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Chapter 4

Can Nanotechnology Shine a New Light on Antimicrobial Photodynamic Therapies?

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Additional information is available at the end of the chapter

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Abstract

Recent developments in light-controlled therapies (e.g., photodynamic and photothermal therapies) provide promising strategies to prevent and suppress bacterial infections, which are a leading cause of morbidity and mortality. Antibacterial photodynamic therapy (aPDT) has drawn increasing attention from the scientific society for its potential to kill multidrug-resistant pathogenic bacteria and for its low tendency to induce drug resistance. In this chapter, we summarize the mechanism of action of aPDT, the photosensitizers, as well the current developments in terms of treating Gram-positive and Gram-negative bacteria. The chapter also describes the recent progress relating to photomedicine for preventing bacterial infections and biofilm formation. We focus on the laser device used in aPDT and on the light-treatment parameters that may have a strong impact on the results of aPDT experiments. In the last part of this chapter, we survey on the various nanoparticles delivering photoactive molecules, and photoactive-nanoparticles that can potentially enhance the antimicrobial action of aPDT.

Keywords: bacterial infections, biofilm, antimicrobial photodynamic therapy, laser, nanotechnology

1. Introduction

"*It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them, and the same thing has occasionally happened in the body*" Alexander Fleming, 1945.

In the 1940s, the introduction of the *penicillin*, discovered in the 1926 by Fleming, opened the era of the antibiotics, recognized as one of the greatest advances in the therapeutic medicine.

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However, the appearance of resistance phenomena came very quickly: by 1944, half of all clinical *Staphylococci spp.* isolates failed to respond to the so-called "miracle-drug" [1]. The World Health Organization (WHO) has recently recognized the multidrug-resistance (MDR) as one of the most important problems facing human health all over the world [2]. The need to overcome this rising problem has stimulated research into alternative antimicrobial approaches with less potential of developing resistances in microorganisms toward controlling the growing incidence of infectious diseases. An innovative light-based approach to achieve this goal is antimicrobial photodynamic therapy (aPDT). The aPDT involves harmless visible light in combination with nontoxic and light-sensitive dye, the so-called "photosensitizer (PS)," and oxygen that can selectively control bacterial infections [3]. Nanotechnology is an emerging technology that may change the face of PDT by new photoactive molecules, with numerous advantages to gain a successful bacterial infections eradication.

1.1. Photodynamic therapy as antimicrobial strategy: how it works

The photodynamic therapy has gained considerable attention as an emerging treatment modality for many forms of neoplastic diseases [4]. However, the PDT was originally discovered over 100 years ago when Oscar Raab observed that *Paramecium spp.* protozoans could be killed by particular combinations of dyes (acridine orange) and bright light [5]. For many years the potential of this finding was forgotten because of the discovery of antibiotics since the relentless increase in antibiotic resistance worldwide has spurred a migration of PDT research effort to its origin in microbiology. Numerous findings strongly support the hypothesis that PDT can represent a viable alternative since the mode of action of photodynamic sensitizers on microbial cells is markedly different from that typical of most antibiotic drugs [6]. aPDT has been successfully applied *in vivo* and *ex vivo* tissue or in biological materials for blood sterilization, in animal models of localized infections as surface wounds, burns, oral sites, abscesses, and in the middle ear. aPDT is being clinically studied for several dermatological infections, such as leishmaniosis and mycobacteria [7]. As mentioned before, PDT combines the action of three components: the PS, visible light, and molecular oxygen. The absorption of the light by the PS leads to a transition from its initial ground state (PS_0) to an energetically excited state ($^1PS^*$) that can relax to the more long-lived triplet state ($^3PS^*$). This triplet state can interact with molecular oxygen by two mechanism of reaction, letting the PS regain its ground state. Type I photoreactions occurs by an electron and/or proton transfer, where the PS interacts directly with the cellular substrate (i.e., lipids, proteins, nucleic acids, etc.). The generated radicals react with molecular oxygen, yielding several different oxygen intermediates collectively called reactive oxygen species (ROS), such as for instance the superoxide anion (O_2^-), the hydroxyl radical (OH), and hydrogen peroxide (H_2O_2). Alternatively, Type II photoreactions proceed by energy (not electron) transfer, while the oxygen is the primary acceptor. The interaction of molecular oxygen in its ground triplet state ($^3O_2$) with $^3PS^*$ generates a more reactive form of oxygen, i.e., singlet oxygen ($^1O_2$). This nonradical species is highly reactive toward electron-rich substrates such as aromatic rings, amines, and thioesters [8]. The contribution of both Type I and Type II reactions to cell death depends on several factors including, among others, the PS itself, the subcellular localization, the substrate, and molecular oxygen concentration within the target cells. Although the detailed mechanism of

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PDT and the concomitant processes are not yet fully understood, it is generally accepted that Type I and Type II reactions both produce ROS that cause oxidation of biomolecules (lipids, proteins, and nucleic acids) in the cell. For a reason not entirely understood, hyper proliferating cells selectively uptake PS [9]. This, together with the fact that cell death is spatially limited to regions where light of the appropriate wavelength is applied, makes PDT a highly selective and useful modality. Because microbial cell possess very fast growth rate, it was suggested that PDT could be effective against microbial cells (**Figure 1**). In most instances, aPDT predominantly proceeds via Type II processes. However, by comparing PSs that tend to undergo either Type I or Type II mechanism, Huang et al. reported that Gram-negative species are more susceptible to •OH than $^1O_2$ [10]. A Type I reaction is therefore favored when targeting Gram-negative species.

1.2. Antimicrobial efficacy of PDT: the photosensitizers

The photosensitizer plays a crucial role in determining the therapeutic outcome. Accumulating selectively in diseased tissue and, via generation of cytotoxic species, PS provokes the desired biological effect, without causing excessive damage to the host tissue. In general, a PS used for antimicrobial PDT should be endowed with the following properties [11]: (i) high triplet-state quantum yields ($\Phi\tau \geq 0.5$), triplet-state with lifetimes long enough (τ microsecond range), and sufficiently energetic (≥94 kJ/mol) to produce $^1O_2$ ($\Phi\Delta \geq 0.5$); (ii) high-binding affinity for microorganism (positively charged PS for good adherence to negatively charged bacterial cell wall) and low-binding affinity for mammalian cells; (iii) broad spectrum of action, since one photosensitizer can act on bacteria, fungi, yeasts, and parasitic protozoa; (iv) minimum dark toxicity and negligible cytotoxicity in the absence of light; (v) not yield toxic and mutagenic

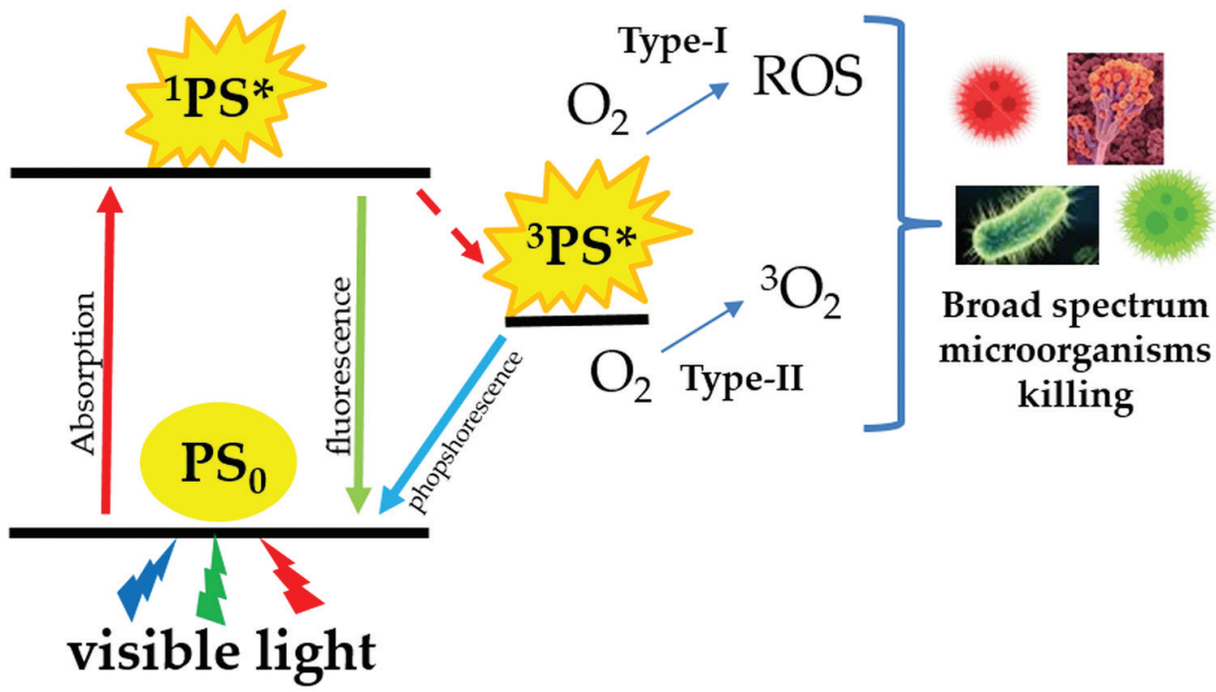

Figure 1. Schematic illustration of photodynamic action.

metabolites; (vi) greater retention in target tissue/cells over healthy ones, and (vii) high molar extinction coefficients, with high absorbance, particularly in the red and near-infrared UV-vis spectral regions (600–800 nm range), for a maximum light penetration and minimum light scattering within the "*therapeutic window*." PSs are usually organic aromatic molecules with a high degree of electron delocalization. They contain a central chromophore with auxiliary branches (auxochromes) which add further electron delocalization to the PS and thus alter the absorption spectra of the PS [12]. As reviewed in [13], different classes of natural and synthetic molecules have demonstrated antimicrobial efficacy against a broad spectrum of antibiotic-resistant microorganisms upon illumination. These include porphyrins, chlorins, bacteriochlorins, phthalocyanines, as well dyes with different molecular framework such as halogenated xanthenes (e.g., Rose Bengal (RB)), perylenequinones (e.g., hypericin), phenothiazinium salts (e.g., toluidine blue oral (TBO), and methylene blue (MB)), and merocianine and cationic fullerenes (e.g., derivatives of C60). PS binding to the bacterial cell and uptake are dependent on the microbial species. In general, aPDT has been more effective against Gram-positive and fungal cells than Gram-negative, especially when neutral or anionic PS was used. Gram-negative bacterial cells are relatively resistant to these compounds [14]. The high susceptibility of Gram-positive bacteria and fungi was explained by their physiology as a relatively porous layer of peptidoglycan and lipoteichoic acid, or beta-glucan and chitin, respectively, surrounds their cytoplasmic membrane and both these structures allow non-cationic PSs to cross [14, 15]. Gram-negative bacteria are less prone to take up exogenous compounds due to the extra outer membrane and the permeability barrier imparted by lipopolysaccharides [16]. This outer membrane provides also an effective permeability barrier and limits the binding and penetration of anionic and lipophilic PS. These critical characteristics guided the research efforts toward approaches that would allow PDI of Gram-negative species [14]. A method adopted by numerous groups is to use a PS molecule with an intrinsic positive charge [17–19]. An increase of the PDI efficacy has been addressed recently both in bacteria using the polycationic biopolymer chitosan [20]. Another method includes the using of metal chelators (ethylenediaminetetraacetic acid (EDTA)) or polypeptide polymixin B [21]. These agents destabilize the lipopolysaccharides coating by removing the Ca^{2+} and Mg^{2+} ions, thereby increasing permeability of the Gram-negative outer membrane and allowing PSs, that are normally excluded from the cell, to penetrate to a location where the reactive oxygen species (ROS) generated on illumination that can execute fatal damage [21]. At present, there is a consensus that aPDT can be effective to kill all known classes of microorganism, including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant (MDR) and pandrug-resistant (PDR) fungi, protozoa, viruses, etc., whether *in vitro* or *in vivo* [13].

1.3. PDT for inactivate biofilm formation

Most important in the chronic infections is the formation of a thick, multilayered biofilm [22]. A biofilm is defined as a microbially derived sessile community surrounded by a self-producing extracellular polymer matrix. The biofilm matrix, a homoglycan composed of β-1,6-linked N-acetylglucosamine residues, is involved in intercellular adhesion and is referred to as polysaccharide intercellular adhesion (PIA) [23]. Biofilm formation includes several sequential steps in which planktonic bacteria initially attach to a solid surface, that may be

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either unmodified or coated with host plasma proteins, followed by cell proliferation, cell-cell interaction, and production of an extracellular polymeric matrix, where bacteria accumulate in multilayered clusters (**Figure 2**).

Biofilms generally do not restrict penetration of antibiotics [24], but they do form a barrier to the larger components of the immune system [25]. As a consequence, biofilm-associated infections can only be resolved by removal of the infected device, determining high-threat care costs. PDT is a possible alternative to inactivate biofilms and may represent a different treatment for several recalcitrant infections. There is a wealth of literature that focuses on PDT-based antibiofilm strategies against a variety of microbial species [26–28]. On the contrary, the effects of PDT on phenotypic biofilm elements (e.g., adhesions and extracellular polysaccharide) are poorly investigated [29, 30]. Staphylococci are one of the most important human pathogens and a major cause of morbidity and mortality worldwide. In particular, *S. epidermidis* and *S. aureus* are emerging as the most important agents of persistent infections, especially in implanted medical devices [31, 32]. The use of tri-meso (N-methyl-pyridyl) and meso (N-tetradecyl-pyridyl) porphine (C14) for inactivation of two structurally distinct *S. epidermidis* biofilms grown on Ti6Al4V alloy has been observed and its photosensitizing efficiency with that of the parent molecule, tetra-substituted N-methylpyridyl-porphine (C1), was compared [26]. In another work, the antimicrobial activity of merocyanine 540, a photosensitizing dye used for purging malignant cells from autologous bone marrow grafts, has been evaluated against *Staphylococcus epidermidis* biofilms. Merocyanine 540-mediated PDT showed a significant inactivation effect on the viability and structure of biofilms of two *Staphylococcus epidermidis* strains, RP62A and 1457, respectively [27]. Moreover, it was found that erythrosine-induced PDT was also more potent than MB, RB, and TBO against *Aggregatibacter actinomycetemcomitans* biofilm

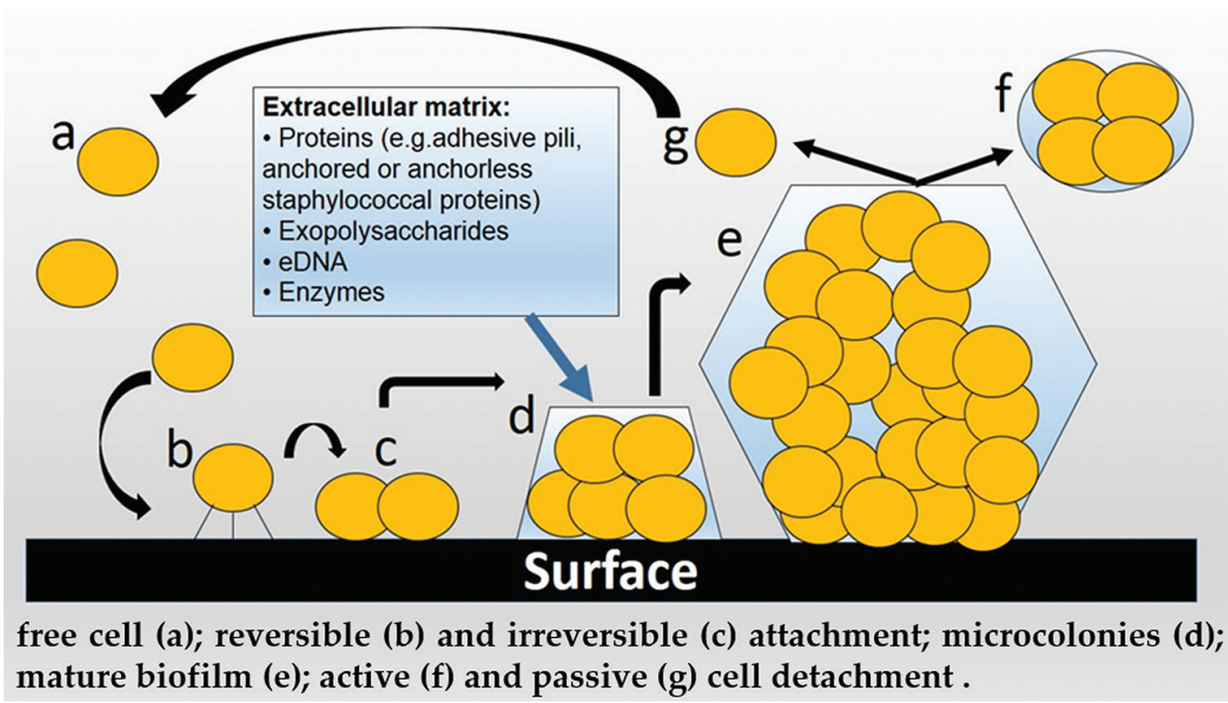

Figure 2. Biofilm development.

[33–35]. A recent paper described the photokilling propensity of the curcumin (a yellow pigment derived from the root of the *Curcuma longa* plant) in *S. epidermidis* biofilm and suspended cells in two *in vitro* models [28]. Finally, although in many systems the PDT seem promising it yields inconsistent results mainly due to the lack of standard reproducible models for assessing PDT efficacy against biofilms, as well as the lack of robustness of the majority of methodologies used in the majority of published studies.

2. Laser source and parameters for PDT in microbial infections

Given the always present variability in biological experiments, and the need to allow for experimental results comparison, we give in this section a guide that will help the reader to understand the properties of laser sources, the physical phenomena occurring in the treated samples, the parameters to be defined in order to set a reproducible experiment, and also the technical aspects regarding the instruments required for a proper optical beam characterization. In order to understand why lasers are generally used to carry out PDT experiments, it is helpful to recall the main differences between the radiation emitted by a lamp and that emitted by a laser. As everybody knows the most "visible" differences between lamps and lasers are the directionality of laser light, and the fact that it is generally "colored" and not white. The directionality aspect of laser light is of fundamental importance when a selective illumination is required, as it allows illuminating certain portions of the sample/tissue, or a specific position of a multiwell plate without irradiating the neighboring area. The laser light directionality thus enables comparing the biological effect in treated versus untreated areas on the same substrate and makes it possible to exploit relatively simple components (lenses and mirrors) in order to control the optical beam properties, as direction and diameter. In particular, the possibility to properly control the beam properties is of great importance in some recently investigated techniques, as two-photons PDT [36] where the PDT is activated only in the focal region of the optical beam, thus making possible to induce PDT in "deep regions" without affecting all the biological material irradiated. From the physical point of view, the fact that the laser light is characterized by a specific "color" means that the emitted electromagnetic radiation has a very specific wavelength. On the contrary, the "white light" emitted by a lamp is given by the simultaneous presence of radiations with different wavelengths, covering the whole spectrum of visible light and generally comprising even radiation in the ultraviolet (UV) and infrared (IR) range. Even if it is always possible to select a well-defined wavelength (i.e., a "color") from a white light source by inserting an optical filter along the light path, the obtained beam characteristics are still quite different, mainly because of two reasons: light "line-width" and the achievable "power." It is worth noticing that the exact line-width value depends on many parameters and in certain cases, it can be reduced to reach values in the kHz range, but an in-depth discussion of the parameters affecting laser line-width is beyond the aim of this section [37]. The origin of these laser beams characteristics is strictly related to the structure of a laser source, which is thus briefly described in the following. The word "laser" is the acronym of light amplification by stimulated emission of radiation [38], thus immediately suggesting that what we call "laser light" is the result of an amplification mechanism, and that a "light amplifier" should be used in order to produce laser light. The laser

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source is composed by two main components: the "active medium," which is the element amplifying the light beam thanks to the stimulated emission process [39], and the "cavity," used to provide the feedback required to transform the amplifier in an oscillator. The "active medium" obviously requires some form of power supply, as otherwise no amplification could occur because of the general energy conservation principle, and the way that power is transmitted to the active medium is generally called "pumping method" (or scheme) (**Figure 3**). As the material used as active medium determines the frequency of the emitted laser light, laser sources are generally identified by describing the active medium; this is the reason why lasers are generally classified as either "solid state," "gas," "fiber," or "semiconductor" lasers. The role of the cavity is that of creating the "selection" of the light components to be emitted. This selection is both a "frequency (or wavelength) selection," and a "direction selection": only those wavelengths that are exact dividers of the cavity length can be in fact emitted by a laser source (the so-called "cavity autofrequencies") and only those rays propagating sufficiently aligned to the cavity axis, so that they can be reflected several times before transmission, are actually selected by the cavity feedback.

The most suitable laser for PDT experiments and applications is probably that of the so-called semiconductor lasers. The first demonstration of the possibility to produce laser light from a semiconductor dates back to 1962 [40, 41], just two years after the first ever demonstration of a laser source. The main advantage of semiconductor lasers with respect to the other laser types (gas, solid, and fiber lasers) is the possibility to electrically pump the active medium, without requiring the use of additional light sources, thus significantly increasing the system efficiency. Because of the combination of high efficiency (and thus small power supply requirements) and small size (generally <1 mm^3), semiconductor lasers are the ideal choice to realize portable, and maybe even battery-operated, hand-held laser devices. Regarding the optical wavelengths that can be emitted by semiconductor lasers, these are related to the so-called "energy-gap" of the semiconductor, which in turns depends on the semiconductor lattice composition. As an example, considering an $Ga_xAl_{1-x}As$ semiconductor it is thus possible in principle to tune the emission wavelength between 570 and 850 nm by changing the value

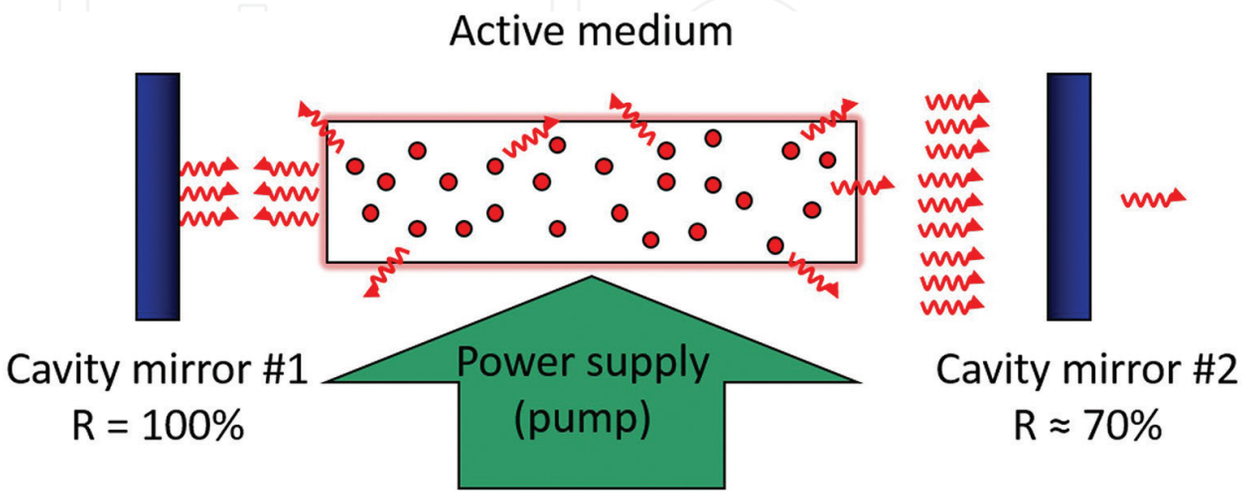

Figure 3. Example of the generation of laser beam into a cavity.

of the *x* parameter (while keeping it between 0 and 1) in the composition formula. From the practical point of view, commercial products, because of fabrication issues, cover not all the theoretically available wavelengths. The "unpleasant side" of semiconductor lasers is related to the properties of the optical beam. The small cross-section of the active medium makes the optical beam to be extremely small directly at the laser output, and in general with an elliptical shape. The very small size induces the beam diffraction phenomenon, making the beam profile broader and broader during propagation.

2.1. Laser wavelengths and irradiation system setup

A fundamental point in the preparation of the PDT experiments is the wavelength of the laser used in the system. In particular, it is useful to recall that the absorption spectrum of the photosensitizer (PS) is not the only parameter to be considered, but other elements, such as the light absorption due to the culture medium or the depth at which the target cells are located (when performing *in vivo* experiments), may impose significant constraints to the choice of the wavelength to be used, and hence on the laser choice. As a first step for the wavelength determination, it is necessary to measure the absorption spectrum of the PS, paying attention to the fact that slight modifications of the PS absorption curve may be observed when changing the medium where the PS is dispersed. After having determined the PS absorption peak, it is important to check if the corresponding wavelength may induce local medium/sample heating, generally checking if the absorption peak lies within the "biological window" (a wavelength range roughly going from 650 to 1300 nm) [42]. If the PS absorption peaks is within the biological window it is possible to assume a good penetration (>1 cm) of the light beam in a standard sample; conversely, if the absorption peak is out of the window a more detailed analysis is required, in order to understand which are the components that could cause light absorption, and the consequences of their presence in terms of heat production, light scattering, and penetration. When the desired wavelength has been selected, it is then necessary to find a suitable laser source. Limiting the discussion to semiconductor lasers, the most used in PDT, it is interesting to notice that even if several different sources are available inside the "biological window" they do not uniformly cover the required biological range and some "dark ranges" are present. This implies that even if a small (generally <1%) tuning of the emission wavelength is possible thanks to temperature tuning of the semiconductor chip, some "dark" wavelength range still exist, and thus custom solutions may be required. Additionally, from what has been reported it is probably now obvious that when multi-PS studies are considered it would be ideally required to have different laser sources, whose emission wavelengths correspond to the PS absorption peaks. As an alternative, if the absorption spectra of the tested PS all have a common "absorption region," it is possible to use a single laser, by fine-tuning the optical power to balance for the different absorption coefficient. As an additional possibility, if the laser system offers this possibility, the emission wavelength can be slightly changed (e.g., by controlling the source temperature in semiconductors lasers) to match the PS absorption peak. In order to realize a suitable irradiation system for PDT experiments, it is important to notice that the availability of the laser source is required but definitely not sufficient. We thus give a short list and description of the elements required for a proper setup preparation. The

semiconductors lasers are generally sold as a "component," implying the use of a suitable mounting and right drivers for controlling both the current injected to the semiconductor (to tune the emitted-beam power) and the temperature. It is thus important, when planning a PDT experiment to: (i) acquire the right drivers, which can be relatively expensive, even if they can be often reused in future experiments simply changing the semiconductor sources; (ii) consider the set of lenses/mirrors to control and steer the optical beam, and the corresponding mechanical mounts, allowing to keep the optical elements in a stable and well-defined position. Moreover, for an accurate characterization three elements are required: (i) an optical spectrum analyzer, for guarantee the stability of the wavelength emitted by the laser source over time (as it may drift in case of nonaccurate thermal control); (ii) a power meter to verify that the laser operating conditions remain stable even after months of usage [43], and (iii) a properly calibrated camera to acquire the spatial intensity distribution and to analyze the obtained images so as to guarantee that the beam uniformity requirements are met on the whole surface.

2.2. The "light-treatment" plan

Finally, it is helpful to highlight which are the light-treatment parameters that may have a strong impact on the results of PDT experiments. While the idea of "light-dose" (measured in J/cm^2) is generally accepted and used in the scientific literature in this field, careful analysis must be performed before comparing results of experiments using the same light-dose on identical samples. A first parameter is the beam wavelength: even very small beam wavelength variations (e.g., <1 nm) can have a strong impact on the ability of the beam to excite the PS, especially if the considered beam wavelength is close to the absorption edge and not exactly in the middle of the absorption spectrum. A second parameter, which is often overlooked, is the beam intensity (i.e., the ratio of the optical power over the irradiated surface) impinging on the sample and measured in W/cm^2. As an example, a 1 W laser beam and a 10 MW beam impinging on the same surface may be used to apply the same "light-dose" by simply scaling (by a factor of 100) the irradiation times between the two beams. Nevertheless, in the first case the beam intensity will be two orders of magnitude higher, leading to a significantly different interaction between the light beam and the biological sample. As a consequence, this means that in order to properly define a suitable light-irradiation plan, it is not sufficient to keep the laser intensity at a fixed level and to investigate the role of different doses, but it is instead necessary to vary both the light intensity and the exposure-time parameter, so as to create a "data-grid" allowing to optimize both parameters (**Figure 4**).

Additionally, the role played by localized thermal heating, due to absorption, may become relevant in PDT experiments, with two consequences: it is necessary to properly evaluate the produced temperature increase, and if the heating is nonnegligible, it is important to model the thermal situation of the experiment. Furthermore, when very high intensities are considered (for example, by using pulsed lasers), several other aspects must be considered, such as the possibility to induce photoablation and two-photons light absorption (i.e., the light beam is absorbed even if the medium absorption at the beam wavelength is very low).

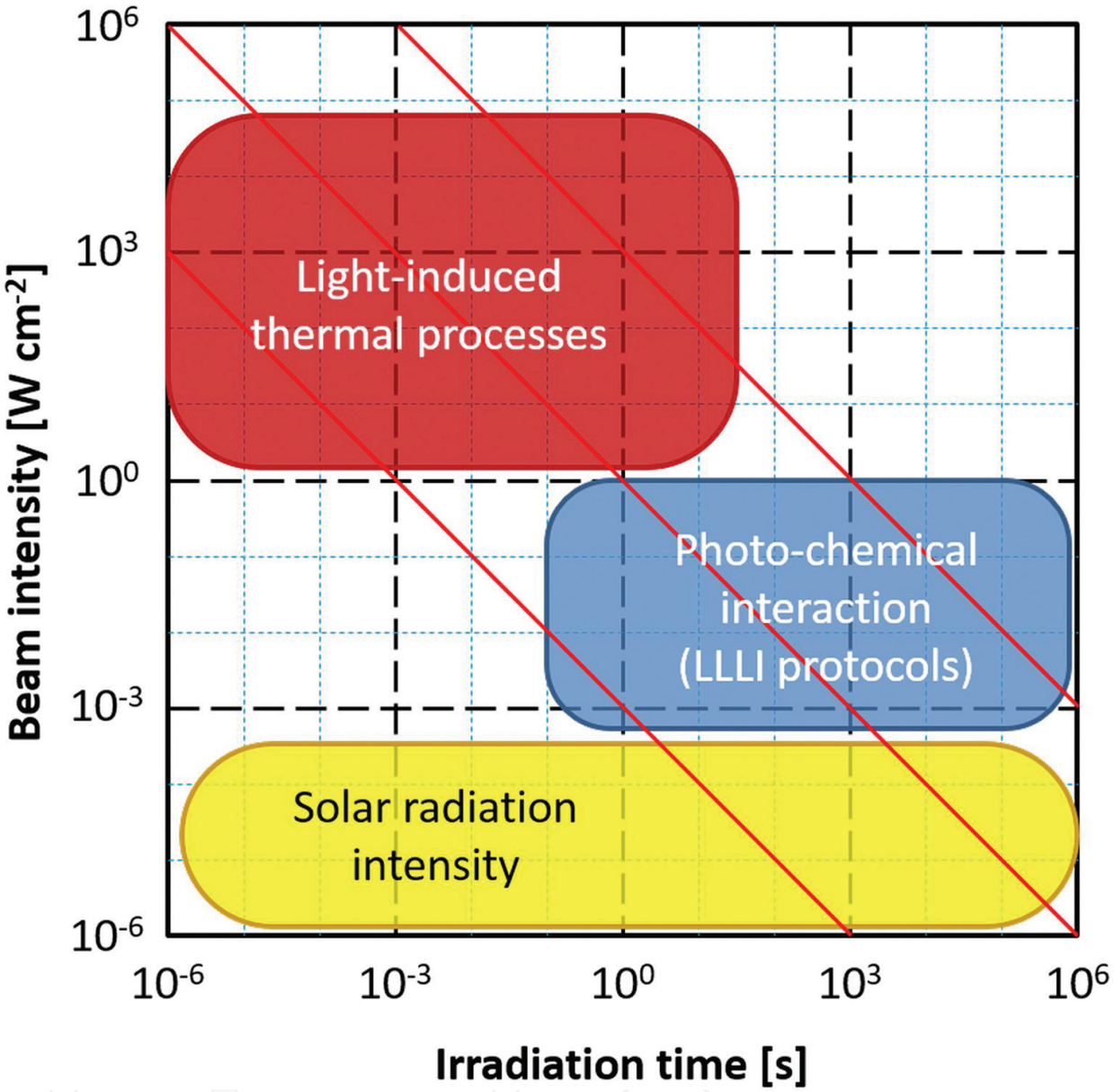

Figure 4. Log-log chart describing the interactions produced as a function of the beam intensity and of the irradiation time.

3. Nanotechnology shines a new light in antimicrobial PDT

Although the efficacy of PDT has been recognized, inefficient PS uptake by bacteria could result in insufficient therapeutic index. It is now clear that a nanotechnology-driven approach using nanoparticles can overcome this problem, increasing the efficiency and efficacy of aPDT. Several types of nanoparticle (NP) systems have been studied to potentiate antimicrobial PDT with the aim of improving photosensitizer solubility, photochemistry, photophysics, and targeting [44]. There are two different ways to combine nanoparticles and PDT for antimicrobial applications: (i) the noncovalent encapsulation or incorporation of PS in nanosystems, and (ii) the covalent binding of the PS to the surfaces of the nanoparticles. Furthermore,

the nanomaterial itself can take part in the optics, physics, and chemistry of the photodynamic process, capable of photodynamically inactivate microorganisms. As reviewed in [44], compared to free the encapsulation of PS in nanoparticles show several advantages: (i) transport a larger concentrations of PS for the production of lethal reactive oxygen species; (ii) enhance the solubility of nonsoluble water PS; (iii) a controlled release of PS, concentrating the PS in inflammatory and infectious locations by virtue of their enhanced permeability and preservation; (iv) increase the targeting to specific cells and tissues and reduced ability of the target cell to pump out the PS, hence reducing the possibility of multidrug resistance; (v) stopping the PS from dimerizing and trimerizing as it occurs in the free state, forms that are ineffective; and (vi) a selectivity of treatment achieved through either passive targeting or by active targeting (charging of the nanoparticle surface). We will give some examples of nanostructures that have been investigated as PS-delivery systems. As the main application of aPDT is likely to be in the medical (wound and surfaces sterilization) and environmental fields (food industry and water purification), particular interest has been placed on biocompatible and biodegradables nanomatrix, such as polymeric nanoparticles [45], micelles [46], and liposomes [47]. The use of biodegradable polymeric nanoparticles as PS-delivery nanoparticles has been recently reported. Polylacticglycolic acid (PLGA), polyacrylamide (PAA), and calcium phosphate have been used as PS polymeric carriers. For example, see in [48], it has been demonstrated how preparation of poly(lactic-co-glycolic acid) nanoparticles loaded with the PS methylene blue (MB) is effectively not only against biofilm formation, but is also able to kill cells already formed in the biofilm. Moreover, calcium phosphate nanoparticles can be used as efficient carriers for MB and porphyrin, against *S. aureus* and *Pseudomonas aeruginosa* bacteria [45]. Hypericin (a natural potent photosensitizer) can be embedded in amphiphilic block copolymers to form Hypericin-NPs, that when light activated demonstrated better inhibition of biofilm cells compared with planktonic cells [49]. Therefore, the encapsulation of PS in nanoparticles opens a new door for the treatment of infections with minimal side effects. Recently, Chlorine 6 (Ce6), a potent PS used in cancer therapy, has been encapsulated in charge-conversion polymeric nanoparticles (NPs) for efficiently targeting and killing pathogenic bacteria in a weak acidic urinary tract infection environment [50]. Additionally, naturally occurring polymers, such as chitosan and cellulose can be used as novel starting material for the preparation of nontoxic nanoparticles with photobactericidal action [51]. Liposomes nanoparticles are also employed as antimicrobial drug delivery vehicles because their lipid bilayer structure imitates the cell membrane and can readily fuse with infectious microbes. The hydrophobic center of these bilayers can accommodate hydrophobic drugs or PS, while the hydrophilic central region or core can accommodate water-soluble drugs or PS [52]. Liposomes exert their antimicrobial activity through different mechanisms: (i) the fusion with the cell membrane and the release of PS into the cytosol; (ii) the increase in solubility and stability of PS; or (iii) the engulfment of these liposomes in phagocytic cells and their disintegration inside the endosomes or lysosomes, thereby releasing the active PS into the cell [51]. However, the properties of liposome influence mostly the action of liposomes to alter PS distribution. For example, their zeta potential is a determinant parameter influencing their aggregation. It has been showed that values close to zero induce their aggregation, thereby reducing the antimicrobial activity [53], on the contrary if these values are too high (>40 Mv), dark toxicity is present [54], whereas negative potentials result in repulsion between bacterial

cells and nanoparticles. An important parameter is the surface charge of liposomes [55]. In particular, cationic liposomes are more effective than neutral or anionic ones in aPDT because of the establishment of electrostatic attraction with the negatively charged cell wall, which facilitate the interaction of liposome to microbial wall, and then the delivery of the PS into the microbial cells. Cationic liposomes for aPDT have been formed from different lipids including the lipid *N*-[1-(2, 3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate (DOTAP), the DL-a-dipalmitoyl-phosphatidyl-choline (DPPC), and the L-a-dimyristoyl-phosphatidyl-choline (DMPC) [55, 56]. As mentioned before, nanoparticles can also improve the efficacy of aPDT either increasing the $^1O_2$ yield of the PS and by covalently binding the PS to the surface of the nanoparticles. In this design, the PS appears to remain on the surface of the NP, but the NP itself still dictates pharmacokinetics [57]. Theoretically, the singlet oxygen would be more available when generated from the surface than from diffusing with a NP [58]. Rose Bengal is one of the most frequently used PS due to its availability, high water solubility, high singlet oxygen quantum yield, and low rate of photodegradation, and has been linked to polystyrene for inactivating *E. coli* and to silica nanoparticles to inactivate Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* [59]. In another study, *S. aureus* has been inactivated with porphyrin bound to carbon nanotubes [60], while the toluidine blue oral (TBO) has been bound to the surface of Au nanoparticles and have been shown to be effective against *S. epidermidis* [61]. What promises to be even more exciting, are those applications where the nanomaterial can acts as PS. The carbon nanomaterials (fullerenes, nanotubes, and graphene) are being discovered to be photoactive in their own right, exhibiting intriguing photo-induced electron transfer properties. Such molecules are particularly attractive due to their long wavelength of absorption, the high quantum yield, and lack of acute toxicity, except in rare cases, in the absence of light. Fullerenes (C60) are the third type of carbon structure; they consist of 60 carbon atoms arranged in a spherical structure that can absorb light and be active PS [62], they generate different ROS according to the solvent, and in polar solvents they produce superoxide and hydroxyl radicals, while in nonpolar solvents they predominantly generate singlet oxygen. As shown from recent studies, their functionalization (with multiple attached cationic groups) make them more soluble in water or other biological fluids and more active for targeting and killing different bacterial species [63]. Titanium oxide (TiO_2) has been more widely studied as a PS among metal oxide nanoparticles in a process termed "photocatalysis," which has been proposed as an antimicrobial strategy for disinfection of surfaces, air, and water [64, 65]. Photocatalysis is the acceleration of a light-mediated reaction in the presence of a catalyst (usually an inorganic semiconductor) [64]. The advantage of photocatalysis is having sunlight or UV-radiation to trigger the disinfection process using a catalyst (TiO_2) [66, 67]. The process has been shown to be capable of killing a wide range of organisms including Gram-negative and Gram-positive bacteria, endospores, fungi, algae, protozoa, and viruses, and has also been shown to be capable of inactivating prions [68]. However, the use of UV-region is also the main obstacle to the use of TiO_2 nanoparticles for medical applications. As a consequence, the research efforts has been in shifting the absorbance spectrum of TiO_2 toward the visible region through doping the titanium surface with other elements, such as ytterbium (Yb^{3+}), erbium (Er^{3+}) [69], and argent [70] ions. Furthermore, the coating with argent and copper, both antimicrobial agents, can enhance the killing activity of TiO_2 [71, 72]. Metal nanoparticles usually are of very small size (i.e., ranging from one to a few nanometers)

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and are characterized by a high monodispersity. Most applications of metallic NPs stem from the principle of their surface functionalization (unprotected metal NPS are highly sensitive to air), which allows loading them with large PS doses. Typical metals employed for this purpose include gold, silver, platinum, and palladium. Gold (Au) is not intrinsically antibacterial but gold nanoparticles possess two or more localized surface plasmon resonances (LSPR) that undergo thermal relaxation upon irradiation. This property has been employed to potentiate the photodynamic inactivation. Using different methods of preparation, Au-based nanomaterials such as Au nanospheres, Au nanostars, and Au nanorods can be obtained to inactive bacteria by a photothermic process [73]. Thus, Au-nanoparticles can be conjugated with specific antibody [74], PSs [75], and antibiotics [76], or have intrinsic antibacterial activity [77, 78]. Furthermore, it has been observed that the coating of glass materials with gold nanoparticles proved to be very efficient in photothermal biofilm laser treatment against *S. aureus* biofilms, suggesting the possibility of fabricating medical devices with the same coating: once internalized, they would not need to be removed if a biofilm is formed on their surface but may be treated *in situ*, i.e., through tissues, avoiding surgical removal (**Figure 5**) [79].

Finally, recent studies have proposed that rare earth mineral nanoparticles (the so-called upconversion nanoparticles, consisting of sodium yttrium fluoride ($NaYF_4$) codoped with ytterbium and erbium ions [80]) can be used to transduce near-infrared light into required short wavelength light for activate powerful photosensitizers and for a better penetration of PS into the tissues.

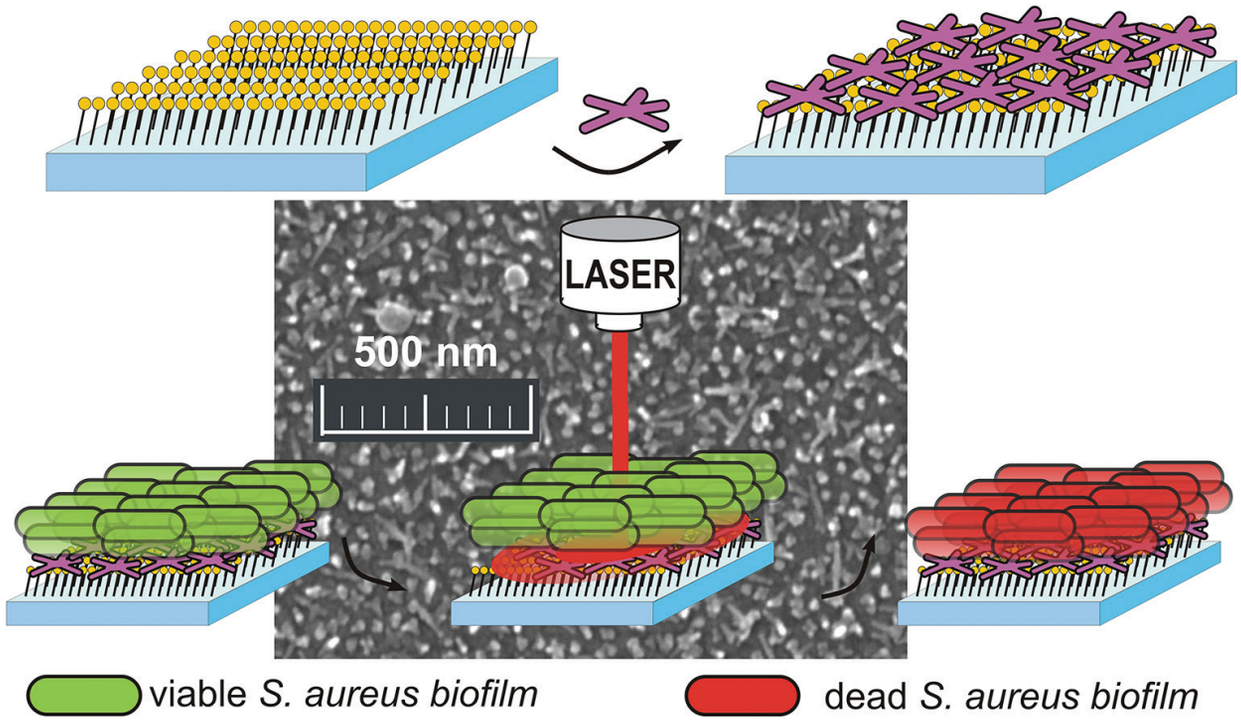

Figure 5. Monolayers of gold nanostars (GNS) grafted on mercaptopropyltrimethoxysilane-coated glass slides for aPDT application.

4. Conclusion

This chapter provides a state-of-the-art analysis about the use of phototherapy and nanotechnology to resist or counteract implant infections, together with a glimpse of the future possible applications and main trends occurring in the field. Progress in the field will correlate with a better understanding of photophysics, chemistry, materials science, biology, and clinical practice, which will allow a rational design of the whole investigation protocol, ranging from optimized formulations to the development of suitable tools for photosensitizers and light beams delivery. Nanotechnology is one of the most rapidly growing fields of translational medicine, and its potential impact on photodynamic therapies is extremely wide. The convergence of phototherapy and nanotechnology may provide new therapeutic modalities (e.g., new nanophotosensitizer formulations) that are easy to apply throughout the body in a targeted manner. In conclusion, exploring the current and possible future interactions between nanotechnology and PDT will offer new outlooks on their bactericidal potentiality.

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Figure 2 is adapted from [31]. **Figure 5** is reproduced from [79] with permission from the Royal Society of Chemistry.

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