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ANTIMICROBIAL PHOTODYNAMIC THERAPY (APDT), AN APPROACH TO FIGHTBACK AGAINST ANTIBIOTIC RESISTANCE: A SHORT REVIEW

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Antimicrobial photodynamic therapy (APDT), a novel tool for combating the drug resistant microorganisms which is combined with modern technologies and tools. The versatile and wide range of available photosensitizers (PS) and different wavelength light combinations opened so many ways to kill potential disease-causing pathogens. The research is developing so fast with the help of photochemistry, photobiology and photophysics. This is the beginning of new era of another antimicrobial solutions compared to conventional antibiotics. Many articles have published regarding studies on APDT and its applications. This method has shown successful eliminations of pathogenic microorganisms in skin, dental and foot infections as well as tumor or cancer treatment. The findings shared the knowledge of safe and resistance free alternative treatment of antibiotics which has clinical importance globally. This review highlights the concept, history, mechanisms, applications and the advantages of APDT.

Keywords: APDT, photosensitizers, Infectious diseases, application of APDT, ROS, antibiotic resistance, *C. elegans*

INTRODUCTION

Antimicrobial resistance (AMR) and Multidrug resistance (MDR) are very common term in public health which are drawing attention globally. The resistance is increasing day by day because of the inappropriate and misuse of antibiotics. This resistance is spreading among nosocomial pathogens known as ESKAPE that includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp*. (1-7). This deliver a message that sooner we will face a huge drug resistance problem by 2050 and the estimated death rate will be 10 million per year (1, 2). The dependency on antibiotics is risky because the alternative of antibiotics are not established yet industrially. Under the current situation it is necessary to find out alternatives to antibitocs to combat drug resistant microorganisms. Scientists are looking forward to establish methods or technologies to treat infections without antibiotics. Inventing new antibiotic compounds are challenging task as a result scientists are trying to modify the old antibiotics with different combination formula (2). Phototherapy has been used as a common practice in the last few countries in ancient Greece, Egypt and India for treatment of skin diseases and showed remarkable positive results. In the early 20th century phototoxicity was revealed and some effective photosensitizers were reported for successful photodynamic therapy (PDT) for cutaneous diseases. The method of PDT was discovered accidentally in 1900 by a medical student named Oscar Raab in Munich. He was doing experiments on acridine red dye on *Paramecium* spp. He failed many times to reproduce the activity and found inconsistent results. Later his supervisor Hermann von Tappeiner found that the result varied with daylight and then they did a clinical treatment with these findings and named this method as "photodynamic phenomenon" (2). APDT gained acceptance to be used in various medical sector for killing disease causing agents with no resistance occurance. Also PDT showed successful results in case of treating cancer cells with the assurance of not recurring attack anymore (8-10). After finding very effective results on elimination of pathogenic microorganisms such as *Staphylococcus aureus* (6) and others such as *Streptococcus mutans*, *Porphyromonas gingivalis*, *Candida albicans*, and *Enterococcus faecalis* (11, 12).

Antimicrobial photodynamic therapy (APDT)

A specific wavelength of light and photosensitizer (PS) made an effective method for many infectious diseases and later on it was found in the cancer treatment. Interestingly the MDR or AMR was found susceptible with antimicrobial PDT (APDT). In early 1990's APDT notified as effective antimicrobial treatment against drug resistant infections and the new world with new approaches begun (1, 3). The modern era of PDT started in the 1970s in the U.S.A., largely due to the efforts of Dr. Thomas Dougherty working at Roswell Park Cancer Institute in Buffalo, New York. The first photosensitizer (PS) that was introduced by Dougherty and co-workers was a water-soluble mixture of porphyrins that was named 'haematoporphyrin derivative' (HpD), and a more purified preparation later became known as Photofrin. Although Photofrin is still the most frequently used PS throughout the world today, it has many acknowledged disadvantages including skin

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photosensitivity that can last for weeks or months and can be highly troubling for patients, and a relatively small absorbance peak at 630 nm making it somewhat inefficient in use, especially for bulky tumors where light penetration is problematic. Since then, medicinal chemists have attempted to synthesize and discover molecules that could act as improved PSs, and several hundred compounds have now been proposed as potentially useful to mediate PDT for tackling cancer, infections and many other diseases. In recent years PDT has returned to its earliest roots, and antimicrobial photodynamic inactivation (APDI) has made new beginning with many combinations of PS and light. The structures of antimicrobial PSs have some features in common with anti-cancer PSs, but there are also major differences among natural Ps. For example, Hypericin and hypocrellin are both Perylenequinone but their structure and origin are both from different sources. Hypericin and Hypocrellin both has the anticancer and antimicrobial properties with different light exposure, 570 and 470 nm wavelength. (Table 1) (13, 14,15).

Generation of ROS

This method depends on the exogenous compounds which is called photosensitizers (PSs). In case of APDT, the visible wavelength lights are used, range 400-700 nm. The bacterial surface usually absorbs the PS and after exposure to the specific wavelength of light it triggers to the excited singlet state (^{1}PS). ROS or reactive molecules are produced by the excited electrons produced with lower energy by undergoing to a system and later converts to triple state (^{3}PS) (8). There are 2 types of mechanisms of making ROS molecules, type 1 and type 2 (Figure 1). From type 1 reactions, free radicals and radical anions or cations such as $O_2^{\cdot -}$, H_2O_2, OH are produced, especially O_2- can produce enough cytotoxic ROS like OH radicals oxidize biomolecules and cause cellular damage to cell death. In type II, there is direct reaction occurs between PS and molecular oxygen (O_2) and produce highly active zwitterionic $^1O_2$. These reactions occur simultaneously in APDT but depends on the specific PS applied. It is referred that in case of many photosensitizers type 2 (singlet oxygen) mostly responsible for the biological events and type 1 occurs in low oxygen concentrated environment or polar environment. However, in any mechanism ROS is produced which oxidizes various cellular compounds for example amino acids: cysteine, methionine, tyrosine, histidine, tryptophan and DNA that cause cell death (1, 2).

Mechanisms of ROS

Photosensitizers are one of the most important elements in PDT. They can be natural or synthetic which use the light energy to create ROS for killing the microorganisms (Figure 2). There are several photosensitizers that had been checked for *in vivo* and *in vitro* PDT experiments until now, only few had been identified as potential photosensitizers. Generally, the photosensitizer should have some easy properties for use, for example worldwide commercially available, pure and suitable for hydrophilicity to get in to the cells and clinical use. The photosensitizer should have lipophilicity to bind properly to the target. Moreover, the ideal photosensitizer should have the chemical and physical stability, good selectivity, low dark toxicity but should have high photo toxicity, activation at a long wavelength and rapid removal from the body. For successful PDT process, sufficient light is required to reach into the target cells for the completion of the mechanism. There are many available light sources including the xenon lamp, light emitting diode (LED), laser beam and fiber optic devices with advantages and disadvantages. The xenon lamp can illuminate a wide tumour area in one burst and the illuminated range can be easily changed; unfiltered xenon light has a peak spectrum in the wavelength range of 450 to 550 nm. Additionally, a laser beam does not have a wide wavelength spectrum. LEDs and fiber optic devices are also used in PDT to treat clinical diseases. Therefore, the choice of an optimal combination of PS, light source and treatment parameters is crucial for successful PDT (2, 13).

Most of the photosensitizers weight range is 1500-1800 Da. Usually the outer wall of the Gram-positive bacteria is not complex rather easily permeable whereas the cell envelope of the gram-negative bacteria has inner cytoplasmic membrane and an outer membrane which are separated by the peptidoglycan layer. The outer wall has a highly heterogeneous composition, including proteins with porin function, lipopolysaccharide (LPS) trimers, and lipoproteins. It gives the outer surface a quasi-continuum of densely packed negative charges. These properties are organized and inhibits the penetration of several compounds: even hydrophilic 600-700 Da molecules can diffuse through the porin channel. It can be guessed that the outer membrane of Gram-negative bacteria forms a physical and functional barrier to the environment. For Gram-positive bacteria and yeasts, the photosensitizer accumulates in the cell wall. After irradiation by visible light, ROS create rapid destruction of the cell wall. ROS can interact with many biological molecules such as unsaturated fatty acids, amino acid residues (cysteine, histidine, tryptophan) and nucleic acid bases of DNA, particularly guanine and thymidine (18). There are various targets where different photosensitizers bind and helps to destroy the bacterial intra and extra cellular components (Table 2).

Effect of APDT on Gram positive and Gram negative bacteria

APDT works initially on external microbial structures such as the cell wall and cell membrane. The mechanisms work during the planktonic cells or in biofilms but in case of biofilms the PS diffusion can be little difficult due to dense cells matrices or their virulent factors. The diffusion potential of ROS depends on (i) the maximal time-limited diffusion length, especially for $^1O_2$ that possesses a shorter half-life compared with other ROS, (ii) the photostability in a given environmental medium, and (iii) the chemical properties of PSs (e.g., molecular size,

charge, lipophilicity, stability), which influence the interactions of the latter with target microorganisms. In case of photoinactivation, photochemical reactions in bacterial cells depends on the PS and its charge. This mechanism found effective against Gram positive bacteria but for Gram negative bacteria it requires cationic PS, or different combination of a neutral PS with membrane damaging agents (1, 2, 18).

Advantages of APDT

APDT showed many advantages although there are also some limitations and APDT has several advantages over antibiotics (Figure 3). The first important advantage is that APDT is considered triply site-specific due to

1. The APDT is site specific.
2. Mostly applicable for the skin/oral treatment.
3. No drug administrated orally.
4. The cellular damage by APDT cannot be recovered by the microorganisms easily.
5. Antibiotic resistant strain can be treated efficiently with APDT
6. No resistance found for photosensitizers (17).

The main advantage of APDT is triple site specific. The uptake of photosensitizers is specific to target cells, not to non-target cells; the pharmacodynamics criteria of non-irradiated photosensitizers, the binding of photosensitizer and irradiation is in infected areas only. The photosensitizers cannot work without the light irradiation for creating ROS. It is totally an environment friendly technique. There are no resistance generation phenomena after APDT treatment (16). So, using APDT several times does not produce any resistant strains. The reasons behind this are firstly the treatment (photosensitizer + light) time is very short to develop any resistance (18, 19).

Next, photosensitizers do not have any dark toxicity effect and sometimes microorganisms cannot sense or understand the ROS mechanisms for their death. So, they cannot produce any adaptive or protective mechanism against this stress. After the treatment, the cell and its major parts are so damaged that they often fail to recover that damage (18). It is also difficult for bacteria to 'sense' that the oxidative stress emanates from the otherwise non-toxic PS, so any metabolic adaptations are directed elsewhere (e.g., antioxidant defense machinery). Third, the cells are too damaged after PDT, disabling them to confer cross-generation adaptively. Lastly, APDT does not target a single site in bacteria, much different from conventional antibiotics (18-20).

Limitations

I) The extra and intra cellular targets

Traditional antibiotics often utilize a key-hole mechanism, where the compounds target one specific membrane- or (intra)cellular component in bacteria, be it proteins, lipids, or DNA, to either stop growth or kill the organism. For example, penicillin binds to the penicillin binding proteins and inhibits the crosslinking of the peptidoglycan multi-layer. Vancomycin binds to the D-Ala-D-Ala residues of the peptide side chain of the peptidoglycan precursor lipid II and deters downstream peptidoglycan synthesis steps. Daptomycin is believed to insert into the membrane of Gram-positive bacteria, where it forms aggregates that modify the curvature of the membrane and cause cavitation, ion leakage, and ultimately cell death. In contrast, the PSs used for APDT typically distribute to multiple extracellular or intracellular compartments and/or produce radical intermediates that can migrate away from the formation site. As a result, various components of cell metabolism are disrupted, culminating in cell demise when sufficiently afflicted (18).

II) Lipopolysacchrides of Gram negative bacteria

Lipopolysaccharides (LPS) are the major component of the outer membrane of Gram-negative bacteria that impart structural integrity and protect the membrane from attacks by chemicals. LPS forms the outermost physical and electrostatic barrier that exogenous compounds must transgress to reach the lipid bilayer of the outer membrane. Its presence is therefore a hurdle for intracellular PS targeting. Although the LPS layer obstructs easy entry of PSs into Gram-negative bacteria, the layer may also serve as a target for APDT. The surface structures are vitally important in bacterial cell physiology. To underscore the importance of LPS: when LPS is structurally modified or removed, the bacteria die. Because of its highly anionic nature, LPS is considered a primary target of cationic PSs (17).

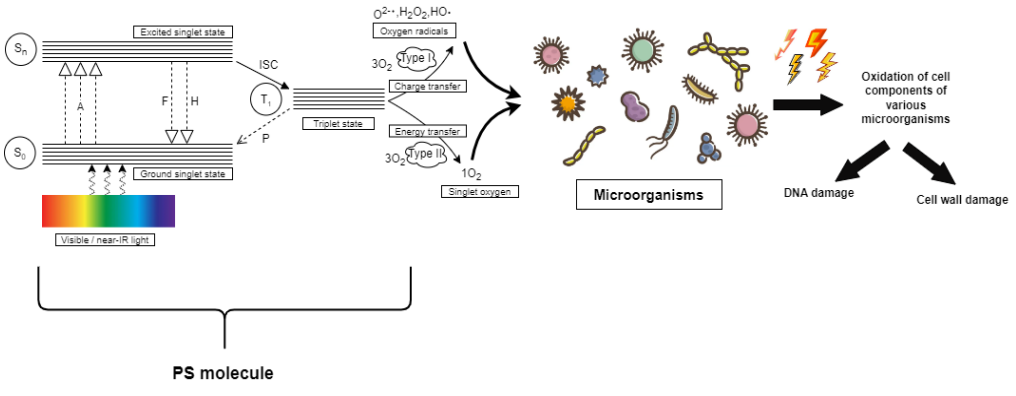


Figure 1: Photochemical and photophysical mechanisms leading to ROS production during PDT.

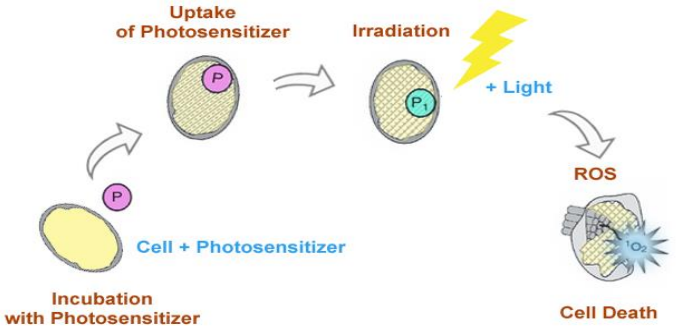


Figure 2: Mechanism of photosensitizer absorption: P, photosensitizer; P1, excited state of photosensitizer after absorption of light; $^1O_2$, singlet reactive oxygen.

Table 1. Some common natural photosensitizers and their applications (13).

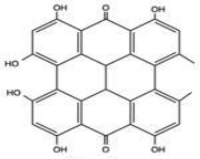
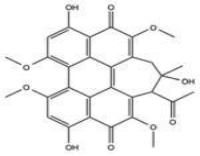
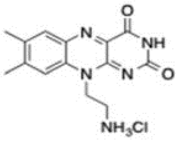
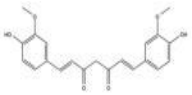
Class	Name	Structure	λ_{max}	Application
Perylenequinone	Hypericin		570 nm	Cancer, antimicrobial, *in vitro*, *in vivo*
Perylenequinone	Hypocrellin		470 nm	Cancer, antimicrobial, *in vitro*, *in vivo*, clinical
Flavin	Cationic riboflavin		UVA/440 nm	Antimicrobial, *in vitro*
Curcuminoid	Curcumin		420 nm	Antimicrobial, *in vitro*, *in vivo*, clinical

Table 2. The extra- and intra-cellular targets of some common photosensitizers (18).

Class	Name	Extracellular target	Intracellular target	Bacteria
Phenothiazinium	Methylene blue (MB)	Cell wall surface and membrane protein	Chromosomal DNA	*E. faecalis*
	Rose Bengal (RB)	Cytoplasmic membrane	DNA*	*E. coli*
	Toluidine blue O (TBO)	Lipopolysaccharides and outer membrane	ND	*P. aeruginosa*
Porphyrin	5,10,15,20-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (Tetra-Py^+-Me)	Lipopolysaccharides and outer membrane lipids	DNA*	*E. coli*, *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Rhodopirellula sp.*, *S. aureus*, *Truepera radiovictrix*, *Deinococcus geothermalis*, *Deinococcus radiodurans*
	5,10,15,20-tetra(4-N,N,N-trimethylammo-niumphenyl) porphyrin	Cell wall and cytoplasmic membrane	Plasmid DNA	*E. coli*
	5,10,15,20-tetrakis(N-methyl-4-pyridyl): 21H,23H-porphine (Tetra-Py^+-Me)	Outer membrane	ND	*E. coli*
	Hematoporphyrin monomethyl ether (HMME)	Cytoplasmic membrane	ND	*S. aureus*
Phthalocyanine	Zinc(II) phthalocyanine (ZnPc)	Outer membrane and cytoplasmic membrane	ND	*E. coli*
Fullerene	N-methylpyrrolidinium C_{60} fullerene iodide salt	Cytoplasmic membrane	ND	*S. aureus*

Note: *DNA as target of APDT still requires further investigation. In most studies, it is not distinguished whether the DNA damage comprises chromosomal DNA or plasmid DNA; ND = not detected/not discussed.

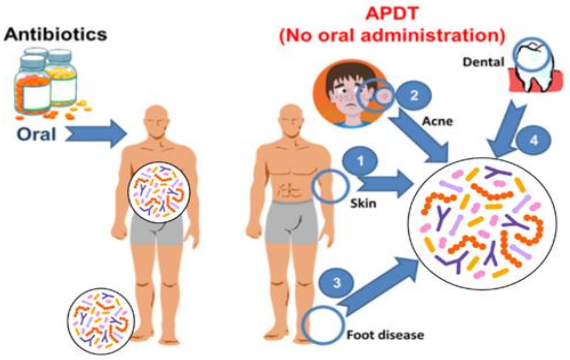


Figure 3: Advantages of APDT compared to antibiotic administration (modified and redrawn from Dias *et al* 2020).

Application (Antibiotic and APDT)

Numerous *in vivo* and APDT tests were directed with animal model to treat different infections including skin and wounds, endodontic infections, burns, oral diseases, osteomyelitis, gastrointestinal, tuberculosis, and diverse fungal diseases (18-34).

Oral and dental infections

In dentistry APDT can be used for oral and dental diseases. Many photosensitizers for example methylene blue and toluidine blue O were found non toxic and can be used easily for these oral problems. They can easily penetrate to the gram negative bacteria and can be killed. Now days nano technology also been involved for using APDT for better results and efficacy. Many oral diseases like periimplantitis, halitosis, recurrent herpes labialis, root canal disinfection, oral plaque and carries control can be treated with APDT (11). Oral plaque biofilm production is a challange in case of treating the infection but APDT application in dentistry made it effective. *E. faecalis* is the causative egent of root canal infections. APDT can be applied by using polyethyleneimine and chlorine (e6) PEI-ce6 under 660-nm laser. There was a synergistic impact for TBO based APDT to the infection and found 82.59% reduction of bacteria after treatment (18). In recent studies the effectiveness of PDT found effective with oral diseases, including *Porphyromonas gingivalis* (*P. gingivalis*; as a Gram-negative obligate anaerobe bacterium which is the main cause of periodontal diseases, *Streptococcus mutans* (*S. mutans*) that involved in caries development, and *Candida albicans* which is the responsible fungus in the oral microbial communities (11, 12, 19, 21).

Other infections

Pathogenic bacterial and yeast diseases in bone outcome in the arrangement of osteomyelitis. In a rodent model, bioluminescent *S. aureus* biofilms were embedded into the rodent tibial bone. 5-aminolevulinic acid (ALA)-APDT was commonly viable against *S. aureus* biofilms in bone, repetitive contamination and biofilms on inserts in bone (22, 23).The foremost nares are normally started by *S. aureus* and additionally spread to other anatomical locales after surgeries. Already the utility of APDT for nasal MRSA decolonization utilizing a custom nasal repository model was referenced. In the MB-APDT method 670 nm diode laser fiber with light diffusing tip can be used. APDT struggle with MRSA colonization on refined human epithelial surfaces, and can be effective in the nose of people with a treatment times under 10 min. APDT inactivation of nasal MRSA was examined in Canada with an enormous clinical trial. MB-APDT with blend of chlorhexidine gluconate prompted 5.1 $\log_{10}$ immediate decrease in bacterial population and 5.9 $\log_{10}$ decrease after 24 h. APDT additionally decreased number of MRSA in nasal swabs and also the number of post-usable surgical site contaminations. Superficial fungal skin infections affect millions of people, where *C. albicans* and *Trichophyton rubrum* are the most frequently encountered fungal pathogens. In previous study, it was employed that a new MB-based model of APDT in a mouse model of skin abrasion infected with *C. albicans* APDT initiated at 30 min or at 24 h post infection could reduce 95.4 and 97.4% cells of *C. albicans* in the skin abrasion wounds respectively (18).

Gastritis, gastroduodenal ulceration in people and stomach disease can be caused by *H. pylori* (HP) and can cause gastric cancer. Conventional treatment for *H. pylori* infections are antibiotics which has side effects such as epigastric pain, nausea and diarrhea and antibiotic resistant strain of *H. pylori* made it difficukt to treat the infection (18). APDT showed significant and promising findings to combat with *H. pylori* infections.In a study, illumination of gastric antrum with blue light (~405 nm) without photosensitizer was enough to kill *H. pylori*. In case of APDT, laser probe can be used endoscopically and

of APDT, laser probe can be used endoscopically and *H. pylori* can be inactivated in patients. In a small pilot study no photosensitizer was used as *H. pylori* can produce natural photosensitizer named protoporphyrin IX and coproporphyrin. This results 99% of inactivation of bacteria but still the mechanism was not fully understood (18, 25-28).

Tuberculosis is caused by *Mycobacterium tuberculosis* infection, and the death rate in drug-resistant infections is among the highest for infectious diseases worldwide. Multidrug resistance was found in *Mycobacterium* which made it more difficult to inhibit and made it worse for treatment. Extreme drug resistance tuberculosis (XDR-TB) had developed resistance to all effective antibiotics including fluoroquinolones, and the injectable drugs for example kanamycin, amikacin, capreomycin. APDT had been applied to treat *Mycobacterium* infection in a mouse model, through injecting PS into the lesion and illumination using a fiber-optic. The subcutaneous granulomas formed from collagen gels were infected with *M. bovis*. Benzoporphyrin derivative (BPD), activated by a diode laser, led to 0.7 $\log_{10}$ reduction in viable bacterial numbers, and another PS 5-ethylamino-9-diethylaminobenzo[*a*]phenoselenazinium chloride (EtNBSe) resulted in at least a 2 $\log_{10}$ decrease (18, 29-31).

Otitis media (OM) is a very common childhood infection that responds poorly to standard antibiotics and around 50-80% of children get infected in US by 1 year age which was found to be highest in 3 years old children. *Streptococcus pneumoniae* is the major causative agent (around 95%) of OM. Later antibiotic resistance occured and introduce challanges to combat with OM and the formation of biofilm was another issue to treat effectively (32). It was investigated the preclinical effect of photogem-APDT. Two days after injection of *Streptococcus pneumoniae* or *Haemophilus influenzae* cells into the bullae of gerbil ears to produce a model of OM, photogem was injected into the bullae, followed by transcanal irradiation with a 632-nm diode laser. APDT was effective in eradicating *S. pneumoniae* in 87% of the infected bullae with OM, and *H. influenzae* in 50% (33).

Wounds and burns are truly susceptible for simple diseases brought about by numerous nosocomical microorganisms for instance the cutaneous barrier destrcution caused by multidrug resistant *S. aureus.* APDT is effective for wound healing and ulcer infections. Using the phenothiazinium color PP904 {3,7-bis (N, N-dibutylamino)-phenothiazinium bromide} and tetra-cationic phthalocyanine RLP068nas photosensitizers, two clinical preliminaries were completed for the control of non-healing chronic injuries like venous ulcers and diabetic foot ulcers. PPA904 therapy for 15 min and red-light illumination at 50 J/cm^2 were applied to 16 patients with persistent leg ulcers and 16 patients with diabetic foot ulcers and there was no side effect noted and there was a significant amount of bacterial biofilm reduction (34).

Application of APDT on Food industry

Food and Agriculture Organization of United Nations (FAO) addressed the whole food system involed with many sub divisions including production, aggregation, processing, distribution, consumption and disposal of food. The main purpose is to deliver safe food to the people but within the food system there are many ways of contamination that hampers the quality of food as well as the hyegiene. So, in result, one in 10 people suffered from food borne illness which is 600 million illness and 420,000 deaths. The majority of the contaminants are biological, chemical and physical hazards. The microorganisms are one of the major sources of food borne illness worldwide by damaging and spoilage of food. Ususally the food rich in nutritions and water content such as milk, meat and sea foods are quickly get rotten. In case of nuts, breads, dried fruits are contaminated by yeasts and molds. Antibiotic resistance bacteria and fungicide resistant fungi were found difficult to kill by conventional method and introduce new challange to inactivate them. Some species of bacteria became multidrug resistant, for example *Salmonella*, *L. monocytogenes*, *A. hydrophila* were frequently detected in fish and fish products. APDT showed promising results for inhibiting these microorganisms and solved the challanges as an alternative method in various sectors of agriculture and food systems. The most amazing part of APDT in food system is no negative impact on food quality. It is also reported that in case of using APDT for food increased the shelf-life. Moreover other benefits including preservation of sensory qualities, and reduction of microbial spoilage. Curcumin based APDT showed higher sensory scores for oysters in odor, color, mucus appearance, texture, pallium gill filaments and shell muscles after 14 days of storage at 4°C (18, 24, 35). Visible light eradiation with TiO_2 nanoparticles coated and co-doped with nitrogen and fluorine for 2 days inhibited the fungal infections on tomatoes compared with coontrol samples (36).

Application of APDT on animal studies

Animal models have become standard tools for the study of a wide array of antimicrobial therapies of wound infections, including antimicrobial PDT. Mice are by far the most frequently used species for wound infection models. However, the principal disadvantages of mouse models relate to the small size of these animals. The number of sequential sampling of blood, other fluids and tissues that can be performed without compromising the mouse is also limited. As a result, *in vivo* studies of PDT utilizing mouse infection models suffer from difficulties in monitoring the development of the infection and its consequent response to the treatment. Standard microbiological techniques used to follow infections in animal models frequently involve the sacrifice of the animals, removal of the infected tissue,

homogenization, serial dilution, plating and colony counting. These assays use a large number of animals, are time consuming, and are often not statistically reliable (21).

Caenorhabditis elegans is a self-reproducing nematode that has been used for almost four decades in various fields of biology. It is a well-known animal model that has frequently been studied for bacterial pathogenesis, host immunity, and drug discovery, among others (37). This tiny worm, approximately 1 mm long and large scale population of 300 genetically identical worms is easy to make within 3-5 days (38, 39). The maintenance of *C. elegans* is easy and inexpensive. The transparent body helps to observe the cells inside the body.The advantage of this model is its short lifespan and various uses in the field of neuroscience, development, signal transduction, cell death, aging, and RNA interference. In recent years this model was used for biomedical and environmental fields. The another plus point is *C. elegans* conserved many basic physiological phenomenon and genetic makeup in higher organisms for example in humans. There are many studies with pathogens such as gram-positive, gram-negative bacteria, and fungi that were conducted by *C. elegans* model, especially *P. aeruginosa* which was the first gram-negative bacteria that infected *C. elegans* and killed.In case of pathogenesis check or host immunity and drug discovery *C. elegans* is gaining more and more acceptance for observation and analysis of many biological effects (37). *C. elegans* model is an excellent tool for initial screening of APDT effects establishment with many parameters such as growth rate analysis, reproduction toxicity, gut purmeability dysfunction analysis etc. The APDT effects with hypericin, ampicillin and orange light were successfully demonstrated in the inhibition of *P. aeruginosa* PAO1 and recovered from the infection in *C. elegans* model by the growth rate analysis. The light toxicity measurement also showed clearly in the reproduction analysis. In addition, the gut permeability dysfunction also showed the improvement while treated with APDT killed *P. aeruginosa* PAO1 bacterial cells. In another study we have evaluated the effect of the well known metabolite 3,3-diindolymethane (DIM) on the intestinal permeability and lifespan in *C. elegans* fed with *P. aeruginosa* (38, 39).

CONCLUSİON

APDT is a vast field of research in terms of cancer treatment or inhibition of bacteria but still there are many more to develop and upgrade the current methods. In case of skin infections caused by bacteria such as acne, foot diseases by fungi or dental and oral infections can be treated in Bangladesh as we are facing increasing problems for antibiotic resistance. As mentioned above there are many limitations are remaining still and need to solve those, scientists are paying attention in this method and searching for new possibilities for a better treatment option against pathogenic microorganisms. Different combinational treatment or nanotechnology can also be applied to overcome the current limitations of APDT.

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