The fight against microbial growth "in the light" of photodynamic therapy

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ABSTRACT
With the emergence of several strains of antibiotic-resistant bacteria, there has been a demand for new therapeutic alternatives to combat the growth of microbial pathogens. PACT (Photodynamic Antimicrobial Chemotherapy) is a therapeutic modality, inspired by the treatment of tumors, that aims to kill microorganisms by photodamage, by employing the combination of a photosensitizer and light irradiation, usually of the wavelength of visible light. Thus, PACT can be applied in different contexts, with Dentistry as one of the greatest applications, but PACT can be used in the treatment of skin infections and even disinfection of surfaces in hospital and industrial environments. This paper addresses historical and technical aspects of PACT, in terms of mechanism of action, sensitive microorganisms and future perspectives. This document is

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INTRODUCTION

The use of light in medicine is dated to records that are more than 4,000 years old. In different parts of the world, such as Egypt, India, and Greece, the use of sunlight was already known in the treatment of numerous conditions, for example psoriasis and vitiligo (Silva *et al*., 2018), as well as in the process of microbiological treatment of drinking water, based on religious precepts (Azevedo Netto, 1959). Since antibiotic therapy, society's quality of life and life expectancy have changed substantially, however, the use and abuse of antibiotics over the decades has allowed the emergence and dissemination of resistant pathogens that pose a great threat to health (Aslam *et al*., 2018).

Thus, new strategies are proposed and the use of light to combat microbial growth has emerged as a potential solution to the problem. PACT, the universal acronym for *Photodynamic Antimicrobial Chemotherapy*, is a simple technique that is based on three pillars: a compound, natural or synthetic, that is sensitized by light irradiation and will form free radicals with molecular oxygen, resulting in the death of the microorganism (Derikvand *et al*., 2020).

The technique is safe and can be applied against bacteria, fungi, viruses and parasites. In this work, advantages, applications, mechanism of action and a brief history of PACT are presented. In addition, the perspectives concerning photosensitizers are presented, as well as the types of light irradiation for each treatment.

HISTORY OF PACT

Based on the premise defined in antiquity about the use of light in medicine, some therapies evolved by associating exposure to sunlight with the consumption of plants, observing that the therapeutic effect was due to the participation of plant pigments activated by light (Silva *et al*., 2018).

During the so-called golden age of microbiology (1860-1930) the idea of cell death stimulated by the interaction of chemicals and light was reported. Oscar Raab was the first to divulge the subject, when he occasionally discovered the lethal action of the combination of acridine red and light on protozoa that cause malaria. A lower lethality was observed on rainy days, while the opposite was seen during bright days. Their conclusion was that the dye was activated by some fluorescence product, postulating a mechanism involving the transfer of energy from light to the chemical compound, similar to what occurs in photosynthesis (Rossin *et al.*, 2020).

In the year 1900, eosin was administered parenterally in a treatment against epilepsy, and dermatitis was observed in the skin regions when exposed to the sun. This observation led to the first application in human medicine of the interaction between light and a fluorescent compound. Herman von Tappeiner and Jesionek tested the application of eosin and exposure to white light for the treatment of skin tumors. In this study, the participation of oxygen in these photosensitization reactions was demonstrated and seven years later the phenomenon was described as "photodynamic action" (Correia *et al*., 2021).

In the following years, much was experimented in the attempt to understand photosensitization, but it was only in the 1960s that studies deepened and PACT was consolidated as a therapy, applied in different treatments, especially local, mucosal or skin infections, as well as widely explored in Dentistry (Takasaki *et al*., 2009). At this time, there was an understanding that the photodynamic action or effect was possible if the light irradiation presents an emission of adequate wavelength with the absorption spectrum of the photosensitizer (dye), in the presence of molecular oxygen (Simões *et al*., 2018).

APPLICATIONS OF PHOTODYNAMIC ANTIMICROBIAL THERAPY (PACT)

PACT is a promising strategy to confront, above all, bacterial resistance to antibiotics, but the generation of reactive oxygen species brings to light different therapeutic possibilities, whose results are promising.

SKIN INFECTIONS

PACT can be used to treat acne, seborrheic dermatitis, and infected wounds. The photosensitizer is applied to the affected area, followed by exposure to light irradiation. Important results have been achieved in *in vitro* and *in vivo assays* against *Staphylococcus aureus* (Karner *et al*., 2020). In *in vivo* models, PACT has also demonstrated an acceleration in the healing process of wounds, ulcers, and infections caused by bacteria. Additionally, the healing process was accelerated because the antimicrobial activity demonstrated by PACT also prevented the development of secondary infections (Sun *et al*., 2020).

ORAL INFECTIONS

Dentistry is the area that benefits the most from PACT. The most commonly used photosensitizers are methylene blue and toluidine blue. Animal models have shown a reduction in the progression of periodontitis, in radiographic terms, with a reduction in bone loss, as well as in histological terms, with a decrease in the extent of the inflamed area (Kikuchi *et al*., 2015). In addition to bacterial periodontitis, PACT can be applied in the treatment of bacterial gingivitis, as well as fungal and viral infections. In the latter, the treatment of cold sores was associated with administration with acyclovir (Gholami *et al*., 2022).

EYE INFECTIONS

In ophthalmology, PACT can be applied to treat bacterial, fungal and viral infections, but more investigations are conducted in *Pseudomonas aeruginosa infections.* The photosensitizer used is rose bengal, whose bactericidal action is against clinical isolates of *P. aeruginosa* (Durkee *et al.*, 2020). The dye has also been used in the laser treatment of bacterial keratitis caused by *Staphylococcus aureus* because, as an advantage of the technique, there is no selectivity for the infectious agent (Paiva *et al*., 2022).

RESPIRATORY INFECTIONS

Important results have been achieved in the therapy of sinusitis and bronchitis, as well as respiratory infections in patients with cystic fibrosis, especially caused by antibiotic-resistant bacteria. *In vitro* assays using methylene blue and toluidine blue and laser irradiation showed a significant reduction in the number of clinical isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa.* The study and others like it suggest that PACT mediated by these photosensitizers represents a promising approach in the treatment of infections with multidrug-resistant respiratory pathogens (Kashef; Yahyaei, 2014).

CANCER TREATMENT

The photosensitizer is administered to the patient, and then the cancer-affected area is exposed to light irradiation, leading to selective destruction of the cancer cells. PACT has been employed as a primary or adjuvant treatment for some cancers, e.g., skin, lung, esophagus, and thyroid cancers (Kim *et al*, 2018).

COVID-19

During the most critical period of the pandemic (2020-2022), PACT was also investigated as a possible form of treatment. In this case, PACT was combined with photobiomodulation (PBMT), a technique that allows the reduction of oxidative stress through the interaction of light with biological tissues. Methylene blue-mediated PACT was evaluated in oral lesions caused by SARS-Cov-2, with laser application to the injured area. This allowed relief from painful symptoms and rapid healing in patients (Pacheco *et al.,* 2022).

SURFACE DECONTAMINATION

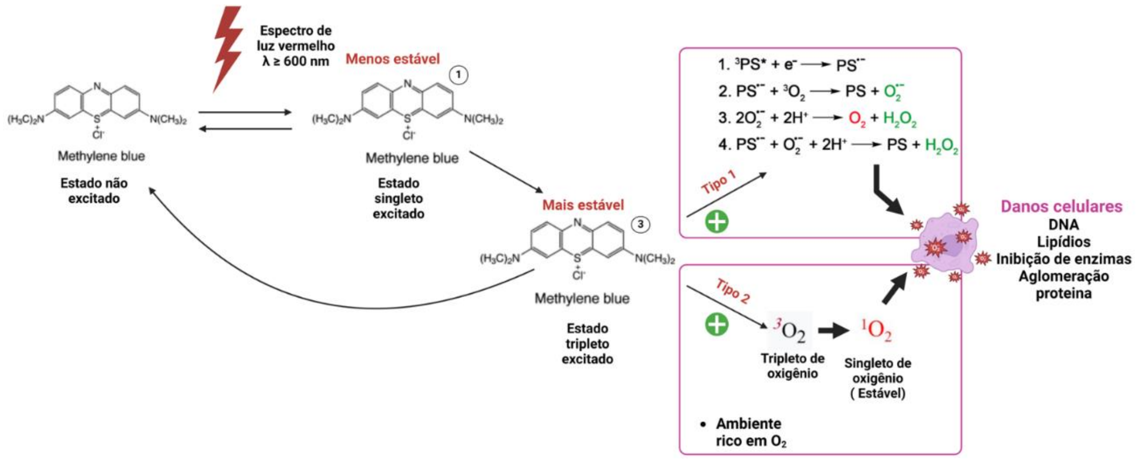
In addition to the treatment of infections in humans and animals, PACT also has applications in the decontamination of surfaces in hospitals, laboratories and other facilities whose disinfection is crucial to prevent the spread of pathogenic microorganisms. Applied to solid surfaces, water, and air, methylene blue-mediated PACT has been shown to be effective, safe, and low-cost (Almeida *et al*., 2020).

PACT MECHANISM OF ACTION

PACT activity is based on the combination of a low toxicity photosensitizer and an appropriate wavelength of light, which in the presence of oxygen in the medium, is activated and generates reactive oxygen species. The phototoxic response produced causes damage to biomolecules and/or oxidation of cellular structures, leading to death (You; Li, 2017).

There are two types of mechanisms in PACT. In both types, the photosensitizers enter the single excited state (type I mechanism), but due to the atomic stability of the molecule, there may be conversion from the single excitation state to the triplet excitation state (type II mechanism). In the type I mechanism, hydrogen is abstracted with the production of free radicals and radical ions, which are highly reactive with molecular oxygen, generating reactive oxygen species, such as superoxide anions or hydroxyl radicals. As a result, oxidative damage is produced in the cells. In the type II mechanism, the reaction with molecular oxygen generates singlet oxygen, which, being extremely reactive, can interact with the cell and produce oxidative damage. The type II mechanism is more common, however, the type I mechanism is important under low oxygen concentrations (Carrera *et al*., 2016). Figure 1 summarizes the process involving the two mechanisms.

Figure 1 – Mechanisms of formation of reactive oxygen species in Photodynamic Antimicrobial Chemotherapy. In the type I mechanism there is excitation formation in the single state, while in the type II mechanism there is singlet oxygen formation.

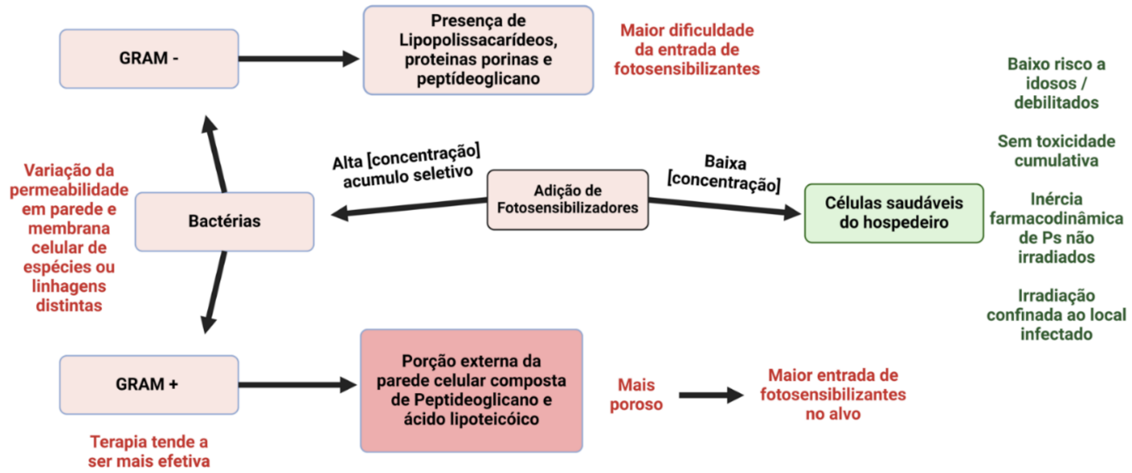


Source: authors

PACT is more effective at inactivating Gram-positive bacteria because the cell wall is thicker, composed of 90% peptidoglycan in addition to lipoteichoic acid. In addition, the porosity of the wall allows the passage and accumulation of the photosensitizer to the plasma membrane (Huang *et al*., 2012). On the other hand, the morphology of Gram-negative bacteria hinders the penetration of the photosensitizer. However, lipopolysaccharides, lipoproteins, and proteins with porin function end up being target molecules (Biyiklioglu *et al*., 2019), as shown in Figure 2.

Different molecules present in microorganisms are targets of PACT. Reactive oxygen species can induce structural changes in nucleic acids, such as DNA-protein cross-linking and single or double-strand breaks, however, it is suggested that DNA damage may not be the main cause of cell death (Sabino *et al.* 2023).

Figure 2 – Influence of cell constitution on the accumulation of photosensitizers.



Source: authors

On the other hand, significant oxidative damage can be observed in lipids, causing lipid peroxidation and plasma membrane disruption (Hu *et al*., 2018). This promotes increased permeability and loss of membrane integrity, resulting in death (Alves *et al*., 2013). Proteins, on the other hand, can be oxidized, leading to the inhibition of enzyme systems, as well as agglutination of critical proteins (Oliveira Silva *et al*., 2024). In this sense, PACT can also be effective against the formation of biofilm by the presence of polymers. However, biofilm is a more resistant structure and the different phenotypes found inside it hinder the action of reactive oxygen species (Melo *et al*., 2013).

PHOTOSENSITIZERS

Regardless of the mechanism of action of PACT, its efficacy of PACT will depend on the interaction of the photosensitizer and the target cell. The most important factors involved in this interaction are photosensitizer concentration, as well as hydrophobicity and light absorption characteristics (Biyiklioglu *et al*., 2019). On the other hand, of the target cell, the main factors are the morphology and cellular constituents (Figueiredo-Godoi *et al*. 2022).

New PACT models search for molecules with potential photosensitizing activity studies in the field of photodynamic therapy. In addition to the property of generating free radicals, the photosensitizer needs to show extra activities such as antioxidant activity, recruitment of immune cells, and action in more than one apoptosis induction pathway (Polat; Kang, 2021).

NATURAL COMPOUNDS

There are two classes of photosensitizers. Natural ones have the advantage of being biodegradable and sustainable. In the search for these substances with varied bioactive profiles, there is a direction in the prospection of drugs of natural origin since they offer a safer and less toxic alternative compared to traditional synthetic photosensitizers. Chart 1 presents a selection of some photosensitizers of natural origin, highlighting their PACT applications in the fight against microorganisms. The summarized examples were chosen based on clinical studies, promoting potential entry into the future pharmaceutical market, with special attention to cancer chemotherapy.

Table 1 – Natural photosensitizers

Molecule	**Fonte natural**	**λmax nm**	**Activity**	**Reference**
Curcumina	*C. Long*	405-547	*S. mutans, L. acidophilus, L. monocytogenes, Salmonella* sp., *P. gingivalis, A. actinomycetemcomitans, S. pyogenes, E. coli, Pseudomonas* sp., *A. baumannii, E. faecalis, C. albicans*	Silva, Vasconcelos (2021) Polat et al. (2021) Youf et al. (2021)
Ys. amniolyco	Endogenous amino acid	630	*B. cereus* (MDR), *E. faecalis* (MDR), *L. monocytogenes, S. aureus* (MDR e MRSA), *S. epidermis* (MDR), *S. faecalis* (MDR), *M. marinum, M. smegmatis, M. phlei, A. baumanni (MDR), A. hydrophilia (MDR), E. coli (MDR), H. pylori, P. acnes, P. aeruginosa (MDR), S. enterica*	Felicio et al. (2008) Harris et al. (2012) Shinoda et al. (2021) Youf et al. (2021)
Hypericin	*H. perforatum*	593	*S. aureus, E. coli, E. faecalis*	Kashef et al. (2013) Dong et al. (2020)
Ác. cynamine	*Cinnamomum* sp.	262-270	*E. coli*	Ferenc et al. (2010) Oliveira et al. (2021)
Ác. Gallic	Chestnut, plum, mango and grapes	320	*S. aureus, E. coli*	Dechsri et al. (2024)
Resveratrol	*Vitis* sp.	307-321	*S. aureus*	Zhang et al. (2019)
Ác. Ferulic	Rice, soybeans, apples, bananas and oranges	215-328	*E. coli*	Shirai; Automotive (2019)

Aloe-Emodin	*A. vera*	370-500	*A. baumannii, E. faecalis,* (M D R) *S. aureus, S. pneumoniae*	Wang et a. (2021) Otieno et al. (2021).
Quercetina	Onion, red wine, fruit	577	*E. coli, L. monocytogenes*	Lee et al. (2023)
Chrysanthemin	Rye and Peach Tree	550	*P. gingivalis*	Teerakapong et al. (2017)
Silibinina	*S. marianum*	550nm	*E. coli*	Lee; Lee (2017)
Polifenol do chá verde	*C. sinensis*	274	*S. aureus* (MDR), *E. coli* (MDR)	Hu et al. (2019)

MDR= Multi drug resistent/Multi resistant to antibiotics
Source: authors

SYNTHETIC COMPOUNDS

The synthetic compounds applied in PACT are classified as first to third generation, according to the structural modifications made to the molecule in order to optimize the properties, with a reduction in adverse effects (Kwiatkowski *et al*, 2018). First-generation photosensitizers today are more limited due to low chemical purity, poor tissue penetration, and hypersensitivity reactions (Gunaydin et al, 2021). In this context, second-generation patients are more selective (Zhang *et al.*, 2018) and third-generation carriers seek greater selectivity through carrier systems such as nanosystems (Mfouo-Tynga *et al*, 2021).

At PACT, in addition to natural bioactives, photosensitizers are classified into three more families, according to the origin and structure of the molecule. Thus, the molecules are classified as synthetic dyes, tetrapyrrole structures, and nanostructure systems (Ghorbani *et al*, 2018). Table 2 summarizes the main molecules.

The group of synthetic dyes is part of the class of first-generation photosensitizers studied using PACT. Because they have a cationic charge in their molecules, they exhibit a high affinity rate in the wall of Gram-negative and Gram-positive bacteria, making them still widely used (Abrahamse; Hamblin, 2016).

Table 2 – Synthetic photosensitizers

Family	classes	Activity	Reference
Tertrapirrol	Porphyrins, phthalocyanins	*P. acnes*, *P. gingivalis*, *Prevotella* sp., *A. actinomycetemcomitans*, *P. aeruginosa*	Chen et al. (2022) Abrahamize; Hamblin (2016)
Synthetic corantes	Phenotizines (methylene blue and derivatives, toluidine blue); Xanthenes (Rose Bengal, Fluorescein)	*S. aureus* MDR, *K. pneumoniae* MDR, *Candida* sp., SARS-CoV 2, *P. aeruginosa*, *N. keratitis*	Chakraborty et al (2024) Adre et al, (2022) Halili et al. (2016)
Third generation	Nanosystems with methylene blue, borodipyrromethane nanoparticles,	*C. thracomatis*, *S. aureus*, *E. coli*	Qi et al. (2023) Wang et al. (2022)

MDR= Multi drug resistent/Multi resistant to antibiotics
Source: authors

Tetrapyrrole structures have the property of producing, for the most part, type II singlet oxygen, but the classes included in this group differ in relation to the region of light absorption. Phthalocyanines have an absorption band in the 670 nm region while porphyrins are active in the 400 nm up to 630 nm region (Kou *et al*, 2017).

LIGHT SOURCES IN ANTIMICROBIAL PHOTODYNAMIC THERAPY (PACT)

The correct definition of the light source is crucial for the efficiency of the PACT as well as for the minimization of harmful effects. For this, some requirements are considered, namely: i) knowing the absorption spectrum of the chosen photosensitive molecule; ii) accurately assess the characteristics of the area to be treated; iii) establish the intensity of light necessary to activate the photosensitizer applied; iv) look for sources that emit light uniformly; and, v) consider the structural, financial and human resources issues of the place where the light source will be used. In this context, the main light sources used in PACT are LASER (*Light Amplification by Stimulated Emission of Radiation*), LED (*Light-Emitting Diode*) lamps and sunlight (Piksa *et al*., 2023).

The LASER is the most widely used light source because it is considered more versatile, having been used since 1960. The structure of the LASER is made up of three main parts: the active medium, which can be solid, liquid or gaseous, and contains the electrons that are stimulated. The second part is the energy source, responsible for providing photons that stimulate the electrons of the active medium; and finally a pair of mirrors arranged on opposite sides, responsible for reflecting the photons released in the process, causing the system to be retrostimulated, allowing only a small percentage of the light to escape (Williams, 2008).

This results in a directed, monochromatic type of light, i.e., with a single wavelength and with high intensity, capable of exciting photosensitizers without affecting adjacent tissues or areas (Piksa *et al*., 2023). The main types of LASERS used in PACT are argon ion, Nd:YAG (neodymiumdoped yttrium-aluminum-garnet), diode and metal vapor pumped dye lasers (Brancaleon; Moseley, 2002; Mang, 2004; Stájer *et al*., 2020). The main disadvantage is the cost of acquisition and maintenance, followed by the small area of reach, however, it becomes advantageous in the application in Dentistry (Asnaashari; Safavi, 2013; Theodoro *et al*., 2021; Rathod *et al*., 2022, Wawrzyk *et al*., 2021; Ying *et al*., 2023).

LED lamps, on the other hand, overcome the limitations of using LASER. They were developed in 1962 and only in the 1990s did they begin to be applied in PACT (Opel *et al*., 2015; Palucka, 2012). LED bulbs are produced with high-efficiency semiconductor materials that produce light when electricity passes through them (Jagdeo *et al*., 2018). The centrality of an LED is the P-N junction, formed by a combination of two types of semiconductors: the P-type (positive) and the N-type (negative). The most commonly used materials are InGaN, AlGaInP, AlGaAs, and GaP; and the emission wavelength can vary from the ultraviolet to the infrared region, depending on the material (Brancaleon; Moseley, 2002; Prasad *et al*., 2020).

In addition, LED technology is very effective, as electrical energy is transformed into light energy with low thermal energy production (Hasenleitner; Plaetzer, 2019). Because of this, LED lamps have high durability, intense brightness and, compared to other light sources, low cost (Pereira; Carvelli, 2018). In addition, LED irradiation is more appropriate for deeper lesions (Doix *et al*., 2018).

Sunlight is most commonly used in disinfection (Amichai et al., 2014). It has a wide spectral range ranging from ultraviolet to infrared, allowing the use of different photosensitizers, with ultraviolet being the one with the best results (Leanse *et al.*, 2023)tag. However, it can result in a therapy with a low degree of control, being less recommended, although it is applied for wound healing, reduction of microbial contamination, and disinfection of household items (String *et al*., 2023).

As an alternative, systems that are less efficient than LED light sources and safer than sun exposure, other irradiation sources are proposed, such as tungsten filament, xenon arc, metal halide, sodium and fluorescent lamps. These fonts are low-cost and easy to handle compared to LASER. Another positive aspect is related to the use in large areas, without the need for coupling to fibers (Brancaleon; Moseley, 2002).

CONCLUSION

PACT presents itself as a good alternative to antimicrobial therapy, especially at a time when microbial resistance is a global threat and new strategies are mandatory in the fight against pathogens. Much of the photodynamic therapy is based on anti-cancer treatment and the optimization of molecules, as well as the search for natural bioactives in a context of sustainability, place PACT at a potentially important level, whether in the treatment of infections or in the disinfection of surfaces, industrial, hospital and home environments.

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