Chapter

Light and Phages on Tackle of Infectious Diseases

Felipe de Paula Nogueira Cruz, Andréa Cristina Bogas and Cristina Paiva de Sousa

Abstract

There has been an important increase in the emergence of resistance in microbial population worldwide. This trajectory needs, necessarily new approaches to treat infectious diseases. The ability to detect and prevent the evolutionary trajectories of microbial resistance would be of value. Photodynamic inactivation (PDI) represents an efficient alternative treatment for diseases caused by viruses, which can cause infections well documented in various mammals. PDI can kill cells after exposure with the appropriate photosensitizer (PS), light of adequate wavelength combined with the presence of oxygen, without inducing resistance. Cytotoxic reactive species formed interaction with vital biomolecules leading to irreversible microbial inactivation. Bacteriophages can act on delivering antimicrobial agents into bacteria, which consist in a likely instrument for the treatment of infectious diseases. Non-enveloped bacteriophages are more difficult to tolerate photoinactivation than enveloped phages, which makes them an important model tool to evaluate the efficiency of PDI therapy against viruses that cause diseases in humans. Combination of photosensitizers and bacteriophage therapy can be employed to eradicate biofilms, contributing to control of infections also caused by drug-resistant bacteria.

Keywords: bacteriophages, biofilm, microbial resistance, photodynamic therapy, reactive oxygen species

1. Introduction

Despite the remarkable progress in human medicine, infectious diseases of microbial origin are one of main global concern to public health [1] worldwide. The relative unavailability of efficient drugs the misuse and/or excessive use of antimicrobials, are some factors that make infections harder or impossible to treat, increasing the risk of spreading diseases and deaths [2]. The gap in the discovery of new antibiotics over the decades [3, 4] also contributes to the increased risk of infectious diseases.

The emergence of antibiotic-resistant "superbugs" and their rapid global spread are alarming [5]. These microorganisms are members of a group known as nosocomial ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.), associated with major risk of mortality in immunocompromised patients [6, 7]. Other highlights in the global list of drug-resistant priority pathogens are the third generation cephalosporins (3GC) resistant *Escherichia coli*, fluoroquinolone-resistant *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Salmonella* spp. and *Candida auris* [8, 9].

Drug-resistant pathogens can be transmitted through the hospital environment, increasing the severity in relation to Health Care-Associated Infections (HAIs) [10], also causing an important economic impact especially in developing countries. In these countries, the infectious diseases are more prevalent, and the prevention measures requires the use of drugs that maximize costs [7]. Given this scenario, effective antibiotics and strategies to combat antimicrobial resistance, prevent the high number of deaths each year and an economic crisis worldwide become urgent [11, 12]. This chapter describes photodynamic treatment, bacteriophages utilization and the combination of both as alternative therapies for minimize the excessive exposure of patients to antibiotic and risks of multi-resistant strains development.

2. Photodynamic therapy: past

The therapeutic potential of light has been used for hundreds of years by the ancient civilizations in Egypt, China, and India. Also, over 3000 years ago, light was used in conjunction with reactive chemicals to treat various conditions such as vitiligo, psoriasis, and some types of skin cancer. In China, it was introduced by Lingyan Tzu-Ming in the first century b.C., and, four centuries later, became a ritual practice in which it was based on exposing a piece of green paper containing a red dye and exposed to sunlight, then it was soaked in water and ingested right after [13, 14].

In the last few decades, Photodynamic therapy (PDT) has emerged as a promising intervention treatment for cancer therapy. However, it is widely used in the removal of small vessels and in the treatment of microbial infections [15]. Still, the first concepts of the nature of light emerged in the 17th century. Preliminary work on the properties of light, such as that of Christiaan Huygens, who used wave theory to explain the reflection and refraction of light in 1690, and, later, the discovery of the properties of electricity and magnetism, in the early 19th century [16, 17].

In fact, quantum theory started when Max Planck, in 1900 published an article that explained the spectral distribution of Blackbody Radiation, which perfectly fitted the laws of thermodynamics with the laws of electromagnetism. And in the same year, Oscar Raab was scientifically proven to have the beneficial effects of light. In his experiment, it was observed that the combination of light with the acridine dye (**Figure 1**) was lethal for Paramecium species. In the same year, the French neurologist, Jean Prime, discovered that oral eosin, used to treat patients with epilepsy, could cause dermatitis when exposed to sunlight [16, 18].

According to electrodynamic theory, light consists of an oscillating electromagnetic field that propagates as a wave through a vacuum or through a medium [16]. This means that when light propagates through space, it behaves like a wave, while when interacting with matter, it behaves like particles [16, 17, 19]. This concept was described by Einstein in 1905 based on the theories of Planck and Hertz for the explanation of the photoelectric effect (Eq. (1)). For that, Einstein assumed that light had

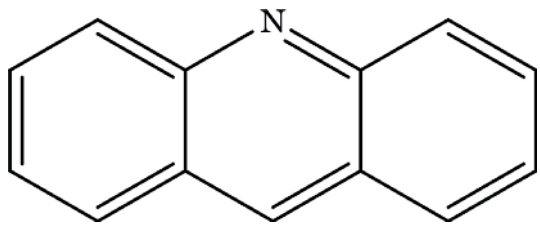

Figure 1.
Chemical structure of acridine.

a corpuscular nature, that is, it would be formed by small bundles of energy (quanta) called photons. Einstein also proposed the existence of a dependency relationship between the photoelectric emission and the frequency of incident radiation. For this theory to be valid, light could not be considered as a wave, but as a particle [17, 20].

Finally, in 1924, de Broglie created the hypothesis of wave-particle duality, which was soon recognized by Erwin Schrödinger who developed the wave propagation equation in matter in 1926 (Eq. (2)). Still during this period, other important scientists contributed to the establishment of quantum mechanics such as, for example, Max Born (Matrix Quantum Mechanics), Paul Dirac (Movement of sub-atomic particles), Werner Heisenberg (Uncertainty Principle) and Wolfgang Pauli (Principle of Exclusion) [20].

Equation 1: According to Einstein, each photon has an energy proportional to the frequency of light.

$$\mathrm{E}_{photon} = hc / \lambda \tag{1}$$

E = de um quantum energy of light
h = Planck constant = 6,63 x 10–34 J.s
c = Speed of light (3×10^{10} cm/s)
λ = Frequency of light (Hz)

Equation 2: Erwin Schrödinger allows to determine to find the wave function of a particle, from the knowledge of the potential energy to which it is submitted.

$$-\frac{\hbar^2}{2\mathrm{m}}\nabla^2\Psi + V\Psi = \mathrm{i}\hbar\frac{\partial\Psi}{\partial t} \tag{2}$$

ħ = Planck constant (6,63 x 10–34 J.s) squared reduced
m = Particle mass
∇ = Ψ Laplacian – Spatial variation of the wave function
Ψ = Wave function
V = V potential that acts on the particle
i = Imaginary number given by the square root of −1
∂t = Variation of wave function Ψ over time

In the same year, Policard [21] conducted a study where he detected the presence of porphyrins in high concentrations in malignant tumors. These, completely non-toxic, were able to destroy the tumor tissue in the presence of visible light and oxygen [21].

Later, in 1950 Schwartz demonstrated that the long-lasting phototoxic effect was not promoted only by hematoporphyrin. The action occurred due to an oligomeric mixture together with it. Since hematoporphyrin is eliminated quickly from the body, Schwartz enriched the oligomer mixture and this preparation was called hematoporphyrin derivative (HpD), which contains in addition to monomers, oligomers containing two to nine units of porphyrin [14, 22].

In the study conducted by Weishaupt [23], it was demonstrated that the destruction of tumor cells was due to the formation of singlet oxygen molecules.

Finally, in 1993, Photofrin ® (Axcan Pharma Inc., Canada) was approved for the treatment of superficial bladder cancer by the Canadian Health Protection Branch. Subsequently, in 1998 the Food and Drug Administration (FDA) authorized PDT in the treatment of cancer [24].

3. PDT fundamentals

PDT consists of a photochemical reaction between a photosensitizing agent and the oxygen that selectively destroys the target tissue, constituting an alternative modality clinically approved by several health agencies in many countries [25–29]. The photodynamic effect consists of causing a powerful and sustained photochemical reaction between light at a given wavelength, the photosensitizer (PS) and oxygen in the target tissue. Consequently, after the irradiation of light, PS converts O_2 into cytotoxic reactive oxygen species (ROS), where cell death can occur through mechanisms such as apoptosis, necrosis, or autophagy (**Figure 2**). However, recent studies have demonstrated the existence of other mechanisms with characteristics of necrosis and apoptosis. These new pathways of cell death, collectively called regulated necrosis, include a variety of processes triggered by different stimuli [14, 30].

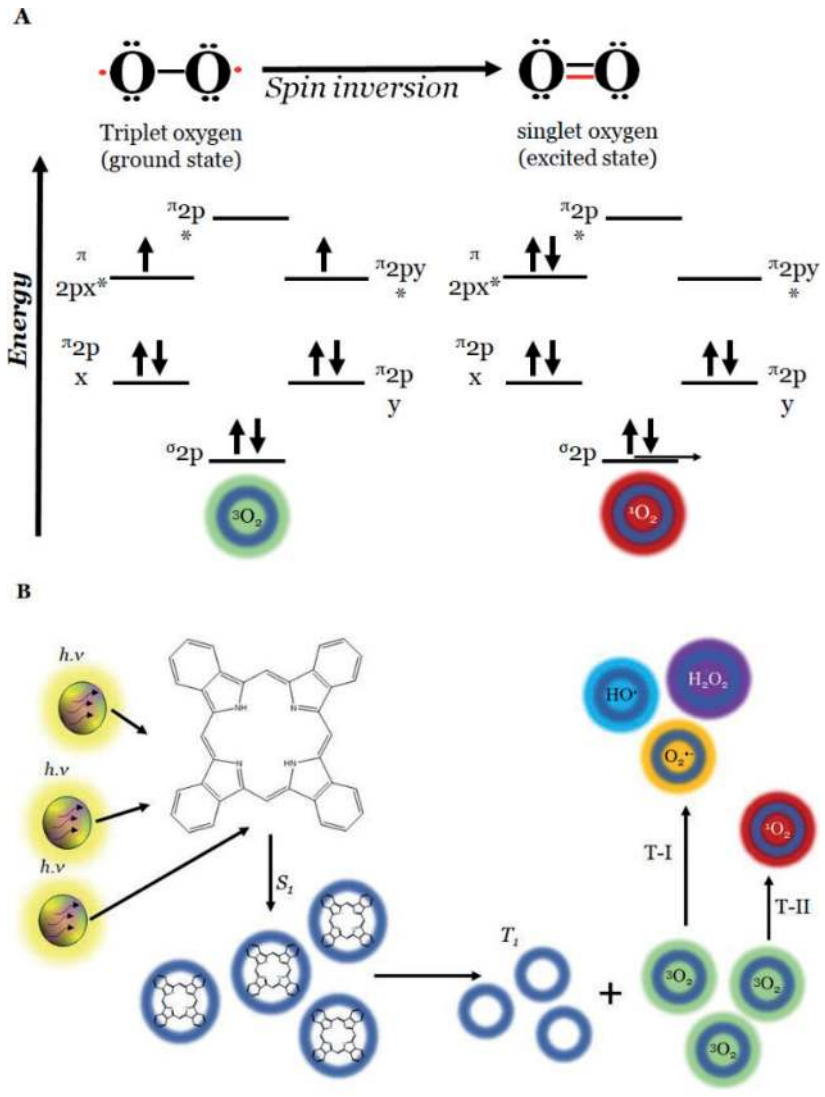

Figure 2.
Basic scheme of the photodynamic reaction. (A) Formation of ROS. (B) Porphyrin group of photosensitizer absorbs a photon that excites it to the short-lived singlet state and may decay by non-radioactive relaxation with heat emission or fluorescence emission to the long-lived triple state. In this triplet state, PS can interact with molecular oxygen in two ways, type-1 and type-2, leading to the formation of oxygen radicals and singlet oxygen [31].

The light is formed by subatomic particles given off by atoms and are endowed with high luminous energy, and energy differences result in different colors called photons. The laser (Light Amplification by Stimulated Emission of Radiation) consists of a monochromatic, non-ionizing and highly concentrated beam of light. Each wave has identical coherence in size and physical shape along its axis, producing a specific form of electromagnetic energy. This wave is characterized by spatial coherence, that is, the beam can be well defined. The intensity and amplitude of the beam follow the curve of the Gaussian beam bell as most of the energy is in the center, with a rapid drop at the edges. There is also a temporal coherence, which means that the emission of the single wavelength has identical oscillations over a period. The final laser beam starts in a collimated form and can be emitted over a long distance in this way. However, bundles emanating from optical fibers generally diverge at the tip. When using lenses, all the beams can be precisely focused, and this monochromatic and coherent beam of light energy can achieve the treatment goal [16, 32, 33].

Photosensitizing agents (PS) consist of molecules in the singlet state in their fundamental state because they have two electrons with opposite spins that allow the transport and transfer of light energy for a chemical reaction, where each PS has unique characteristics for successful activation such as wavelength and creep intensity [34–36].

Most of them are derived from endogenous dyes and are characterized by not being toxic to cells. The molecular structure of most PSs used in PDT is based on a tetrapyrrol skeleton. This type of structure occurs naturally in several important biomolecules, such as heme, chlorophyll, and bacteriochlorophyll, being called "pigments of life" [36]. Therefore, PSs based on porphyrin structures satisfy most of the desirable properties of PSs, such as the high efficiency of singlet generation ($^1O_2$), absorption of the higher wavelengths of the electromagnetic spectrum and a relatively greater affinity for malignant cells, in addition, due to the internal dimensions of the macrocycle cavity and the chelate effect, the porphyrin macrocycle can coordinate transition metals in various oxidation states [36].

4. Bacteriophages and PDT

Resistance to antibiotics spreads rapidly in relation to the discovery of new compounds and their introduction into clinical practice. In addition, the increase in bacterial adaptation can be directly correlated to the scarcity of new classes of antimicrobial agents. In the last decades, synthetic tailoring has been the main strategy to improve the nuclear scaffolding established through analog generation. Although this approach has been beneficial, this research has faced a 'Discovery Void' for 30 years, of which no new class of drugs effective against problematic ESKAPE pathogens. In addition, pathogenic microorganisms generally have the ability to form biofilms. This cellular superstructure may exhibit greater resistance to antibiotics and cause serious and persistent health problems in humans [37–39].

Bacteriophages (phages) are ubiquitous viruses which cause no harm to human or animal cells but are capable to specifically infect, replicate, and kill bacteria [40, 41]. Bacteriophages have been described for delivering successfully antimicrobial agents into bacteria, which consist in a potential alternative for the treatment of infectious diseases [42, 43] caused by bacteria.

The first *in vivo* evidence of effective phage therapy against *Klebsiella pneumoniae*, one of Gram-negative bacterium of ESKAPE group listed in the critical priority tier [44, 45] as serious opportunist in nosocomial infections in the respiratory

and urinary tracts, wound sites and blood [46], was demonstrated by Anand et al. [47]. The authors observed significant reduction in the lung lesion severity in the mouse model, suggesting the efficacy of a novel lytic phage VTCFPA43 therapy against virulent *K. pneumoniae* infection by the intranasal route.

According to [48, 49], the delivery systems based on a phage-carrying PS exhibit increased effective killing by the concentrated fluence at the bacterial cell wall, and consequently, reduced side damage to the indigenous microbiota by the site singlet oxygen. Moreover, they investigated the photodynamic effects of the photosensitizer tin (IV) chlorin e6 (SnCe6) (**Figure 3A**) covalently linked to phage 75 on several strains of *S. aureus*, including methicillin- and vancomycin-intermediate strains. Pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSa) show that antibiotic resistance rates are surpassing 50% in 5 out of 6 world regions of the World Health Organization (WHO) [37, 50]. Results showed that the phage 75 conjugated with SnCe6 was not capable to damage human epithelial cells whereas potently showed bactericide effect against vancomycin-intermediate and MRSa. Additionally, other exogenous photosensitizers (protoporphyrin IX and protoporphyrin diarginate) have been successfully *in vitro* evaluated against clinical strains of MRSa [51].

Acinetobacter baumannii is other important Gram-negative bacterium multidrug-resistant involved in nosocomial infections [51]. Due its capacity to form biofilms, they have the capacity to survive and persist in intensive care unit environment and medical devices [52], what also make of *A. baumannii* one of critical-priority pathogens encompassing the ESKAPE group, for which new antibiotics and combating strategies are urgently needed [11, 12]. In this way, [53] used for the first time the strategy of combining of cationic photosensitizer (NB), structurally modified to produces ROS, and bacteriophages (APB)-based photodynamic antimicrobial agent (APNB) for eradication biofilm formed by multi-drug resistant *A. baumannii*. Both *in vitro* and *in vivo* assays demonstrated that APBN was efficient to treat *A. baumannii* infection, including being more efficient than some antibiotics when evaluated *in vivo*. These results demonstrated the potential of APNB in combating multidrug-resistant bacteria and biofilm ablation.

Candida albicans and more recently *C. auris* are opportunistic polymorphic fungal pathogens, which exhibits almost 40% mortality rates for superficial and systemic infections in humans [54–57]. Likewise, the increasing occurrence of antibiotic-resistant among *C. albicans* strains, demands new approaches to control this life-threatening pathogen [58]. In [59], it reported the photodynamic inactivation of *C. albicans* by the Pheophorbide A (PPA) (**Figure 3B**), a chlorophyll-based

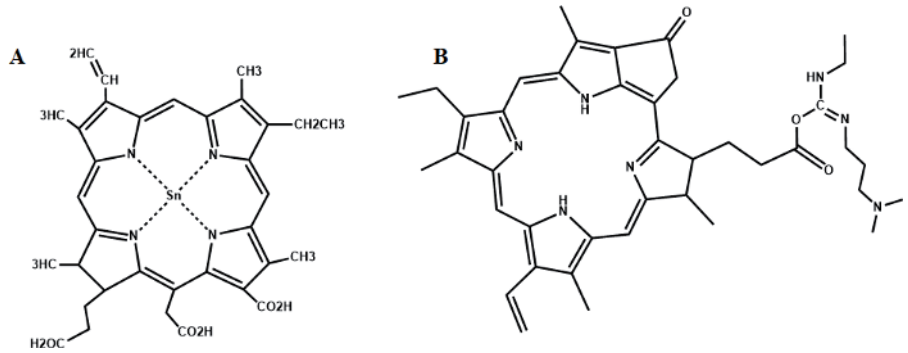

Figure 3.
Chemical structure of (A) SnCe6 and (B) PPA.

photosensitizer crosslinked associated with single-chain variable-fragment phage (JM), which possesses high affinity to β-glucanase mannoprotein (MP65), an essential cell-wall mannoprotein of *C. albicans*. The complex PPA-JM-phage was capable to induce a caspase-dependent apoptosis pathway in *C. albicans.*

The second-generation of PSs exhibits improved photophysical properties in relation to first-generation PSs, which halogens or other substituents are added in the meso- positions of the porphyrin macrocycle. These porphyrins present a better activation of the ring, since the halogens act as removers of electronic density of the ring [18]. Chlorins, which are essentially reduced porphyrins derived from chlorophyll bacteria fetophorides, stable derivatives of chlorophyll varieties that are found in bacteria, are related to porphyrins and are simple to produce. Phthalocyanines and naphthalocyanines, which are derived from azaporphyrin, have high stability, and selectivity [36].

On the other hand, light absorption capacity is an important factor, since most tissues present a comparatively low absorption in the spectral range that extends from 500 nm to about 1500 nm (**Table 1**). This wavelength range is popularly known as the therapeutic window or the diagnostic window (**Figure 4**) [26, 61].

Tissue	**Wavelength (nm)**				
	630	**632.8**	**675**	**780**	**835**
		Optical penetration depth (mm)			
Blood		0.19	0.28	0.42	0.51
Mammary tissue		2.59	2.87	3.12	3.57
Brain (postmortem)		0.92	1.38	2.17	2.52
Brain	1.6				
Lung		0.81	1.09	1.86	2.47

Table 1.
Capacity penetration (mm) of light in different tissues. Adapted from [60].

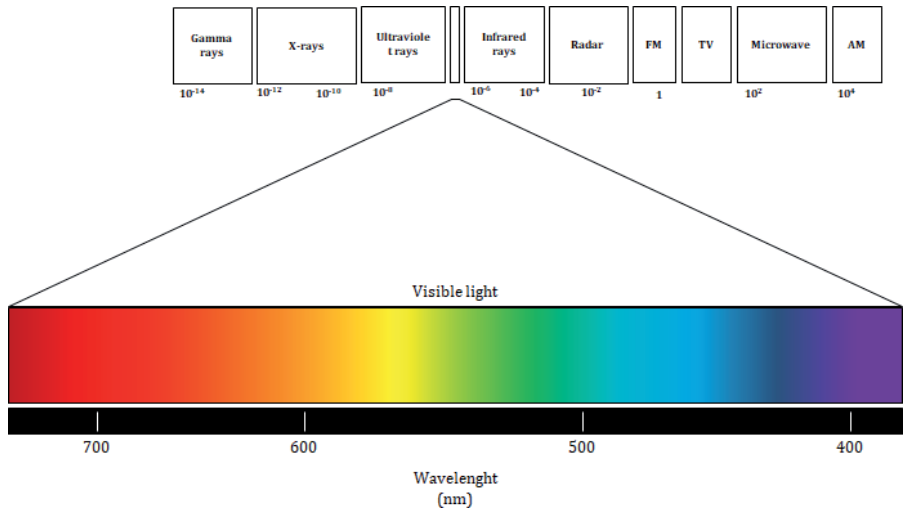

Figure 4.
Electromagnetic spectrum and their respective wavelengths in the region of visible light as a function of the different types of LASERs.

5. Concluding remarks

According to the theoretical bases discussed here, PDT deserves a more central position in the treatment of infectious diseases, since several studies report its enormous potential and applicability on several fronts.

Bacteriophages can act for delivering antimicrobial agents into bacteria, which consist in a potential and efficient alternative for the treatment of infectious diseases.

Delivery systems based on a phage-carrying PS exhibit increased effective killing by the concentrated fluence at the bacterial cell wall, and consequently, reduced side damage to the indigenous microbiota by the site singlet oxygen.

The discovery of new PSs and formulations based on nano structures, in addition to the use in conjunction with already established protocols, PDT shows itself as a strong alternative to conventional treatments.

Conflict of interest

The authors declare no conflict of interest.

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