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Antimicrobial Photodynamic Inactivation and Photosensitizers: A Succinct Review

Maha Fadel[a], Maha Nasr[b], Sarah Salah Thabet[a*]

[a] Pharmaceutical Technology Laboratory, Department of Medical Applications of Laser, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Cairo, Egypt, [b] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Abstract

Purpose: The growing rise in the development of multidrug-resistant strains of bacteria towards conventional antibiotics necessitates exploring alternative techniques such as antimicrobial photodynamic inactivation (aPDI). aPDI relies on the activation of a photosensitizer (PS) by a specific wavelength of light with the production of excess reactive oxygen species (ROS), which have the ability to successfully eradicate a wide range of human pathogens like bacteria (either Gram-positive Gram (+) or Gram-negative Gram (-)), fungi, protozoa, parasites, viruses, and even bacterial biofilms. One of the notable advantages of aPDI is that it doesn't lead to bacterial resistance or be affected by the already established resistance to antibiotics. The characteristics of the photosensitizer used have a major impact on how effective aPDI is. The best PS for selective aPDI is thought to have a strong positive charge, be safe in the dark, and produce a large quantity of ROS when activated by red light. Various PSs, either natural or synthetic, have been proven effective in aPDI. The synthetic dye methylene blue and the natural PS curcumin have been extensively explored. Moreover, tetrapyrrole structures like porphyrins and phthalocyanines have been extensively investigated because they are easily chemically modified.

Conclusion: Nanocarriers played a significant role in aPDI, as some nanocarriers function as PSs by themselves, like fullerenes, while others bind PS to their surfaces or embed it within their matrix. Nanocarriers have been demonstrated to enhance the antibacterial activity of the PS, protect it, and improve its delivery to the target site.

Keywords— photodynamic therapy, photoactive molecules, nanocarriers, multidrug-resistant bacteria, photokilling

I. Introduction

Phototherapy began in ancient Egypt, where the Egyptians employed sunlight and herbs to cure various skin conditions. One noteworthy incidence is the use of natural photosensitizers, like psoralens, which are isolated from specific plants like parsley and St. John's Wort, to cure leprosy lesions [1, 2]. Combining light radiation with a medication called a photosensitizer (PS) to kill cancer cells and infectious microbes upon light activation is known as photodynamic therapy (PDT). PDT is a minimally invasive treatment approach in which photosensitive materials are triggered by a particular wavelength of light, often emitted by a laser. When the PS is exposed to light, it is activated and triggers a reaction that harms neighboring cells. Both the light source and the PS are safe on their own [3]. Nowadays, there are numerous PSs available to treat a range of conditions, such as psoriasis, age-related macular degeneration, acne, and multiple malignancies [4]. PDT is also useful in treating viral, bacterial, and fungal infections; for these reasons, it is often referred to as antimicrobial photodynamic inactivation (aPDI) [5]. Furthermore, research has demonstrated that this light-based therapy can activate the immune system, providing the body with an additional tool to aid in the destruction of abnormal cells that may be bacterial, malignant, or precancerous. In PDT-mediated cancer treatment, irradiated cancer cells are directly destroyed, and tumor-specific cytotoxic T-cells are activated, enabling the death of distant, untreated tumor cells. Additionally, PDT promotes the growth of anti-tumor memory immunity, which may be able to stop cancer from returning. Due to increased neutrophil infiltration into the affected areas, which appears to magnify the therapeutic effect, PDT's immunological effects also increase the efficacy of the therapy when used to treat bacterial infections [6, 7]. Though much research has been done, the mechanism underlying photodynamic treatment is still unclear. The process entails administering a dye, known as a photosensitizer (PS), which is a photoactive substance. Afterwards, in the presence of oxygen, the dye is exposed to radiation at a wavelength that corresponds to its absorption band. As shown by the modified Jablonski diagram **(fig. 1)**, PDT includes PS absorbing a photon of light, which excites it from its ground singlet state (S_0) to its short-lived (nanoseconds) excited singlet state (S_1). With intersystem crossing or an electronic transition, this singlet state PS can become a substantially longer-lived (microsecond) triplet state (T_1). Due to its extended lifetime, the triplet PS can undergo one of three distinct photochemical reaction pathways; known as **Type 1, Type 2 and Type 3 reactions**. An electron transfer from the excited PS to an organic cell component is part of the **Type 1 route**. Highly reactive free radical species are the product of this interaction. These

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Corresponding author: Sarah Salah Thabet Email: sarahsthali@cu.edu.eg

species will react with oxygen molecules to produce harmful reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals. Through lipid peroxidation of the constituents of the cell membrane, ROS molecules assault the membrane and cause an irreversible rupture. Furthermore, throughout the process, membrane-bound peptides and enzymes may become inactive [8–10]. Singlet oxygen ($^1O_2$), a highly reactive form of oxygen, is created via a direct interaction (energy transfer) between the excited PS and oxygen molecules in the **type 2 pathway**. Singlet oxygen molecules cause oxidative damage to the cell membrane or cell wall as a result of their interaction with many biomolecules, including proteins, lipids, and nucleic acids [8–10]. Singlet oxygen has the ability to eradicate a variety of microorganisms, such as bacteria, viruses, fungi, and protozoa, presenting a promising antibacterial modality known as antimicrobial photodynamic inactivation (aPDI) [11, 12]. Furthermore, a **type 3 photodynamic pathway** has recently been proposed for deeply seated lesions and other hypoxic tissues. These types of lesions have extremely low levels of oxygen (the essential component of PDT); therefore, a special type of PS should be utilized. This PS could transmit energy directly to tissue without the need for oxygen. The excited triplet state of this oxygen-independent PS has the ability to target proteins, nucleic materials, and other subcellular components with their subsequent destruction. Interestingly, it was hypothesized that this pathway may take place in both hypoxic and non-hypoxic conditions. Unfortunately, this unique oxygen-independent PS is rare, so there is little data available about this type of reaction [13].

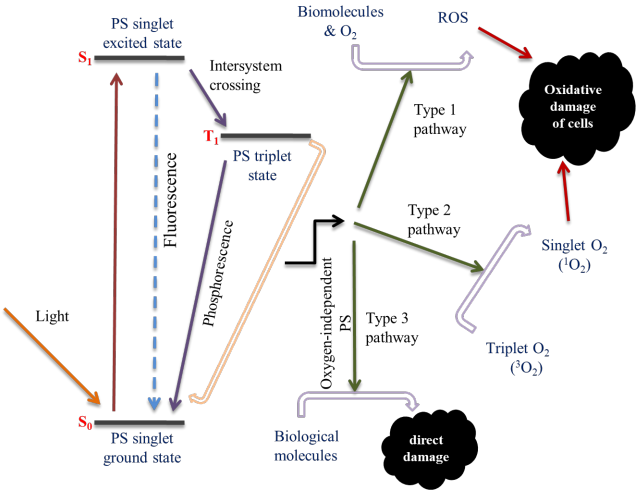

Figure 1: Modified Jablonski diagram showing the mechanism of PDT. PS: photosensitizer, ROS: reactive oxygen species

Despite the fact that PDT can operate through either pathway, singlet oxygen has been claimed to be the primary cytotoxic agent responsible for PDT's biological effects. Hence, the type II reaction is the main mechanism that causes the antibacterial effect of aPDI [10, 14].

II. Antimicrobial Photodynamic Inactivation

2.1. Historical background

PDT was discovered more than a century ago (1900) by the coincidental observation that microorganisms (Paramecia) were killed when exposed to both sunlight and a photosensitizing dye (acridine hydrochloride) at the same time, even though PDT has been researched and developed as an anti-cancer therapy rather than an antimicrobial therapy [15]. In 1960, aPDI was first introduced when toluidine blue was employed to combat germs like bacteria, algae, and yeast. 99% of the bacteria were observed to be eliminated in 30 minutes after being exposed to 21–30 mW of continuous-wave gas laser light at 632 nm [16]**.** Since then, it has been determined that PDT has potent antibacterial effects as well. However, the development of penicillin and its amazing bactericidal qualities, along with other antibiotics, has slowed the advancement of aPDI.

2.2. Advantages of aPDI

The widespread development of resistant strains of bacteria to antibiotics and the emergence of multidrug-resistant species necessitate the need for an alternative modality to conventional antibiotics. aPDI has gained attention in response to its superior advantages over conventional antibiotic regimens [16]**.** The advantages of aPDI include:

2.2.1. Broad-spectrum nature of aPDI

Various human pathogens, such as bacteria (either Gram-positive (Gram (+)) or Gram-negative (Gram (-)), fungi, protozoa, parasites, and viruses, have been successfully eradicated by aPDI, as illustrated in **fig. 2**. This implies that therapy can begin prior to the identification of the infectious agents. [16, 17]**.**

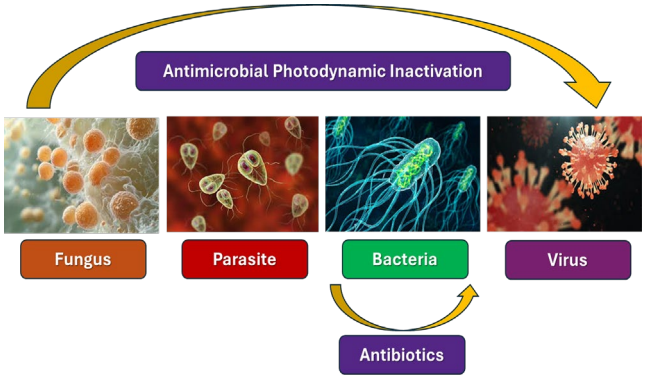

Figure 2: Broad-spectrum antimicrobial photodynamic inactivation versus antibiotics

2.2.2. Selective microbial binding over a short incubation time

The lifespan of ROS and singlet oxygen ($^1O_2$) produced by aPDI is extremely short. With a 0.04 ms lifespan in a biological environment, singlet oxygen has an action radius of only 0.02. Because of this, the generated radicals are quite potent only at the site of their generation. The fact that aPDI has no effect on distant tissues is a benefit [8]. Therefore, in order for the PS to bind selectively to microbial cells rather than host mammalian cells, it must be given locally to the target region in a safe manner [18]**.** Since microbial cells generally have a more pronounced negative charge than mammalian cells and positively charged aPS will bind selectively to them, it was determined that the best way to achieve this goal of aPDI was to make sure that the antimicrobial photosensitizer (aPS) had a pronounced cationic charge [16]. Furthermore, when a brief drug-light

gap (a few minutes) is used, the cationic aPS binds to microbial cells rather quickly while being absorbed slowly by mammalian cells, offering significant selectivity [19-21].

2.2.3. Effectiveness against resistant microbial strains without developing microbial resistance

The fact that aPDI functions just as well regardless of the microbial cells' level of antibiotic resistance strengthened its benefits as a possible clinical antimicrobial therapy [15]. Furthermore, even after 20 cycles of partial death followed by regrowth, aPDI has not been demonstrated to induce bacterial resistance [15, 22]. The photosensitized inactivation processes at the microbial membrane level are usually multi-targeted, involving multiple membrane proteins and lipid domains. This prevents the expression of potential protective factors, such as the biosynthesis of stress proteins, thereby reducing the likelihood of the emergence of resistant strains [23].

2.2.4. Effectiveness against microbial biofilms

Antibiotics given systemically are unable to break through the microbial biofilms that accumulate in many chronic illnesses. It has been demonstrated that aPDI destroys biofilm-grown cells in vivo and in vitro [24].

2.2.5. Topical or local application of PS to the infected area

This is especially helpful when burn infections or injured tissues with low blood flow occur. In these cases, systemically administered antibiotics are unable to reach the infection site in high enough concentrations. Topical aPDI may kill microorganisms quickly—it can start working in just a few seconds—while antibiotics can take hours or days to start working. This suggests that aPDI may be advantageous for treating infections that spread quickly, including necrotizing fasciitis [15].

2.2.6. Application in deep infections

Almost any anatomical location can now receive light through the use of endoscopes, fiber optics, and interstitially inserted needles with a tiny diameter [25].

2.3. Properties of an appropriate antimicrobial photosensitizer (aPS)

One important factor influencing the result of aPDI is the kind of aPS that is employed [16]. In order to be suitable for usage in aPDI, PS needs to have specific characteristics, as illustrated in **fig. 3**. The aPS must, first and foremost, not be hazardous to mammals, especially when incubated in the dark. Second, the aPS should have high molar absorption coefficients and good quantum yields of ROS at a wavelength that aligns with the tissue optical window, which is the region of the spectrum where tissue light penetration is most effective (red and near infrared) [26]. Thirdly, during short incubation times, aPS should show selectivity for microbial cells over host mammalian cells [19–21]. The fourth and most crucial factor is that an aPS should have cationic charges [5, 16].

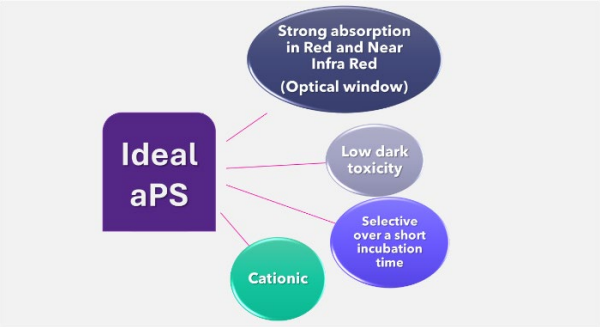

Figure 3: Properties of ideal antimicrobial photosensitizer (aPS)

2.4. Classification of aPSs

aPSs can be mainly classified into three groups based on their structure and origin: synthetic dyes, natural PSs and tetrapyrrole structures [16].

2.4.1. Synthetic Dyes

Phenothiazinium is a class of artificial dyes. The phenothiazinium dyes toluidine blue (TB) and methylene blue (MB) are the most commonly used aPS [27]. They are usually employed in aPDI in clinical settings because of their inherent cationic charge, which renders them efficient against a wide variety of bacteria. However, because of their low penetration into the biofilm, numerous investigations have demonstrated their relatively modest antibacterial activity on bacterial biofilms. Novel MB compounds, including dimethyl methylene blue, have been investigated lately. These compounds are more potent against bacterial cells because of their strong cationic charge [28–30].

Additional artificial dyes include Rose Bengal (RB), Eosin Y, and Erythrosine (ERY), which are anionic xanthene dyes made from fluorescein. The green wavelength region (480–550 nm) is where the absorption peak of each of these dyes is located. Because anionic PSs are less likely than cationic PSs to bind to and be absorbed by bacterial cells, these dyes have weaker antibacterial activity [31].

2.4.2. Natural aPS

Many naturally occurring substances, including coumarins, furanocoumarins, benzofurans, anthraquinones, and derivatives of flavin, are isolated from plants and other creatures and function as PSs. Two natural substances that have been thoroughly investigated as PS over the years are hypericin and curcumin. Hypericin is an anthraquinone derivative extracted from Hypericum perforatum, also referred to as St. John's Wort, which has long been used to treat burns and other skin lesions. At a wavelength of 600 nm, which is perceived as orange light, hypericin is best absorbed. Due to its non-cationic nature, it has been demonstrated that hypericin-mediated aPDI is more pronounced on Gram (+) bacteria than on Gram (-) bacteria [32, 33]. As a result, the creation of noble cationic hypericin derivatives is likely to increase the efficacy of aPDI against Gram (-) bacteria. Another naturally occurring PS that was extracted from the root of the Curcuma longa plant has an optimal absorption range of 405–435 nm. This compound is called curcumin. Apart from its beneficial effects on wound healing, curcumin is a safe PS that possesses anti-oxidant, anti-inflammatory, and anti-microbial characteristics.

Although it has been thoroughly studied for the treatment of cancer, recent studies have shown that curcumin can suppress drug-resistant bacterial strains by photo-inactivation [34]. Furthermore, curcumin has shown some antimicrobial qualities when exposed to no radiation [35, 36]. Research suggests that curcumin has 300 times greater photo-killing efficacy against Gram (+) Staphylococcus aureus compared to Gram (-) Escherichia coli (E. coli) and Salmonella typhimurium [37]. Curcumin has a high therapeutic effect, but its poor water solubility and photo-labile characteristics, which lead it to rapidly degrade at physiological pH, limit its application. Consequently, polyvinylpyrrolidone curcumin (PVP-C), a novel derivative of curcumin, was created and tested for its aPDI on Staphylococcus aureus. The results demonstrated total bacterial eradication [38].

2.4.3. Tetrapyrrole Structures

One of the biggest and most recently discovered PS groupings is tetrapyrroles. The tetrapyrrole nucleus is the basis for the majority of PS used in the previous 100 years to treat tissue disorders and cancer, with a strong reliance on porphyrin usage. In aPDI, phthalocyanines and porphyrins are the most commonly utilized PSs. Because of their ease of chemical modification and high rate of reactive oxygen species (ROS) formation, porphyrins are among the most widely used PSs. They absorb light between 405 and 550 nm in wavelength. Certain anaerobic bacteria that generate black pigments have a tendency to amass a lot of porphyrins, which makes them vulnerable to UV or blue light radiation [39–41]. As a result, these bacteria with endogenous PS can be killed by aPDI without the need for PS administration [42, 43].

Cationic porphyrins, such as TMPyP (meso-tetrakis(4-N-methylpyridiniumyl) porphyrin), have been synthesized with a fourfold positive charge; however, their effectiveness against bacterial biofilms is debatable because TMPyP has been reported to be effective against some types of bacterial biofilms and ineffective against others [44, 45]. Today, cationic antimicrobial peptides, or cell penetrating peptides, are conjugated to porphyrins to increase their efficiency. These conjugated porphyrins exhibit a great degree of cell inactivation during aPDI [46].

Phthalocyanines (Pc) are a class of diverse agents with a peak absorption in the red region at 670 nm [47]. Of these agents, zinc phthalocyanine (ZnPc) is the most studied phthalocyanine for aPDI [16]. This phthalocyanine, when used in conjunction with cationic and anti-membrane agents like polymyxin B or EDTA (ethylene-diamine-tetraacetic acid), can become effective against Gram (-) bacteria.

Additionally, the structure of phthalocyanine offers a wide range of options for designing different derivatives. So functionalizing ZnPc with cationic groups improves its binding affinity to bacterial cells without the need for polymyxin B [48, 49]. Numerous studies were conducted in an effort to improve ZnPc's aPDI efficacy by substituting or chemically altering its structure to create cationic and water-soluble derivatives [50]. However, the majority of these modifications made ZnPc extremely hydrophilic, necessitating additional chemical modifications in certain situations to improve its amphiphilicity [50, 51]. Widely ranging pathogens, including Gram (+) methicillin-sensitive Staphylococcus aureus and methicillin-resistant Staphylococcus aureus strain (MRSA) [20], Gram (-) Aeromonas hydrophila [51], *E. coli* [52,53], and fungi such as *Candida albicans* [54], have all been demonstrated to respond well to derivatives of ZnPc.

2.5. Nanocarriers and aPDI

2.5.1. Nanocarriers as aPDI mediators

As was already established, aPSs have a distinctive antibacterial action on pathogens, but their low solubility, bioavailability, and biocompatibility prevent them from being widely used. In this sense, aPDI has been transformed by nanotechnological intervention to fight microbial infections and the health effects they cause. Nevertheless, the bulk of research has concentrated on examining the efficacy of nanocarriers to improve PDT's anticancer properties, with very few examining their antimicrobial properties [55].

Because they enhance the delivery and release of PS at the intended location, nanocarriers enhance the effectiveness of PDT as compared to PS alone. This can be explained by the way ROS are dispersed; the ROS generated by free PS were less effective because they were uniformly distributed in the medium, whereas the ROS generated by PS-nanocarriers were locally concentrated. Moreover, PS attached to nanocarriers more effectively crosses the membrane than unbound PS does [16].

Additionally, nanotechnology has been used to introduce the positive charge required for aPDI into the PS by conjugating to polycationic polymers like poly-L-lysine, which promote strong binding to the negatively charged exterior of pathogens and permit the PS to pass through the permeability barrier of Gram (-) bacteria [56, 57]. Excisional wounds infected with the lethal Gram (-) bacteria Pseudomonas aeruginosa have been shown to respond favorably to aPDI mediated by the polycationic poly-L-lysine-chlorin p6 conjugate (pl-cp6) in terms of bacterial load reduction and wound healing [56]. Similarly, when compared to the anionic rose Bengal (RB) and the weak cationic toluidine blue O (TBO), the polycationic poly-L-lysine-chlorin e6 conjugate (pL-ce6) was found to be the most effective antimicrobial photosensitizer on three classes of human pathogens: E. coli (Gram (-) bacteria), Staphylococcus aureus (Gram (+) bacteria), and Candida albicans (yeast). When compared to RB and TBO, pL-ce6 showed the greatest bacterial reduction at significantly lower concentrations and light fluences [58].

The conjugation of chlorine e6 with polyethyleneimine (PEI) is another example of a conjugation with a polycationic moiety. It was thought that this conjugation was better than poly-L-lysine-PS conjugates for the aPDI of localized infections [57]. The interaction between nanocarriers and PS used in aPDI can be expressed as nanocarriers themselves act as the PS, PS is either bound to the surface of nanocarriers or embedded in nanocarriers, nanocomposites and smart nanocarriers. **Table. 1** summarizes nanocarriers used in aPDI.

Table. 1: Summary of nanocarriers used in aPDI.

Nanocarrier	PS	Main characteristics	Indication	Effect
Polycationic poly-L-lysine-chlorin p6 conjugate [56, 57]	Chlorin p6	Polycationic	-Excisional wounds infected with the lethal Gram (-) bacteria Pseudomonas aeruginosa	-Bacterial load reduction -Wound healing
Polycationic poly-L-lysine-chlorin e6 conjugate [58]	Chlorin e6	Polycationic	-E. coli (Gram (-) bacteria) -Staphylococcus aureus (Gram (+) bacteria) -Candida albicans (yeast)	Effective aPDI
Polycationic polyethyleneimine (PEI) chlorine e6 conjugate [57]	Chlorin e6	Polycationic	Three classes of human pathogens; -E. coli (Gram (-) bacteria), -Staphylococcus aureus (Gram (+) bacteria) -Candida albicans (yeast)	Better than poly-L-lysine-chlorin e6 conjugate for the aPDI of localized infections
Fullerenes [61]	No PS	-Act as a PS -Neutral	--	Weak bactericidal action
Cationic fullerene N,N-dimethyl-2-(40-N,N,N-trimethyl-aminophenyl) fulleropyrrodinium iodide ($DTC60_{2+}$) [62]	No PS	- Act as a PS -Cationic	-E. coli	Significantly hindered E. coli proliferation
Semiconductors zinc oxide (ZnO) and titanium oxide (TiO_2) [63]	No PS	-- Act as a PS -Irradiated by UVA	--	-Not employed in medical settings.
Graphene quantum dots (GQD) [65, 66]	No PS	- Act as a PS	-Staphylococcus aureus -E. coli	-Decrease count after 10 second irradiation
CdSe/ZnS quantum dots (QD) [71]	Toluidine blue (TBO)	--	Staphylococcus aureus and Streptococcus	-Enhance aPDI
Gold nanoparticle [72, 73]	toluidine blue	--	Staphylococcus aureus	-Enhanced aPDI
Multiwalled carbon nanotube (MWNT) [74]	Protoporphyrin IX (PpIX)	--	Staphylococcus aureus	-Enhance aPDI with visible light
Protein Cage [75]	--		Staphylococcus aureus	-Enhance aPDI
Nanoemulsions [82]	Chloroaluminum phthalocyanine (ClAlPc)	Cationic vs anionic	-Methicillin-susceptible Staphylococcus aureus -MRSA	-Cationic NE has better aPDI than anionic NE
Nanoemulsions [83]	Chloroaluminum phthalocyanine (ClAlPc)		-The highly resistant, potentially fatal Cryptococcus neoformans melanized cells	-Enhance aPDI
Nanoemulsions [84]	Chloroaluminum phthalocyanine (ClAlPc)	Cationic vs anionic	-Candida albicans planktonic cultures and biofilm	-Cationic NE has better aPDI than anionic NE
Nanoemulsions [77]	Zinc phthalocyanine (ZnPc)	--	Leishmania species	-Enhance aPDI
Nanoemulsions [85]	zinc phthalocyanine (ZnPc)	--	-Enterococcus faecalis -MRSA	Enhance aPDI
Nanoemulsions [86]	Zinc phthalocyanine (ZnPc)	Cationic	-MRSA -Multidrug-resistant E. coli	-Enhancd aPDI -Wound healing
Polymeric nanocomposite of ethylcellulose/chitosan [89]	5,10,15,20-tetrakis(m-hydroxyphenyl)porphyrin (mTHPP)	Cationic	-Multi-drug resistant Pseudomonas aeruginosa, -Staphylococcus aureus, -Candida albicans	-Enhance aPDI
Metallic nanocomposite of zeolitic imidazolate framework-8 (ZIF-8) [90]	Chlorin e6		MRSA	-Enhance aPDI -wound healing
Nanocomposite of gold nanocluster within chitosan polymer matrix [91]	Protoporphyrin IX (PpIX)		Gram (+) and Gram (-) bacteria and biofilm	-Enhance aPDI -Biofilm removal

Upconversion nanocomposites [92]	chlorin e6	Irradiated with near-infrared (NIR) light (980 nm), then the UCNPs can emit strong red light (655 nm)	-E. coli -Staphylococcus aureus	-Enhance aPDI by self-oxygen replenishment -Regulate inflammation
Enzyme-sensitive smart nanocarrier ex; Lipase-sensitive methoxy poly (ethylene glycol)-block-poly(ε-caprolactone) (mPEG-PCL) micelles [95]	Hypocrellin A (HA)	Selective and triggered release of HA by bacterial lipase enzyme	-MRSA	-Selective aPDI
PH-sensitive, surface charge switchable smart nanocarriers ex.1 pH-sensitive polydopamine (PDA) NPs of RB, coated with polymyxin B (PMB) and gluconic acid (GA) [96]	Rose Bengal (RB)	Nanocarrier exhibit negative charge at physiological pH and turned into positive at acidic pH of bacterial biofilm	-Bacterial biofilm	-Selective biofilm penetration and eradication
pH-sensitive, surface charge switchable smart nanocarriers ex. 2 pH-sensitive poly (ethylene glycol) (PEG) block polypeptide copolymer [PEG-(KLAKLAK)2-DA] linked with α-CD-Ce6 prodrugs [97]	chlorin e6	Nanocarrier exhibit negative charge at physiological pH and turned into positive at acidic pH of biofilm	-MRSA biofilm	-Selective biofilm penetration and eradication
Dual-responsive smart nanocarriers: H_2O_2-responsive block copolymer of POEGMA-b-PBMA assembled with a surface charge-switchable photosensitizer, 5,10,15,20-tetra-{4-[3-(N,N-dimethyl-ammonio) propoxy]phenyl} porphyrin (TAPP) into NPs [98]	Surface charge-switchable photosensitizer, 5,10,15,20-tetra-{4-[3-(N,N-dimethyl-ammonio) propoxy]phenyl} porphyrin (TAPP)	Selective and triggered release of TAPP by overexpressed peroxides at infection sites and biofilm, followed by changing surface charge of TAPP into positive.	-Bacterial biofilm	-Selective and enhanced aPDI with less self-quenching
Smart nanocarriers linked to responsive linkers to bacteria ex. hyperbranched PEG linked with Zinc porphyrin via disulfide and benzacetal linkers [99]	Zinc porphyrin	Selective and triggered release by glutathione (GSH) and acidic		-Selective and enhanced aPDI

2.5.2. Nanocarriers themselves as aPSs

Fullerenes are recognized as one of the most significant nanocarriers that can act as PS [59]. Other nanocarriers in this group are semiconductors [60]. Fullerenes have a spheroidal structure made up of pentagonal and hexagonal rings, such as C60, C70, C84, etc. The weak bactericidal action of these compounds can be attributed to their neutral charge and lipophilic nature [61]. In order to make fullerenes cationic, many alterations have been made to them using various cationic chemicals. In contrast to the negligible killing effect of non-charged fullerene N-methyl-2-(40-acetami- dophenyl) fulleropyrrolidine (MAC_{60}), aPDI mediated by the cationic fullerene N,N-dimethyl-2-(40-N,N,N-trimethyl-aminophenyl) fulleropyrrodinium iodide ($DTC60_{2+}$) significantly hindered E. coli proliferation [62]. More research is necessary because of this PS's great efficacy and selectivity. Semiconductors, or photocatalysts, are materials with semi-conductive qualities, such as zinc oxide (ZnO) and titanium oxide (TiO_2). Following irradiation of these materials by UVA, ROS are produced due to the excitation of the electron in the valence band and shifting to the conductance band. Due to their absorption in the UV spectrum, TiO_2 nanoparticles are not employed in medical settings. TiO_2 nanoparticles are mostly utilized to disinfect water and produce clean, hygienic water when sunlight is the light source [63]. Researchers have concentrated on doping TiO_2 nanoparticles with other elements to change their absorbance spectrum from ultraviolet to visible light in order to make them useful in clinical applications [64]. Furthermore, current research suggests that graphene quantum dots (GQD) can be used alone in aPDI without the need for conjugating PSs [65, 66]. Using GQD as the photosensitizer, a decrease in both Gram (+) and Gram (-) bacteria, S. aureus and E. coli, respectively, was observed after a 10-second irradiation [67].

2.5.3. aPDI using nanocarriers

Biodegradable matrices, like silica, have the ability to entrap a wide variety of PSs, produce a monodisperse distribution, and sustain antibacterial activity over an

extended period of time. Because of the permeability of these matrices, ROS and other types of molecular radicals produced during irradiation can easily migrate through the matrices and kill nearby bacteria. In addition, the entrapment of PSs inside the matrices guards them against microbial attack and keeps them stable despite pH changes [68, 69]. Quantum dots (QD), like cadmium selenide quantum dots (CdSe QD) and zinc sulfide quantum dots (ZnS QD), enhance the efficacy of PS in aPDI. These molecules absorb photons with certain energies (wavelength less than 480 nm) and release longer-wavelength photons (about 642 nm). Through QD, the energy of light with the proper wavelength is transmitted to a nearby PS [70, 71].

The antibacterial capabilities of PS are enhanced when it is attached to the surface of nanocarriers. Different PSs have been shown to bind to distinct nanocarriers in a number of studies. For instance, TB has a tendency to bind to the surface of gold nanoparticles [72, 73], whereas porphyrin tends to bind to carbon nanotubes [74]. It was discovered that PS bound to nanoparticles had significantly higher antibacterial activity than PS in its free form. An alternative strategy involves using a viral protein cage to deliver PS was conducted. The genetic construct of the viral protein cage used in this strategy introduced two advantages: enhanced inactivation of bacterial cells and the ability to target specific sites with the aid of antibodies [75].

A variety of different nanocarriers, including emulsion-based systems, have been employed as PS delivery vehicles, including tetrapyrroles, natural products, and phenothiazinium dyes. Nanoemulsions (NE) are biphasic systems on the nanoscale that hold unique advantages for being used as novel carriers for aPS, especially because of their ease of preparation, improved stability, high solubilization of drug molecules, and enhanced biocompatibility [76]. Oil-in-water nanoemulsions (o/w) can be employed to administer hydrophobic drugs—like the majority of PSs—as they will be distributed in the oily phase before being dispersed into the aqueous phase in the form of droplets [77]. These nanocarriers have widely been used as delivery agents for PS with improved safety and efficiency [78–82]. Only a small number of studies have looked into NE's potential as an aPDI nanocarrier; the majority of reported studies have used it for PDT of cancer. After encapsulating chloroaluminum phthalocyanine (ClAlPc) in nanoemulsions (NE), it enhanced the photokilling of the highly resistant, potentially fatal Cryptococcus neoformans melanized cells via aPDI [83].

Additionally, the effectiveness of the cationic chloroaluminum phthalocyanine nanoemulsions (ClAlPc/NE)-mediated aPDI in reducing the metabolic activity of Candida albicans planktonic and biofilm cultures has been confirmed by additional research [84].

Furthermore, ClAlPc/NE-mediated aPDI was successful in photokilling methicillin-susceptible and methicillin-resistant Staphylococcus aureus suspensions and biofilms. The two strains of S. aureus were shown to be particularly susceptible to photokilling by cationic nanoemulsion (ClAlPc/NE) and free ClAlPc, whereas the MRSA strain was not susceptible to photokilling by the anionic formulation of ClAlPc [82]. Additionally, ZnPc was formulated in NE and demonstrated a notable improvement in photokilling of Leishmania species by aPDI [77].

Recently, ZnPc-NE was found to exhibit more photobiological activity on Enterococcus faecalis and MRSA than free ZnPc [85]. One of the most recent studies to create ZnPc in the NE system proved that ZnPc nanoemulsion has improved antimicrobial photodynamic inactivation of resistant bacterial infections in vitro with almost complete eradication of MRSA and a multidrug-resistant strain of E. coli. It also provided a promising therapeutic means of treating serious infections and promoting wound healing in vivo [86].

2.5.4. Nanocomposites for aPDI

Nanocomposites (NC) are multiphase materials with nanoscale additions in one of the phases. These phases are dispersed in such a way that they offer properties that neither of the individual phases can provide [87]. Polymeric nanocomposites have gained great attention in recent years for their bioavailability, biodegradability, sustainability, and non-toxicity [88]. For aPDI, various polymeric nanocomposites, composed of a wide range of biopolymers have been investigated. One of the studies loaded the cationic PS 5, 10, 15, 20-tetrakis (m-hydroxyphenyl) porphyrin (mTHPP) on the surface of ethylcellulose/ chitosan nanocomposite with a significant eradication of multi-drug resistant Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans [89].

Furthermore, it has recently been postulated that metallic nanocomposites, such as metal-organic framework (MOFs) nanocomposite, can enhance aPDI. Metal ions and organic linker molecules are used to create MOFs, which are nanoporous materials with a high surface area, adjustable pore size and porosity, high drug loading capacity, and good biocompatibility. The FDA approved Ce6 was attached to the surface of zeolitic imidazolate framework-8 (ZIF-8) and showed an enhanced photokilling of MRSA with enhanced wound healing [90].

Metal and polymers were combined in a different investigation to create a nanocomposite for aPDI. This study integrated the non-toxic gold nanocluster protected with mercaptopropionic acid, and protoporphyrin IX (PpIX), within a chitosan polymer matrix. In response to exposure to white light, this nanocomposite demonstrated a twofold increase in ROS production, a notable eradication of both Gram (+) and Gram (-) bacteria, and improved penetration and biofilm removal [91].

Interestingly, a novel near-infrared triggered, multifunctional upconversion nanocomposites were developed with a strong ability to photokill bacteria. They consisted of up-conversion nanoparticles, Ce6 and Manganese pentacarbonyl bromide. When this nanocomposite is subjected to near-infrared (NIR) light (980 nm), the UCNPs can emit strong red light (655 nm), which further initiates the aPDI of Ce6. The resulting reactive oxygen (ROS) subsequently breaks the metal carbonyl bond of Manganese pentacarbonyl bromide, producing carbon monoxide (CO) molecules as well as manganese ions (Mn^{2+}). This further breaks down hydrogen peroxide (H_2O_2) in the microenvironment to oxygen (O_2). Consequently, this nanocomposite not only offers significant self-oxygen replenishment for improved aPDI, but it also makes it easier to effectively regulate inflammation through CO across a variety of deep infections [92].

2.5.5. Smart nanocarriers in aPDI

Smart nanocarriers are drug delivery technologies that can efficiently target bacterial cells and kill them selectively while leaving healthy tissue intact. This can usually be accomplished by either increasing the PS's affinity for particular bacterial components (such as membrane proteins) or by disturbing the pathogen to promote its uptake. Generally, it could be achieved by incorporating either poly-cationic materials, bacterial-targeting peptides, polymers, antibiotics, or antibodies [93]. PS can actively target bacteria by formulating polymeric nanocarriers attaching one of bacterial targeting moieties. Exopolysaccharides (EPS), glycan, and different sugars such as mannose, sialic acid, and galactose have been utilized to target different pathogens [94].

More and more target moieties are emerging as the bacteria are studied and understood in greater detail. Some nanocarriers were designed in such a way that the release of PS was only triggered by specific bacterial enzyme at the infection site. One study formulated the photosensitizer hypocrellin A (HA) into lipase-sensitive methoxy poly (ethylene glycol)-block-poly(ε-caprolactone) (mPEG-PCL) micelles, which once come into contact with bacteria that secrete lipase, the PCL is degraded to release HA. Photoactivation of HA resulted in complete eradication of MRSA [95].

Moreover, smart nanocarriers took advantage of the acidic nature of the bacterial biofilm and developed a bacterial-activatable polymeric delivery system, achieving selective killing of the bacteria while keeping the host normal tissue unaffected. Lack of oxygen in the biofilm milieu causes anaerobic glycolysis, which contributes to the acidic, highly reductive (high glutathione (GSH)) microenvironment, with abundance of ROS. PH-sensitive, surface charge switchable nanocarriers were developed. These systems loading PS exhibit negative charge at physiological pH, enabling it to prolong the circulation time in blood with minimal cellular internalization. Upon exposure to an acidic microenvironment at infection sites and biofilms, the surface charge of the nanocarrier turned into positive as a result of pH-sensitive electrostatic interactions. Hence, positively charged nanocarriers effectively bind to the surfaces of bacteria and enhance photoinactivation.

One investigation created pH-sensitive polydopamine (PDA) NPs of RB, which were coated a layer-by-layer with polymyxin B (PMB) and gluconic acid (GA) to generate functionally adaptive NPs (RB@PMB@GA NPs) that exhibited good biofilm penetration and eradication [96]. Another study developed pH-sensitive, surface charge switchable supramolecular polymeric system with the pH-sensitive poly (ethylene glycol) (PEG) block polypeptide copolymer [PEG-(KLAKLAK)2-DA] which interacted with the α-CD-Ce6 prodrugs through host-guest interaction. Upon light irradiation, this smart nanocarrier synergistic photodynamic eradication of MRSA biofilm (pH 5.5) with minimal harm to healthy tissues [97].

Smart nanocarriers with dual-responsive polymeric nanosystems have been recently designed to be sensitive to both the acidic microenvironment and the overexpressed peroxides of bacterial biofilms. An H_2O_2-responsive block copolymer of POEGMA-b-PBMA was assembled with a surface charge-switchable photosensitizer, 5, 10, 15, 20-tetra-{4-[3-(N,N-dimethyl-ammonio) propoxy] phenyl} porphyrin (TAPP) into NPs. At the infection area with overexpressed peroxide, nanoparticles were disintegrated to release TAPP, which was subsequently protonated in the acidic infection area with enhanced aPDI by making it more hydrophilic and less self-quenching [98]. Other smart systems create nanocarriers with double linkers that react to two aspects of the biofilm microenvironment. For instance, investigators linked the hyperbranched PEG with Zinc porphyrin through disulfide and benzacetal linkers, which react to reductive (GSH) and acidic microenvironments of bacteria, respectively [99].

2.5.6. Limitations of using nanocarrier in aPDI

No doubt that nanocarriers have greatly enhanced the solubility of PS, protected them, prolonged their circulation time, improved their targetability towards microbial infections, and boosted their overall pharmacokinetics. However, few of nanotechnology-based PDT reached clinical applications. Due to the inconsistency between in vitro and in vivo models, dosages, or experimental techniques reported in the literature, there are still a lot of unresolved queries regarding the biological impacts of nanoparticles themselves. It is essential to carefully assess the biocompatibility of nanoparticles in terms of both cytotoxicity and general cellular homeostasis. Better understanding of the cellular and molecular mechanisms that nanoparticles stimulate, such as inflammatory processes, is especially crucial since such actions could have toxicity or long-term impacts, compromising the biosafety of nanomaterials [100]. However, it is worth noting that PS complexation or covalent conjugation with a nanocarrier may drastically change the drug's physicochemical properties, which could impact its ability to cause phototoxicity. Furthermore, high PS concentrations may have a self-quenching effect and reduce phototoxic action. Therefore, before starting the biological research stage, it is essential to fully characterize the innovative delivery system upon drug binding and strike the correct balance between the PS loading and photodynamic action of the prepared formulae [101]. Therefore, techniques for synthesis and chemical characterization must be developed to produce formulations with repeatable structure, purity, and characteristics [100]. Given all those factors, it is reasonable to predict that using nanoparticles as therapeutic delivery systems in PDT still needs a lot of effort. However, the abundance of publications on the biological activities of PS-nanoparticle formulation in vitro and in vivo gives us optimism that, in the future, better drug delivery may enable us to greatly increase the efficacy of PDT.

III. Conclusion

Antibiotic resistance in microorganisms continues to be a serious medical problem that complicates therapy. On the other hand, it has been shown that aPDI in conjunction with nanotechnology is a promising therapeutic approach for the eradication of bacterial biofilms and resistant bacterial infections. With nanotechnology, photosensitizers of various origins can have their characteristics modulated to increase their effectiveness and selectivity.

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