *Shigella flexneri*	(102)
Micelles	Naphthoquinone	*Micrococcus luteus*	(103)
	DTC_{60}^{2+}	*Escherichia coli*	(104)
	Hp	MRSA *S. epidermidis* *S. pyogenes*	(31)
	Ce6	*S. aureus*	(31)
Nanoparticles	Tri (2,2'-bipyridine) ruthenium	*Escherichia coli*	(105)
	Rose bengal	*Escherichia coli*	(105)
	Methylene blue	*S. aureus* *P. aeruginosa*	(106)
	5,10,15,20-Tetrakis (3-hydroxy phenyl) porphyrin (mTHPP)	*S. aureus* *P. aeruginosa*	(106)
	Rose bengal	*S. aureus* *St. epidermidis*	(107)

PDI agents can be useful for the following reasons: (a) non-cationic photosensitizers may be used as efficient killing agents of microbial cells, and (b) the synergic effect of positively charged and highly fluid components of the lipophilic carrier facilitates uptake by microbial cells and enhances its overall photoactivity. A significant challenge to liposome research is that encapsulating drugs in liposomes is not simple and the composition of an optimal formula can only be obtained through tedious experimental trials[31]. In cancer research, for example, many studies do not favor liposomes as a delivery system because phospholipids are expensive, inherently unstable, and the preparation of liposomes is difficult to scale up. In fact, in some applications, less expensive starting materials, such as polymers, can be used instead of phospholipids to encapsulate drugs[53].

II. *Micelles*

Micelles are nanosized, aqueous self-aggregates of amphiphilic molecules with a hydrophobic core, which are capable of solubilizing nonpolar molecules[54]. Therefore, they are attractive carriers for poorly water soluble therapeutic drugs. Micelles composed of Cremophor-EL, Tween-80, etc. have been used in preclinical and clinical studies

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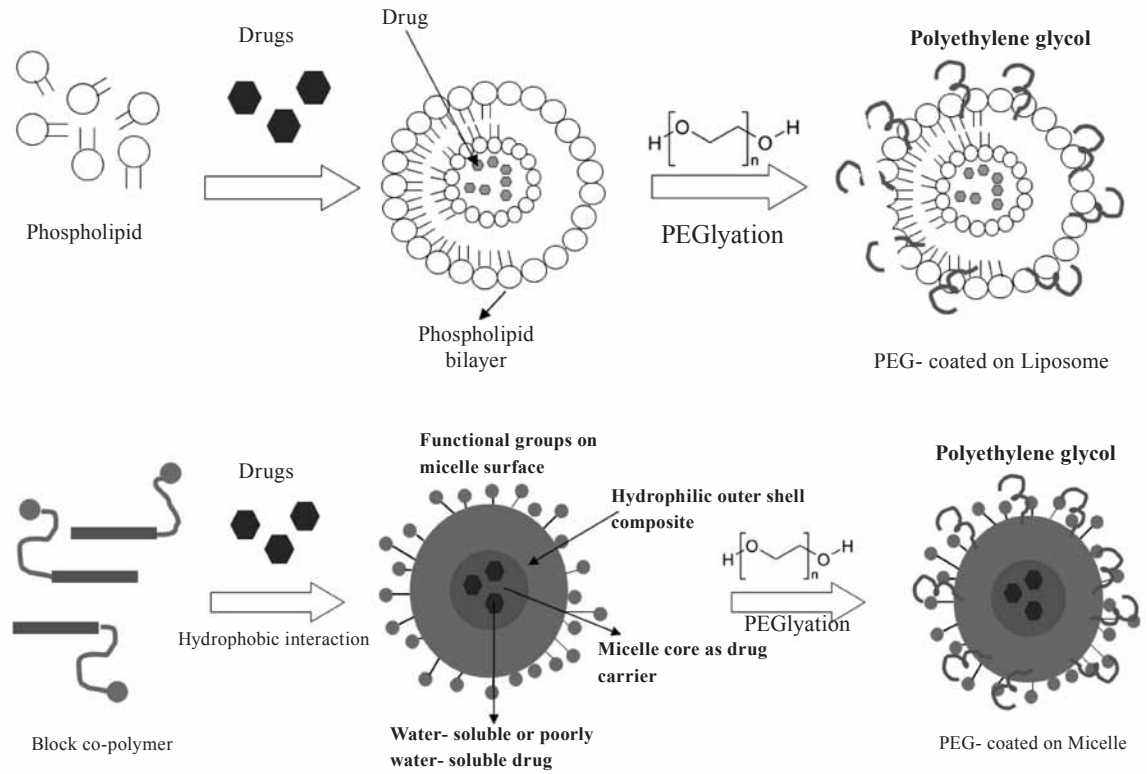

Figure 3. Schematic illustration of liposomes loaded with (A) drugs and (B) polymeric micelles as intelligent nanocarriers for drug delivery, with or without peglation.

for the delivery of PS. However, such micelles disassemble easily upon dilution and are known to be associated with allergic reactions.

An improvement over the surfactant-based micelles, in terms of safety and efficiency, are the polymeric drug carriers, which are made up of biocompatible, hydrophobic-hydrophilic copolymers (e.g., poloxamers, ploxamines, pluronics, etc.) Over the past decade, polymeric drug carriers including polymer-drug conjugates and polymeric micelles have been proven useful in drug delivery[55] and are being increasingly investigated in the preclinical stage for PDT and PDI. In particular, polymeric micelles (Figure 3B) are currently recognized as one of the most promising modalities of drug delivery[56]. They are potent nanocarriers for site-specific drug delivery, which has been shown in several clinical trials[57].

Polymeric micelles are characterized by a unique core-shell architecture in which an inner core, serving as a nanocontainer of hydrophobic drugs, is surrounded by an outer shell of hydrophilic polymers, such as poly(ethylene glycol) (PEG) (Figure 3C). It is well known that block copolymers with amphiphilic character spontaneously assemble into polymeric micelles with a diameter of several tens of nanometers in aqueous media[58]. These systems have demonstrated longevity in the bloodstream and effective tumor accumulation after their systemic administration[57,59]. The biocompatibility of polymeric micelles and their capacity to avoid renal exclusion and their reduced uptake/degradation by the reticuloendothelial system allow for their prolonged systemic circulation time. Drugs encapsulated within polymeric micelles usually exhibit higher passive accumulation in tumors compared to free drugs with reduced distribution in non-targeted areas. This passive accumulation is due to the enhanced permeability and retention effect (EPR) associated with tumor tissues. Furthermore, polymeric micelles have several advantages, such as simple preparation, efficient drug loading without chemical modification of the parent drug, and controlled drug release[60]. Besides, hydrophobic and/or electrostatic interaction between charged block copolymers and oppositely-charged macromolecules has allowed the formation of unique core-shell nanoparticles, which were termed "polyion complex (PIC) micelles"[61].

Ionic dendrimer photosensitizers, another example of a miceller system in which the core of porphyrin or phthalocyanine is surrounded by large dendritic wedges, were developed to solve the inherent problems with conventional PSs[62]. It is assumed that dendrimer photosensitizers elicit effective ROS production even at extremely high concentrations because the dendritic wedges sterically prevent or weaken aggregation of the center dye molecules[63]. Also, ionic groups on the dendrimer periphery allow their stable incorporation into polyion complex (PIC) micelles through the electrostatic interaction with oppositely charged poly(ethylene glycol)-polyelectrolyte block copolymers[62,63].

III. *Nanoparticles*

Various delivery systems have been tested in preclinical studies[64]. Of these are the molecular Drug Delivery Systems (DDS) that have been developed as a way to deliver photosensitizers to the target tumor[65]. Photoimmunotargeting may use monoclonal antibodies that recognize tumor antigens[66] or ligands against receptors that are upregulated in tumor cells[67]. General strategies for the delivery of phtosensitizers via the molecular DDS was reviewed by Konan *et al*.[67] and Wang *et al*.[68]

In general, liposomes and immunoliposomes can be used in conjunction with photosensitizers[69]. They were shown to have good selectivity towards tumor tissue, but their loading capacity is limited[67]. Micellar systems were also shown to possess good selectivity, but severe side effects such as anaphylactic shocks were reported[70]. This led to intensive research on particulate delivery systems, e.g. nanoparticles, which may consist of polymers, metals and ceramics that can incorporate lipophilic photosensitizers and impart selectivity against tumor cells.

Nanoparticles represent an emerging photosensitizer delivery system that has shown a great promise for PDT[71]. The efficiency of nanoparticles in PDT may be attributed to the fact that PDT relies on the production of $^1O_2$. Therefore; it is unnecessary to release the loaded photosensitizers and no time for biodegradation is needed, but it is only essential that the oxygen diffuse in and out of the nanoparticles. For example, the pores in the ceramic particle are 0.50 - 1.00 nm in diameter, which is too small to allow the drug to escape but are large enough to enable efficient oxygen diffusion to and from the particles[72]. The lifetime of $^1O_2$ in aqueous media is in the order of microseconds. As $^1O_2$ reacts so rapidly, PDT-induced oxidative damage is highly localized to regions comparable to the thickness of a cell membrane[38,71]. Thus, the variability in size of the nanoparticles under 100 nm should have a negligible effect on the delivery of $^1O_2$.

Nanoparticles, either biodegradable, from which photosensitizer may be released within the tissues[73], or ‘‘pure carriers” non-biodegradable nanoparticles, which entrap photosensitizers during their activity[74] are commonly investigated[65]. There are several advantages of using either biodegradable or non-biodegradable nanoparticles[71]. Biodegradable nanoparticles, such as the polymeric nanoparticles made from polylactide/polyglycolide copolymers[73], are mostly aqueous in composition, whereas the silica-based non-biodegradable nanoparticles are comprised of totally or mostly inert silica, a medium that is probably not more reactive than water[71]. Also, biodegradable polymer nanoparticles degrade readily to release the photosensitizers, whereas the shells in non-biodegradable particles are difficult to collapse. Compared to biodegradable polymeric carrier systems, however, non-biodegradable nanoparticles are not subject to microbial attack[75] and are stable to fluctuations in temperature and pH[76]. Furthermore, their size, shape, porosity and mono-dispersibility can easily be controlled during their preparation[68].

Based on these concepts, different nanoparticle chemistries have been investigated to determine the optimal components and the simplest structure that may be used in clinics. Most efforts have been focused on developing a carrier with low complexity, void of metabolic and tissue interactions, and which fulfill the following two conditions[65]: (1) is stable, at least for the duration of action during which carriers accumulate in tumor tissue and the photosensitizers produce ROS upon light activation, and (2) requires the simplest synthesis route and avoids the use of targeting agents while taking advantage of the ‘‘enhanced permeability and retention (EPR) effect” offered by tumors[77]. The EPR effect is based on two factors. First, the capillary endothelium in malignant tissues is more disordered and thus more permeable towards macromolecules than the capillary endothelium in normal tissues. This allows extravasation of circulating polymeric nanoparticles within the tumor interstitium. Second, the lack of tumor lymphatic drainage in the tumor bed results in drug accumulation.

CONCLUSIONS

In this review, recent progresses on liposomes, polymeric micelles and nanoparticles as nanocarriers for PS delivery are reviewed. Current studies indicate that nanocarriers as delivery systems may be useful for clinical applications. They have the capacity to encapsulate various drugs, including hydrophobic compounds such as metal complexes, gene and siRNA. Their unique core-shell architecture with a diameter of several tens of nanometers might allow for targeted therapy, enhanced uptake and prolonged blood circulation. The size of these carriers may be further tuned for efficient biodistribution within the nanocarrier range from 10 nm up to 200 nm[65] by adjusting either the temperature of the process or the concentration of co-surfactants. While nanocarriers allow for efficient delivery of PS, their photodynamic activity depends on a multitude of factors, such as the type of photoactive dye, particle size and charge, incubation time and the nature of polymer used. In the future, PS encapsulated in carriers made of different polymers and functional groups with easy-to-use light device may be used in the treatment of localized microbial infection, including the photodynamic inactivation of microorganisms (Figure 4).

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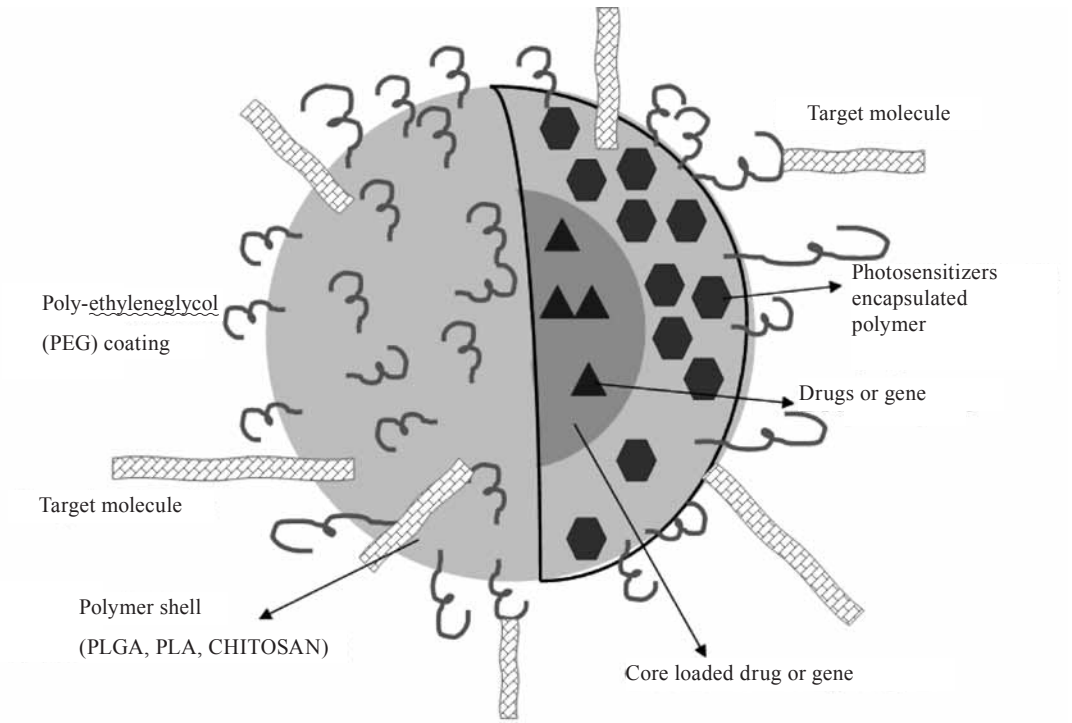

Figure 4. Schematic illustration of an ideal multifunctional nanocarrier.

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