Science and Survival. A focus on SARS-CoV-2 and connections between large-scale risks for life on this planet and opportunities of science to address them

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Photodynamic Therapy in the Context of Covid-19

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1. Introduction

The first reports of a coronavirus animal infection belong to the 1920-1930 decade, when an acute respiratory infection of domesticated chickens emerged in North America.[1] Since then several coronaviruses have been identified and isolated from animals and the first found in humans occurred in the 1960s.[2] Other human coronaviruses were identified in the following decades, including the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), causing the infection named COVID-19 in 2020.[3] The name "coronavirus" derived from the Latin "corona" which means "crown" was introduced by J. Almeida and Tyrrel after their findings that the virus has "*a characteristic 'fringe' of projections 200 A long, which are rounded or petal shaped ... This appearance, recalling the solar corona, is shared by mouse hepatitis virus and several viruses recently recovered from man, namely strain B814, 229E ...*". [4] The name appeared after observation of the infective form of the virus (virions) by electron microscopy, which showed a fringe of large, bulbous surface projections similar to solar corona. COVID-19 has been characterized not only by respiratory infections but also by severe inflammation and damage to endothelial cells in the heart, kidneys, liver and intestines, which points to a strong vascular infection.[5] It is known that the SARS-CoV-2 is a RNA virus enclosed in a bilayer lipid envelope embedded with a number of protein molecules that protect the virus when it is outside the host cell.

These structural features may be one of the main reasons for the high societal human contamination that originated the actual pandemic situation. It is still not clear how and when SARS-CoV-2 was introduced to humans but it is likely to have occurred at Wuhan, Hubei, China in late December 2019, where an outbreak of a mysterious pneumonia characterized by fever, dry cough and fatigue, and occasionally gastrointestinal symptoms occurred.[7] The disease, named as coronavirus disease 2019 (COVID-19), rapidly spread to nearby cities and other countries.[8] Then the pathogen of the outbreak was identified and also classified as SARS-CoV-2.[8] Later, on January 30, 2020, the World Health Organization (WHO) declared it a Public Health Emergency. On March 11, 2020, COVID-19 was classified by WHO as a pandemic. Although the case fatality rate of COVID-19 (estimated at 2%-3%) is lower than those of SARS (approximately 10%) and MERS (approximately 40%), the pandemic associated with COVID-19 has been far more severe[9] and represents a huge challenge for governments, individuals, and society as a whole. COVID-19 has a mean incubation period of 5.2 days (95% confidence interval, 4.1-7.0).[8] Symptoms usually begin with nonspecific syndromes, including fever, dry cough, and fatigue. Multiple systems may be involved, including respiratory (cough, short of breath, sore throat, rhinorrhea, hemoptysis, and chest pain), gastrointestinal (diarrhea, nausea, and vomiting), musculoskeletal (muscle ache), and neurologic (headache or confusion).[8] These symptoms can be present between 6 to 41 days. Currently, there is no validated treatment for COVID-19; however, many strategies are used such as symptomatic and supportive care: keeping vital signs, maintaining oxygen saturation and blood pressure, and treating complications, such as secondary infections or organ failure.[8]

Current epidemiological studies estimate that each infection will give between 1.4 to 3.9 new ones if no members of the community are immune and no preventive measures are taken. Therefore, in alignment with WHO recommendations, we consider that there are three major challenges to combat the COVID-19 pandemic, namely, prevention, vaccine development and early destruction of viral load. It is scientifically accepted that the best way to prevent illness is to prevent contact with this virus once it spreads mainly from person to person who

are in close contact, through respiratory droplets (aerosols) produced by infected people coughing or talking, even if they are asymptomatic.

Regarding prevention, everyone should ***Clean and Disinfect*** by washing hands very often with soap and water (20 seconds) or with alcoholic solutions particularly after being in a public place, before eating, before touching the face and after blowing the nose, coughing or sneezing. Everyone should ***Avoid Close Contact*** with sick people inside and outside the home. Physically distancing is particularly important for people who are of higher risk (70 years old and with clinical pathologies). ***Covering the Mouth and Nose*** with a mask inside and outside when people are around others is also very important, since we can spread COVID-19 to others even if we do not feel sick. Finally, to be alert for symptoms of COVID-19, everyone should ***Monitor Health Daily*** namely monitor temperature, cough and shortness of breath. In respect to production of immunologic response, the vaccine is currently considered as the main tool to solve the current pandemic crisis. Therefore, scientists and politicians are devoting great efforts and funds to try to solve the problem. There are approximately 200 vaccines in development, 24 of which are already in clinical trials. But, when it becomes available, there are great concerns about the possible secondary effects of the COVID-19 vaccine, the immunologic response period, and worldwide spread availability including low-income countries. To make this possible it is crucial that both governments and pharmaceutical companies work together in such endeavor.

Regarding the early destruction of the viral load, we perceive that the development of new techniques for virus inactivation in the nasal tract, to prevent the subsequent development of pneumonia and/or multi-resistant bacteria in the endotracheal tubes of ventilated patients, may be crucial to reduce the number of deaths. From the experience of our multinational and multidisciplinary research team in inactivating bacteria and viruses, we believe that photodynamic therapy may provide a rapid and effective response to the COVID-19 pandemic in the near future. In this chapter, we describe the basic concepts about the technique of photodynamic therapy and its recent advances in the inactivation of viruses and bacteria, particularly those that cause respiratory infections.

2. COVID-19 pandemic: Photodynamic therapy as a potential solution

Societies worldwide are facing enormous challenges in dealing with the ongoing global COVID-19 pandemic. But it is not just society: science also faces some similarly dispiriting situations. This sense of uncertainty derives not just from this current pandemic, but the prospect of many others in the near future, associated with as yet unknown microorganisms or vectors. Furthermore, science is already struggling to deal with infections caused by multi-drug-resistant microorganisms, which are becoming increasingly difficult to treat. The moment calls for innovative techniques and alternative methods for promoting microbiological control while also achieving high efficiency and affordability.

It is worth noting that respiratory tract infections, such as pneumonia, caused by SARS-CoV-2 and/or those associated with fungi and bacteria (mainly *Streptococcus pneumoniae* and *Staphylococcus aureus*), have higher mortality rates among patients infected with COVID-19.[10] Patients diagnosed with pneumonia are typically treated with antibiotics. However, the rise in antibiotic microbiological resistance (AMR) is now a public health concern worldwide, and it is now regarded as essential to urgently develop alternative treatments to antibiotics. As previously mentioned, a promising alternative or auxiliary treatment for pneumonia is photodynamic therapy (PDT), a non-invasive treatment that can be employed in localized cancer treatment and for infected tissues/organs. Due to its antimicrobial effect, PDT is also known as antimicrobial photodynamic therapy (aPDT).

PDT was first described in 1900 by Oscar Raab,[11] and is based on the use of a photosensitizer that preferably accumulates in pathogens. The photosensitizer is then activated by light of a specific wavelength and, in the presence of molecular oxygen, generates reactive species that are toxic to the target.[12] aPDT is of particular interest for treating infections because there is no evidence of cross-resistance with antibiotics, and the response restriction of light exposure at the target tissue offers a further degree of selectivity.[13] Moreover, unlike target-specific antibiotics, aPDT can damage a variety of molecules and so the emergence of resistance to this treatment among previously susceptible strains is described as highly unlikely.[14] Additionally, aPDT has a broad spectrum of microorganism action and is effective against bacteria, fungi, viruses, and protozoa.

PDT is based on three components: light, a photosensitizer, and oxygen. The photodynamic process starts with electron transfer to a higher-energy orbital following the absorption of light.[14] The excited photosensitizer molecule may dissipate this energy by emitting fluorescence, or else go through intersystem crossing to a triplet state.[13b] From there, the energy can be dissipated non-radioactively, or generate toxicity through either of two mechanisms: in the type I reaction, the photosensitizer reacts with adjacent organic molecules and forms reactive oxygen species (ROS); in the type II reaction, there is energy transfer to the molecular oxygen, which is a triplet in its basal state ($^3O_2$), and it goes to a singlet state ($^1O_2$).[13b] Both reactions contribute to the phototoxic effect, and both singlet oxygen and the ROS have short half-lives, thereby restricting their diffusion

and therefore limiting the effect of the PDT to the site of light exposure.[14] This mechanism is illustrated in the Jablonski diagram.

For effective aPDT, light propagation within the infected tissue must be sufficient to ensure that all present pathogens are irradiated with a threshold dose, thereby achieving the photodynamic response. For infections in superficial tissues or decontamination of cavity sites such as nostrils, oral cavity, and pharynx, aPDT can be performed relatively simply through topical application of the photosensitizer and direct illumination of the target tissues. In these cases, photosensitizers with absorbance in the blue-green spectral interval are attractive, since light penetration of the tissues is minimized.

When considering application to internal organs such as in lung infections, it must be ensured that sufficient energy dose reaches the photosensitized pathogens, despite scattering and absorption by the interface layers of tissue between the light source and the target infection site.[12] Therefore, the choice of wavelength should take into account the depth of light penetration, the absorbance spectrum of the photosensitizer, and the location of the infectious agents.[12] Light excitation within the range 650-1000 nm is advantageous because it penetrates deeper into biological tissue, since it is less absorbed by water, melanin, and hemoglobin.[15] Consequently, this range is known as the “optical window” or the “therapeutic window” for photodynamic therapy. However, most photosensitizers do not absorb wavelengths greater than 800 nm, so the usual excitation sources have peaks that range from 650 nm to 850 nm.[15-16]

COVID-19 is a highly complex viral infection. Due to the unpredictability of the infection progression and also the current lack of a vaccine and specific and effective therapies, the best strategy is still to avoid SARS-CoV-2 contamination, minimize infection severity, and prevent related infections. Considering these aspects, aPDT has high potential to contribute to the treatment of COVID-19 patients.

3. Photodynamic therapy for upper respiratory tract

Viral infections are variable in type and, as such, current experimental and clinical applications for treatments are in continuous development. Most antiviral PDT protocols are for the treatment of skin infections and are performed on small areas. Antiviral PDT is still at the early stages of development for clinical treatments, and only a few protocols are applied to non-superficial tissues; however, due to its antiviral response, there is scientific and clinical interest in its application to extensive and deep-infected areas of the body. PDT has successfully treated some localized infections of the upper airway tract, such as pharyngotonsillitis[17] and sinusitis[18] caused by influenza virus, rhinovirus, coronavirus, and enterovirus, the main virus families involved in these pathologies.[19] The PDT antimicrobial effect for virus inactivation relies on the photosensitizer interaction with the viral structures and further infected tissue irradiation.[20] Viral inactivation can occur with the destruction of the viral nucleocapsid, its proteins or lipid envelope, and also of its single-strand RNA. One of the main advantages of antimicrobial PDT is its localized action: during PDT of the respiratory tract, mucosal lighting is controlled, avoiding adverse effects from vascular changes with vasodilation.[21]

The viral load of an infected person is expressed by the quantity of virus per volume of fluid. The number of viral particles per milliliter, sputum samples, nasopharynx smears, or even tracheal aspirates of bronchoalveolar lavage can be evaluated by quantifying the occurrences of known genomes in these sample obtained from the upper and lower air tract.[22] The maximum viral load can be estimated from the proportion of known genome sequences in the obtained samples. However, the accuracy of such estimates is clearly dependent on the quality of clinical samples collected from specific anatomical sites of pulmonary infections.

Knowledge of viral load during the disease course is essential for estimating the infectious period and for defining guidelines on the duration and monitoring of therapies.[23] Viral load reflects a virus’s active replication, which is used to monitor the clinical progress of a respiratory viral infection and its response to therapy, regarding cure or relapse.[23] Viral load can have different implications for an infection’s progression, with higher viral load usually associated with more severe infection and higher virus transmission. In the case of SARS-CoV-2, large variations have been observed in patient viral load, infection progression, and clinical symptoms. In SARS-CoV and SARS-CoV-2, viral loads in the upper respiratory tract are initially higher in the nose than in the throat, reaching peaks of 10 and 1, respectively, three days after onset. Viral loads in asymptomatic patients are similar to those in symptomatic patients, suggesting similar suggesting similar transmissibility.

Antiviral PDT is presented as an attractive auxiliary technique to reduce SARS-Cov-2 viral load in the upper respiratory tract and, consequently, to minimize infection severity and viral transmission. Topical application of photosensitizer solution at the nostrils, and photosensitizer delivery through a maximum viral elimination can occur in the first days after the onset of symptoms and then present a shorter or less intensive period of infection, dependent on the pathology and viral replication location.[24] The evaluation of these data is dependent on adequate clinical samples for maximizing acquisition of the viral genome, which defines the

ideal treatment follow-up.[25] These conditions in the disease's progression can change from fulminant to recoverable, depending on reducing the viral load located with the treatments such as PDT. Therefore, for scientific evidence of efficacy, detailed molecular studies of viral treatment by PDT are crucially dependent on monitoring viral load from clinical samples with adequate amounts of intact viral nucleic acid to enable sequence analysis at specific respiratory tract locations (superior and inferior). In addition to viral load, the presence of the viral envelope, be it double-stranded or single-stranded DNA or RNA, can affect the efficiency of photosensitizer targeting and thus the effectiveness of aPDT.

Considering the use of aPDT against COVID-19, treatment of the upper respiratory tract can be performed in patients with positive diagnosis, whether symptomatic or asymptomatic, aiming to reduce the SARS-Cov-2 viral load at the nostrils, oral cavity, pharynx, and tonsils. Reduction of viral load has the potential to minimize infection severity as well as viral dissemination by the patient. aPDT can also be performed among health professionals who have contact with patients, aiming to reduce the possibility of infection. aPDT can be simply performed through topical application of the photosensitizer, as a curcumin solution, and a few minutes later, the target cavity is illuminated using a fiberoptic light source or via direct illumination using a light emitting diode (LED) device customized to fit to the anatomical site.

4. Antimicrobial-coated endotracheal tube: An approach to avoid ventilator-associated pneumonia

An endotracheal tube (ETT) is a medical device comprising a tube connected to a mechanical ventilator, and is commonly used in intensive care unit (ICUs) when a patient requires mechanical ventilation. Many clinical scenarios may necessitate endotracheal intubation, for example obstructed airways, hypoventilation, unconsciousness (coma), surgery involving general anesthesia, pneumatic shock, muscle relaxation in surgery, polytrauma, respiratory failure, and the need for intermittent positive pressure breathing such as in pathologies of the respiratory system and cardiothoracic surgery. In the context of COVID-19, patients showing low oxygen saturation due to severe respiratory depletion may require mechanical ventilation.

Ventilator-associated pneumonia (VAP) is a frequent complication among ICU patients receiving mechanical ventilation.[26] Microaspiration and biofilm formation are the major factors in VAP implicated in the colonization of the tracheobronchial tree and lungs.[27] In this regard, ETT is considered one of the major risk factors for VAP, acting both as a reservoir for pathogenic microorganisms and as a bridge between the oropharyngeal environment and the bronchoalveolar space by bypassing host defenses.[28] For some years, there has been increased scientific interest in developing antimicrobial ETT coatings that prevent VAP by inhibiting biofilm formation in the lumen of ETTs.[29] These antimicrobial coatings are based on the use of biological (e.g., peptide) and/or chemical entities (e.g., metals, commercial antibiotics, and photosensitizers) on the ETT surface to inhibit biofilm formation and/or destroy biofilm present in the lumen of ETT.[29]

Among the antimicrobial coatings used to date, we highlight the use of photosensitizer molecules on ETT surface, which, in the presence of light of appropriate wavelength, produce ROS *via* photodynamic action that are able to combat biofilm formation in the lumen of ETT.

A functionalized ETT with photosensitizer and the aPDT applied to this medical device may play an important role in COVID-19 treatment by preventing secondary infections of an already compromised patient; furthermore, by reducing the global microorganism load, including SARS-Cov-2 and other nosocomial pathogens, there will be a decrease in environmental contamination at ICU and other sites, with correspondingly lower potential for infection of health professionals when manipulating the infected ETT.

5. Lower respiratory tract infections: Burden and treatment limitations

Acute lower respiratory infections (ALRIs) are the most common infectious cause of death worldwide, accounting for 13.1% of all deaths in children younger than 5 years and 4.4% of all deaths overall in 2016.[30] It is estimated that, during that year, there were more than 336 million ALRIs, leading to more than 65 million hospital admissions globally.[30] Over the past two decades, efforts have successfully reduced the number of deaths resulting from ALRIs.[30] However, in 2020, the COVID-19 pandemic is greatly increasing those numbers and overwhelming healthcare systems worldwide.[31]

The main pathogen associated with mortality in ALRIs is the Gram-positive bacterium *Streptococcus pneumoniae*, which caused more deaths than all other etiologies combined in 2016, despite the existence and widespread use of multiple vaccines.[32] The World Health Organization (WHO) recommends either co-trimoxazole or amoxicillin as first-choice antibiotics for pneumococcal pneumonia in children.[33] National guidelines for the treatment of adults and children vary greatly, but often include aminopenicillins, macrolides, and tetracycline.[33] However, drug resistance often leads to a treatment failure, and therefore the guidelines recommend that severe cases be treated with a combination of two, or sometimes three, different classes of antibiotics.[34] If a COVID-19 patient also presents with infection caused by a resistant microorganism, no existing therapeutic options are available.

aPDT offers a therapeutic option for potentially combatting AMR. The main advantage of utilizing aPDT against lung infections is its broad inactivation action, not specifically related to a single microorganism type. There is an extensive literature describing *in vitro* tests of aDPT against the microorganisms that cause pneumonia, employing different types of photosensitizers and light sources.[35] However, to date, only a few studies have tested aPDT *in vivo* against pneumonia.

Among the prior studies, our group (in Brazil and Canada) has been establishing aPDT protocols for the treatment of pneumonia. In *in vivo* studies of mice infected with *S. pneumoniae*, those that received a single aPDT session showed significantly lower pulmonary bacterial load and also significantly greater survival, compared to controls that did not receive treatment. The protocol employed instillation of indocyanine green (ICG) as PS and infrared light at 780 nm.[36]

This higher survival rate suggests that aPDT did not cause any significant lung damage. To confirm the safety of aPDT, the same protocol was applied to cell cultures that are present in the alveolar microenvironment: pulmonary epithelium, macrophages, and fibroblasts. The treatment was not associated with loss of viability in any of the strains tested. Another study also demonstrated the efficacy of aPDI (with ICG and 808 nm light source) against *S. aureus* in a murine model, further supporting the safety of pulmonary PDT.[37]

In order to improve the delivery of photosensitizers, studies were carried out to adapt pre-clinical treatment to situations more similar to clinical conditions. For this, nebulization was investigated as an alternative to instillation. Although nebulization requires higher doses of PS, instillation generates an unwanted accumulation of the PS in other body organs, such as the stomach. In addition, aspects of the distribution and elimination of ICG when delivered via the pulmonary route were studied. No evidence of lung or liver damage was observed in healthy mice, suggesting that there was no toxicity to the lungs. The protocol presented successfully inactivated two strains of S. aureus, while showing no cytotoxicity to three different mammalian cell lines. Moreover, mice that received the treatment recovered fully and 7 days later showed no signs of tissue damage or inflammation. The light doses proposed in this protocol can be achieved with extracorporeal illumination without limitations, in relatively short times and with complete safety with respect to damage to healthy host cells.

External illumination has been adopted as a good option for treating pneumonia. In addition to the studies already mentioned, other research groups have demonstrated the use of this irradiation method. External illumination combined with new PS utilizing nanotechnology was effective in mice infected with *P. aeruginosa*, using sequential treatment of 5 days using NIR laser (808 nm) and high irradiance (2 W/cm^2) to conjugate the photodynamic and photothermal effects.[38] *Ex vivo* experiments in porcine lungs have also demonstrated that nebulizers and light-diffusion aPDT offer great potential for treating pulmonary infections.[39]

In combination, these results suggest that aPDT combined with external illumination has excellent potential as an alternative route for treating pneumonia. The *in vitro* and *in vivo* results provide strong evidence that aPDT is capable of treating lung infections caused by different microorganisms through different mechanisms of photosensitizer delivery. Nebulization in particular has shown potential for delivering ideal photosensitizers within clinical settings, thereby enabling the beginning of the next stages of pulmonary PDT.

Before defining a clinical protocol for pulmonary aPDT, some challenges still need to be addressed. One of the main concerns is to avoid inflammation and damage to lung cells, thereby demanding highly selective photodynamic action only over pathogen cells or viral structures. Fortunately, microorganisms are more susceptible to photodynamic action than are mammalian cells. Selective action can be further achieved when establishing a customized protocol, taking into account the most appropriate photosensitizer displaying greater interaction with the target microorganism; appropriate drug–light interval, when low concentration of the photosensitizer is within the lung cells; and low fluence (energy dose), avoiding tissue damage. These protocol factors will be investigated in a porcine model to establish the safety and efficacy of aPDT for pulmonary applications.

5. Antimicrobial photodynamic therapy for inactivating HCV in donor lungs for transplantation

Recently, a new technique based on aPDT was proposed for the inactivation of hepatitis C virus in lungs for transplantation.[40] aPDT was applied during normothermic *ex vivo* lung perfusion (EVLP), not directly to the organ but rather to the perfusate (circulating preservation liquid). Methylene blue was used as a photosensitizer, and EVLP & aPDT was performed in HCV-positive porcine lungs for up to 9 hours. The results showed a relevant decrease in HCV viral load at the circulated perfusate measured by qPCR, and no infectivity of the treated perfusate. The non-infectivity response is likely related to unviable virions, which could be still detected by qPCR but did not infect the lung cells.

HCV is an enveloped virus with capsid structure similar to SARS-Cov-2. Even though there are still no reported data on aPDT inactivation efficiency for COVID-19, the results observed for HCV and other viruses support the idea of a highly probable photodynamic action against SARS-Cov-2.

6. Designing photosensitizers and nano-based systems for photodynamic therapy: Perspectives for the treatment of respiratory tract infections

The need to develop new strategies that do not promote antimicrobial resistance is becoming increasingly urgent.[41] In recent years, antimicrobial photodynamic inactivation has increasingly become regarded as a promising technique to treat microorganisms because of its potential to address the problem of antibiotic resistance. However, several technical challenges mean that PDT is still not a first option for the treatment of infections.[42]

Most clinically approved photosensitizers absorb within a region that has poor light penetration (between 400 and 700 nm). Therefore, most PDT treatments are limited to superficial applications unless introducing an optical fiber for direct illumination of the disease site. Other important limitations include the preparation of pharmaceutical formulations since most PS agents aggregate under physiological conditions, and the ability of PS to accumulate in the target tissue, both of which are necessary for PDT to be effective.[42] In recent years, a new generation of photosensitizers and novel nanoscale PS delivery systems have offered solutions to overcome these barriers, resulting in greater effectiveness of PDT. The use of nanoparticles (NPs) also enables the synergic combination of PDT with other treatments (such as photothermal therapy, chemotherapy, or immunotherapy).[43]>

A considerable number of photosensitizers (natural, semi-synthetic, synthetic) have been developed and studied for *in vitro*, *in vivo*, and clinical applications.[44] At present the photosensitizing drugs that are used for PDT are dominated by tetrapyrrolic macrocycles (porphyrins, chlorins, bacteriochlorins), phthalocyanines, xanthene derivatives, curcumin, metal complexes, and bodipy, phenothiazium, each of which have particular optical/chemical properties, advantages, and limitations.[45] In this regard, improvements in their chemical and photophysical properties (e.g., log P, pKa, selectivity, dark toxicity, light absorption) via synthetic strategies pave the way for development of more efficient photosensitizers.[46]

Historically, researchers have classified photosensitizers as first, second, or third generation according to advances in their properties, and have postulated some characteristics as the “ideals” for a photosensitizing molecule,[47]. From that perspective, organic synthesis that allows the modulation of photosensitizers’ structure–activity relationship has introduced new properties to photosensitizers, which maximize their functionality and provide more efficient and safer photodynamic protocols.

As previously described, research into the design of photosensitizers has significantly improved their photophysical, chemical, and biological properties. For instance, the commercially available methylene blue is a widely used photosensitizer because of its good performance, but it does have some limitations (e.g., low uptake due to its high water solubility).[48] To overcome this issue, novel methylene blue derivatives such as phenothiazinium have been synthesized, which have shown far superior efficacy against relevant biological targets and photodynamic action. The use of different metals has improved their efficiency in photodynamic protocols, for example Pd(II)-tetrapyrrolic derivatives (WST11), and Sn(IV) (Purlytin).[49] Analyzing the molecular structure of WST11, the presence of Pd(II) improved its stability and increased the intersystem-crossing rate. Furthermore, the presence of a non-essential metal enables establishing photosensitizer localization and quantification via techniques such as X-ray absorption spectroscopy (XAS), inductively coupled plasma-mass spectrometry (ICP-MS), and confocal microscopy (if the metal complexes are luminescent).[50] Moreover, the development of synthetic methods for stable *meso*-tetraarylfluorinated sulfonamide bacteriochorins allowed the development of a photosensitizer (redaporfin) with almost ideal properties, for head and neck cancer killing. This photosensitizer is currently being evaluated in phase II clinical trials.[51]

The literature includes a few examples of photosensitizers employed in the treatment of upper and lower respiratory tract infections (*in vivo* and clinical trials), including *meso*-tetra (hydroxyphenyl) chlorin (*m*-THPC), methylene blue, 5-aminolevulinic acid (ALA), curcumin, and indocyanine green.[52] All of them showed good results in combatting respiratory tract infections (e.g., pneumonia, respiratory papillomatosis, and pharyngo-tonsillitis) and also presented low toxicity.[52] However, the delivery of photosensitizers via pulmonary administration for lower respiratory tract conditions remains a challenge.

So far, we have discussed the importance of organic synthesis for modulating the photophysical and chemical properties of photosensitizers. However, it is still a substantial challenge to develop clinically suitable photosensitizers for PDT treatment of respiratory tract infections, especially in achieving low toxicity and efficient pulmonary delivery. Herein, we will highlight the use of nano-based systems (e.g., liposome, polymeric

nanoparticles, or micelles) to enhance the therapeutic index of photosensitizers through improvement of their bioavailability, stability, and residency at targeted lung regions.

PDT based in nanomaterials can use nanoparticles as the carrier or as the PS. Compared to traditional PSs, NPs are more stable under illumination and have shown improved optical properties that enable greater penetration of the biological tissue.[53] Among other advantages, NPs improve PS delivery, with greater accumulation in the target region, reducing side effects;[54] increase ROS production, resulting in a greater photodynamic effect;[55] and promote the enhanced uptake, due to modifications on the surface of NPs. These characteristics are possible due to adsorption, covalent bonding, or surface modification with some specific ligand, which make NPs hydrophilic and of appropriate size. Specific changes in the optical properties of nanoparticles enable the use of low doses of light and deeper penetration, as two-photon absorption or upconversion systems.

Nanoparticles have been synthetized with different shapes, sizes, and architectures, and methodological advances have enabled the construction of specific structures, properties, and functionalization. These can improve the solubility of PS and avoid dark degradation, thereby improving bioavailability, biodistribution, and blood half-life.[56] In terms of infection treatment, the search is ongoing for NPs that could help in biofilm internalization for its destruction and could be effective for multi-resistant microorganisms has been used in combination with other therapies, such as photothermal therapy (PTT), using nanoparticles with both capabilities or with the incorporation of other drugs.

Several nano-based systems applied in aPDT have been investigated for efficacy against the primary microorganisms that cause respiratory tract infections. The use of ZnO-NPs and blue light showed effectiveness against the drug-resistant microorganism *Acinetobacter baumannii*.[57] Carbon quantum dots carried by polymers have been described as a good option to reduce *S. aureus*, *E. coli*, and *K. Pneumoniae* under illumination.[58] In addition, methylene blue conjugated with gold nanoparticles also is an option for multi-resistant bacteria such as *S. aureus, E. coli*, and *E. cloacae*.[59] With illumination by near-infrared light and therefore deeper penetration, a combination of nanoparticles and indocyanine green presented a synergic effect between aPDT and PTT, showing very promising results against planktonic and biofilm of *E. coli*, *K. pneumoniae*, and *P. aeruginosa.*[60]

The matrix of nanosystems defines the type of nanoparticles, which can be divided in two main categories: organic and inorganic. Specifically for photosensitizing nanosystems, these may either be a nanoparticle with photodynamic properties that is capable of delivering other molecules (such as a chemotherapeutic agent),[61] or a nanoparticle without photodynamic properties that instead delivers photosensitizers. They can be further divided into non-targeted versus targeted nanoparticles, i.e., photosensitizing nanoparticles that target specific receptors or antigens. When this type of NP is applied in cancer treatment, they are termed ‘active’ and can be further sub-divided according to their mechanism of action.

The convergence of nanotechnology and PDT has introduced numerous possibilities for developing different types of photosensitizing nanoparticles that can overcome the technical hurdles of traditional PDT. However, despite the advances of nanotechnology in PDT, very few have yet been clinically approved because the use of nanoparticles presents a range of unique clinical challenges, such as large-scale synthesis, reproducibility, and biocompatibility.[62] Therefore, it is necessary to emphasize the need for simplification and standardization of the synthesis process; to expand research from *in vitro* studies to more complexes animal models; and to achieve clinical translation of PDT protocols.[63] These efforts by the scientific community towards clinical translation of nanomedicines and the development of nanotechnologies for aPDT will enable significant improvement to PDT and to current therapies against infectious diseases.

7. Future perspectives and conclusions

Microbiological control with photodynamic action has been shown to have excellent microorganism inactivation response and great potential to promote innovative methods for the treatment of various infections that accompany COVID-19. Pneumonia is currently common following lung inflammation, for which photodynamic techniques offer appropriate treatment. Similarly, the intubation process frequently leads to serious infections that, if avoided, would increase patient survival. Finally, the techniques presented here have the advantage of being economically viable, thereby allowing their deployment even in countries with limited health care resources. This economic reality is fundamental to treating respiratory tract infections in general, and specifically to enabling an effective global response to the current COVID-19 pandemic.

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