

Chapter 14

Future Perspectives

**Fábio Parra Sellera, Cristiane Lassálvia Nascimento,
Fabio Celidonio Pogliani, Caetano Padial Sabino,
and Martha Simões Ribeiro**

Abstract Nowadays, it is clear that the activity of different photosensitizers (PSs) has a strong potential for moving photodynamic therapy (PDT) to clinical practice. Present technologies as dedicated light sources, new PSs, and nanotechnology are emerging strategies to promote PDT as a reliable, cost-effective, and safe approach to veterinary medicine. This chapter addresses an overview of emerging clinical applications and recent technologies to encourage veterinarians toward PDT.

F.P. Sellera (✉) • F.C. Pogliani
Department of Internal Medicine, School of Veterinary Medicine and Animal Science,
University of São Paulo, Av. Prof. Orlando Marquês Paiva, 87, Butanta 13635-900, SP, Brazil
e-mail: fsellera@usp.br; fabiocp@usp.br

C.L. Nascimento
Veterinary Unit of Santos Aquarium, Santos Aquarium, Av. Bartolomeu de Gusmão S/N,
Ponta da Praia, Santos 11035-500, SP, Brazil
e-mail: cristianelassalvia@hotmail.com

C.P. Sabino
Department of Microbiology, Institute for Biomedical Sciences, University of São Paulo,
Av. Lineu Prestes 1347, Cidade Universitária, Sao Paulo 05508-000, SP, Brazil

Department of Clinical Analysis, School of Pharmaceutical Sciences, University of São
Paulo, Sao Paulo, SP, Brazil

Center for Lasers and Applications, Nuclear and Energy Research Institute, National
Commission for Nuclear Energy, Av. Lineu Prestes 2242, Cidade Universitária,
Sao Paulo 05508-000, SP, Brazil
e-mail: caetanosabino@gmail.com

Department of Medical Biophysics, Princess Margaret Cancer Institute/University of Toronto,
Toronto, Canada

M.S. Ribeiro
Center for Lasers and Applications, Nuclear and Energy Research Institute, National
Commission for Nuclear Energy, Av. Lineu Prestes 2242, Cidade Universitária,
Sao Paulo 05508-000, SP, Brazil
e-mail: marthasr@usp.br

14.1 Introduction

In the previous chapters, we presented some aspects related to photodynamic therapy (PDT) as its history, mechanisms, and applications that deserve special attention. In recent decades, the number of researches on PDT in veterinary medicine has increased. New photosensitizers (PSs) or functionalized PSs are being developed to optimize PDT (see Chap. 3) as well as dedicated light sources. In fact, innovative light sources for light-based therapies have been widely explored. Recently, Chinese researchers have developed a “3-D bright fabric,” composed of flexible polymer optical fibers and LED at red emission to be used on the human body injuries in the treatment of diseases of various origins [1].

Several studies have demonstrated a wide perspective in clinical treatment of diseases, such as topical infections and cancer. Moreover, environmental applications, as water treatment in fish farming, are promising candidates to reduce antimicrobial residues. As we mentioned above, the number of veterinary PDT studies has increased; on the other hand, veterinary medicine still has difficulties to determine clinical protocols due to a wide variety of animal species with different characteristics and particular diseases. Many well-established studies in other areas indicate applications not yet explored in veterinary medicine. In this chapter, we will discuss some relevant aspects regarding worthy PDT as new frontiers of research.

14.2 Prospects for Clinical Applications

Technological advances in recent decades have improved the diagnosis and treatment of diseases that used to be hardly managed in the past. In previous chapters, we presented a series of PDT studies in veterinary medicine that proved that this technique could be widely investigated in this field. Besides that, the growing concern about the overuse of antibiotics, resulting in the appearance of multidrug-resistant microorganisms, such as the absence of a 100% effective treatment against the various types of cancer, makes PDT a promising candidate for veterinarians.

As previously discussed elsewhere, antineoplastic PDT has been more studied than antimicrobial PDT in dogs and cats. A lack on antimicrobial therapies is not an excuse to slow enhancements in cancer studies which should be more explored by veterinarians. New alternatives are being established by the development of new photosensitizers and irradiation systems that could help on different types of cutaneous tumors treatments, becoming new strategies against this illness. Studies already developed in human medicine stimulate employment in routine dermatological practice, which certainly can be extrapolated for veterinary medicine in the near future.

14.2.1 Skin Diseases

Skin infections are commonly diagnosed in companion animals. Thus, PDT could benefit Veterinary Dermatology. The direct and easy approach, with few physical barriers, makes its application more suitable than for internal organs, given that they are more difficult to be irradiated and accessible for dyes. Furthermore, studies on pathogens from human diseases similar to those that affect animals have proved to be susceptible to PDT, e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus intermedius*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus* spp., *Microsporum* spp., *Trichophyton* spp., *Sporothrix* spp., and *Malassezia* spp. These results motivate the development of clinical trials focusing attention on topical treatments and targeting antimicrobials to systemic therapies.

Localized and integumentary infections and skin cancer are among eligible diseases that PDT can be useful for farm animals. Swapping the usual systemic for topical treatment fulfills the reasons to develop future researches. For instance, habronemiasis (*Habronema* spp.) and pythiosis (*Pythium insidiosum*), a helminth and an oomycete agent, respectively, are severe equine skin infectious diseases with relative high occurrence in tropical countries. In addition, equine sarcoid is the most commonly diagnosed tumor of the skin and soft tissues. These diseases combine surgical and systemic drugs for treatment, but the rate of unsuccessful results is high leading to euthanasia. PDT could be tested as substitute or adjuvant therapy.

Udder and ear helminthiasis in cows, caused by *Stephanofilaria* spp. and *Rhabditis* spp., respectively, still affect many animals worldwide. Protocols of residue control avoid antimicrobial systemic drugs during milk and meat withdrawal periods. In many cases, topical drugs are ineffective. Thus, PDT could be employed as an alternative or combined therapy with topical agents.

Besides the ability to treat infections and cancer, there is indication of PDT to treat macular degeneration in humans, as commented in Chap. 13. Animals also can be affected by disorders involving neovascularization. Lucroy raised a very interesting question not yet investigated [2]. In fact, he suggested that PDT could be applied to combat formation of exuberant granulation tissue in horses. Horses have specific dermal and subcutaneous precursors that predispose to a local exuberant granulation tissue preventing the wound contraction and reepithelialization. A similar principle of selectively destroying functional blood vessels, as described for macular degeneration in humans, could be investigated for exuberant granulation tissue in horses, using appropriate PSs as those reported in Chap. 3.

The wild animal medicine seems to open a huge range to develop researches involving PDT, once the knowledge about different wild species, metabolism, diseases, and their treatments is scarce.

Successful treatment of footpad dermatitis in penguins encourages us to expand the list of species, which suffer from similar diseases as is the case of birds of prey, waders, Galliformes, among others. Similarly, dermatitis caused by poor captive conditions that affects many species, such as bedsores due to contact with urine and

feces, moisture, and imprisonment in restricted areas, can be benefitted from this broad-spectrum treatment against bacteria and fungi.

Until now, there are no reports of PDT in amphibians. Thus, we believe that challenging conditions, such as the chytridiomycosis (*Batrachochytrium dendrobatidis*), which threaten to extinguish several species of amphibians, deserve to be studied as PDT application viability.

14.2.2 Oral Diseases

Veterinary dentistry is another potential area for PDT application. PDT has been widely studied in human dentistry to treat dental, mucosal infections and cancer presenting benefits in clinical practice.

Although dogs have been used as experimental model, PDT was successful to treat induced periodontitis and periimplantitis (see Chap. 12). Nevertheless, veterinary medicine still has not explored this practice, and other diseases could be also treated based on human studies.

An interesting PDT approach may be to treat epulis. Epulis is considered the most common tumor in dog's mouth. In severe cases, it could lead to fracture due to bone involvement. Although histologically benign and with favorable prognosis, epulis must be surgically removed, and in some cases the association with radiotherapy is recommended. Recently, Truschneegg et al. reported an epulis case in human treated by methylene blue (MB)-mediated PDT and red laser [3]. No sign of recurrence of any hyperplastic tissue was observed after 4-week follow-up and even after 12 months. These results are motivating to extrapolate this condition to veterinary's use.

Stomatitis in reptiles is another interesting approach to be addressed in the near future. Our preliminary results (see Chap. 12) suggest that PDT was effective to treat this disease in snakes. Birds are also affected by oral infections, including oral and beak infections. According to our previous experience, we encourage further studies to perform PDT for this purpose.

14.2.3 Diseases Related to Other Organs

Nowadays, there has been growing public concern related to the persistence of drug residues in milk products and their consumption by humans. Diseases like mastitis are the main concern worldwide once the same drugs could be used by human medicine. It is well known that continued or indiscriminate use of antibiotics for mastitis treatment has been favoring the increase of multiresistant microorganisms (MOs) and limiting effective strategies to eradicate this problem in milk farms. New PDT appliances can be designed in order to overwhelm those challenges, and the antibiotic use becomes strict to indispensable medical cases.

Mastitis is one of the most prevalent diseases in dairy cattle. Production losses and early culling rates due unsuccessfully treatments are negative effects related to economic losses. Because bacterial infections are considered the most prevalent cause, antimicrobial therapy is always required.

An unusual type of mastitis caused by algae *Prototheca zopfii*, also a zoonosis, remains with no effective treatment and cure [4]. Likewise the mastitis caused by *S. aureus*, clinical presentation is severe and difficult to control. It is also potentially infectious to other animals in the herd. For both *P. zopfii* and *S. aureus*, the recommendation is culling the infected animals. Recently, we investigated PDT in vitro against different mastitis pathogens, i.e., bacteria and *P. zopfii*. The results were successful (see Chap. 11), but more realistic experiments as in milk samples have to be conducted before clinical trials [5].

PDT is also well renowned in cancer treatment to treat dysplastic lesions and malignant types of cancer by endoscopy. In these situations, diffusing fibers are especially developed to irradiate cavities such as bladder, esophagus, lung, and stomach. At this point, PDT could emerge as an alternative to treat different types of internal cancer. This approach has not been explored, but there is a great perspective for its use.

Another interesting issue to be investigated is the use of PDT to treat osteomyelitis in dogs and cats. Some studies conducted by Tardivo et al. presented successful results in human patients [6, 7]. In those studies, PDT was able to prevent foot amputation in diabetic patients. As is well known, various traumas (e.g., running over) may lead to extensive damage of the skin providing a gateway to infection and contributing to MO proliferation in osteomuscular tissues. Osteomyelitis in companion animals, as in any other species, requires prolonged treatment based on cocktail drugs including anti-inflammatories and systemic antibiotics [8]. Often these drugs are not able to control the infection eventually leading to amputation or euthanasia [9]. In this context, PDT may emerge, if not to replace, in association with other clinical protocols routinely used.

Bacterial and fungal ulcerative keratitis, as well as non-ulcerative fungal keratitis as stromal abscess, is frequent in horses. Environmental and behavioral factors are the main cause to make horses more susceptible to corneal and conjunctival lesions than other domestic animals, since these structures are frequently exposed to bacteria and fungi, especially *Aspergillus* spp. and *Fusarium* spp. Recent study evaluated clinical outcome in equine keratomycosis reporting that surgical intervention was necessary for 54% of the eyes, and 28% of these eyes were enucleated [10].

PDT could be an alternative approach for those cases. Since the 1970s of the last century, researchers use animal models to test PDT in virus-induced keratitis with good outcome [11]. More recently, Shih and Huang used MB-mediated PDT combined with amikacin to treat nontuberculous mycobacterial keratitis in rabbits [12]. The authors indicated PDT as a potential adjuvant treatment for intractable mycobacterial infection. In 2015, Zborovska and Dorokhova presented in the 15th EURETINA Congress, held in Nice, France, data about PDT on fungal inflammatory eye diseases from studies in vitro to human clinical practice. In vitro assays determined the most effective parameters of MB-mediated PDT. Preclinical studies

in rabbits revealed that control group, which received standard anti-inflammatory (AI) therapy, had disease duration higher than PDT + AI group (about 7 days for moderate and severe keratitis). Clinical outcome showed that after 3 months, a proportion of patients with corneal infiltrate and erosion area in PDT group was lower than that of control group.

14.2.4 Environmental Applications

Phototoxicity against pathogens could be enhanced by using sunlight (see Chap. 13). The idea of using compounds activated by sunlight is old, but seems to be an interesting option to control parasites and fungal diseases, mainly in water. In fact, Chap. 13 addressed recent works regarding this issue with encouraging results.

PDT could be investigated to inactivate other parasitic agents as monogenetic trematodes (gill fluke, i.e., *Dactylogyru*s spp., *Gyrodactylus* spp., *Cleidodiscus* spp.), microcrustaceans (*Argulus* spp., *Ergasilus* spp., *Lernaea* spp.), cestodes, and nematodes.

Other fish parasitic diseases could be also explored by PDT as marine (*Cryptocaryon irritans*) or freshwater protozoa (*Ichthyophthirius multifiliis*, *Epistylis* spp., *Ambiphrya* spp., *Trichodina* spp., and *Trichophrya* spp.). Fungi (*Saprolegnia parasitica* on aquatic invertebrates) and bacteria (*Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Flexibacter columnaris*, *Streptococcus* spp.) may be sensitive to PDT as seen in Chap. 12. The main challenge is the technical adaptation to the aquatic environment.

Similarly, sunlight-based PDT appears to be interesting alternative to reduce parasites on pasture. The idea is based on the oral supplementation of animals with nontoxic photosensitizers to break parasite cycle. When the animal eliminates parasites, the sun can inactivate them. The published studies regarding this topic were detailed in Chap. 12.

14.2.5 Biotechnology Applied to Animal Reproduction

As we discussed in Chap. 11, another application that can be envisaged for PDT involves biotechnology applied to animal reproduction. Due to many ethical questions, the investigation of PDT on human reproductive system to treat embryos and semen infections seems to be more limited than veterinary. By the other hand, future researches involving animals could promote an improvement of knowledge and safety, which could be extrapolated for humans.

In poultry, semen samples are usually contaminated with feces and urine. PDT can be performed to reduce microbial load improving the sperm motility and fertilization rate. Furthermore, this application could be extended to conservation of

endangered species, improving animal fertility rate in captivity and, consequently, increasing the success rates in their conservation.

Endemic diseases in cattle are spread worldwide. Thus, artificial insemination could be a high-risk potential vector to infect animals due to the large-scale commerce of cattle semen among countries. Following international recommendations provided by the World Organization for Animal Health (OIE) to evaluate the health status of bulls in artificial insemination stations, several tests are being made in animals and their semen. However, diseases with slow transmission are more difficult to monitor due to their seroconversion [13], and diagnostic tests on semen present some limitations [14, 15]. Therefore, the risk of pathogen transmission in artificial insemination procedures remains and challenges new practices for its containment.

Addition of antibiotics in the media is a satisfactory safety for bacterial diseases, mainly campylobacteriosis [13]. However, other pathogens could lead to infections due to the inefficacy of antimicrobials against them. The main relevant diseases that could be transmitted by semen are viral (foot-and-mouth disease, enzootic bovine leukosis, infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, rinderpest, bluetongue, bovine diarrhea, malignant catarrhal fever, Akabane virus), bacterial (contagious bovine pleuropneumonia, Johne's disease, brucellosis, bovine tuberculosis, leptospirosis, bovine genital campylobacteriosis, Query fever (hemorrhagic septicemia)), protozoan (bovine genital trichomoniasis), and even prion (bovine spongiform encephalopathy). The PDT inactivation of these agents should be evaluated in raw and collected semen from bulls.

In addition to the semen approach, the disinfection of embryos in farm animals also appears to be an attractive strategy to control transmission of reproductive diseases. Embryo technologies are constantly evolving; however, it is imperative to contain the risk factors and assure the health status of the farm, herd, donor cow, and embryo to achieve full success in livestock reproduction programs. Thus, the risk assessment of potential pathogens, preventive measures, diagnosis, and disease control has become a true challenge nowadays.

Sutmoller and coworkers made simulations to evaluate the risks of transmission of viral agents by embryos after following the recommendations for international trade based on epidemiology and surveillance as well as the internationally approved embryo processing protocols [16]. The authors concluded that the foot-and-mouth disease virus, vesicular stomatitis virus, and bluetongue virus are with a very low risk. In another research done by Wrathall and colleagues, they studied embryos produced with infected semen for enzootic bovine leukosis, bovine herpesvirus-1, bovine viral diarrhea virus, and bluetongue virus [17]. After embryo processing, they concluded that even with an extremely low health risk, virus removal from these embryos is difficult with the exception of enzootic bovine leukosis.

The majority of the studies focus on viral infections because viruses represent a great risk of infection of the embryo due to their too small size [18]. Therefore, more studies are necessary to evaluate different types of MOs that could be transmitted by reproduction techniques and probably be prevented by PDT.

Embryo transfer by *in vivo* and *in vitro* techniques is used in cattle, horses, sheep, goats, and pigs. Scientific protocol available cannot be used for different species, even when washing procedures in combination with trypsin treatment are performed to avoid transmission of diseases, mainly those associated with virus that could be attached in the embryo zona pellucida [18]. Therefore, the susceptibility of the different MOs should be addressed by PDT and further applied to semen and embryo technologies.

14.3 Nanoparticle-Based PDT

Nanotechnology involves the creation of any material, system, or device through the manipulation of matter at very small scale, measuring 1–100 nm. Nanomaterials are defined as small objects that behave as a whole unit regarding to their transport and properties. Moreover, nanomaterials have unique electronic, optical, magnetic, and chemical properties distinct of larger particles of the same material.

Recent developments in nanotechnology allowed improving the use of the PDT for both cancer and infections. In fact, mainly for cancer, the accomplishment of PDT may be partial due to the difficulty in administering PS with low water solubility, which compromises the clinical use of several molecules. Nanotechnology is an interesting approach for PDT mainly because nanoparticles (NPs) (organic and inorganic) can be guided to increase PS concentration at the target and diminish toxic effects to normal tissue and cells. In fact, various types of NP as metallic (silver and gold NP), crystalline (upconversion – rare earth doped), superparamagnetic (superparamagnetic iron oxide nanoparticle, SPION), and semiconductor (quantum dots (QD)) can be functionalized (marked with specific molecules) for use in PDT [19]. Besides, particularly for cancer PDT, NP can accumulate at the tumor site due to increased endocytic activity and leaky vasculature in the tumors. NP can also enhance the solubility of hydrophobic PS.

Incorporation of PS in nanostructured delivery systems, such as polymeric nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, gold nanoparticles, hydrogels, liposomes, liquid crystals, dendrimers, and cyclodextrin, is an emergent approach to surpass PDT limitations. Thus, the application of nanotechnology offers exciting possibilities to improve cancer and antimicrobial PDT for humans and veterinary medicine.

Different NPs have been used in PDT with distinct interactions between NP and PS. Some examples were presented in Chap. 3. Depending on interaction, NP can be active (NP acts as PS) or passive. Four interactions are described by literature [20] (Fig. 14.1):

1. The PS is embedded in a polymeric NP. In this case, nanoparticles are loaded with PS and are used as carriers to deliver the PS into the target, incorporated on biocompatible and biodegradable matrixes such as liposomes or synthetic and natural polymers (e.g., poly (lactic-co-glycolic acid) (PLGA), chitosan, and cellulose).

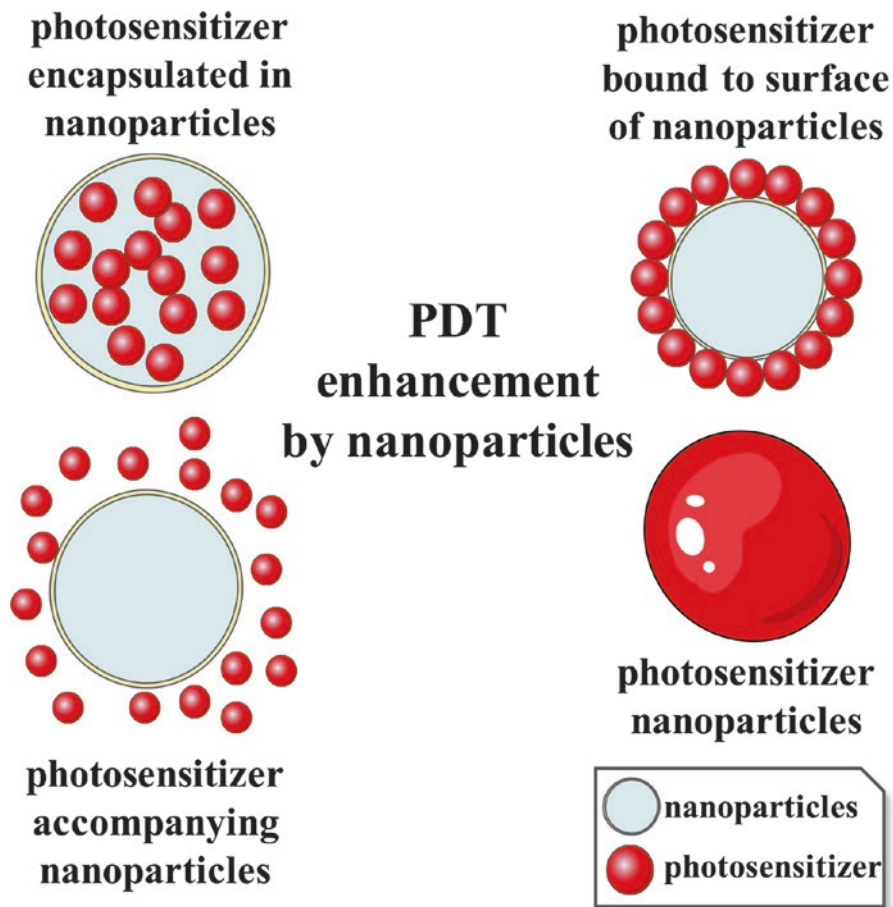


Fig. 14.1 Schematic illustration of the interaction between nanoparticles and photosensitizers to enhance PDT

2. The PS is bound to the NP surface. In this case, the new PS presents better properties compared to original PS.
3. The PS is accompanied by NP. In this case, nanoparticles are used to enhance the photodynamic effect. Metallic NP (gold and silver) and quantum dots have been reported to enhance PDT efficiency in both cancer and antimicrobial PDT [21, 22].
4. The NP acts as the PS. In this case, NP is itself photoactive and able to generate reactive oxygen species (ROS).

Here, we address some works that encompass nanotechnology-based PDT with potential application to veterinary medicine. Our intention is not to provide the peculiarities behind the NP development but to present some studies involving different NPs that were successfully combined to cancer and antimicrobial PDT.

14.3.1 PS Encapsulated in or Immobilized to NP

As abovementioned, NPs can be used as a vehicle to improve the PS delivery at specific sites. For cancer treatment, recently European researchers developed poly-methylmethacrylate core-shell fluorescent nanoparticles (FNP) loaded with the photosensitizer tetrasulfonated aluminum phthalocyanine (Ptl) and carried in vitro and in vivo assays using a human prostate tumor model [23]. Their data showed that Ptl@FNP is internalized by tumor cells and intracellular accumulation of Ptl is favored. Upon irradiation with $\lambda = 680$ nm, they observed ROS production, which triggered cell death. In a murine model, the engineered NP was able to reduce tumor growth with higher efficiency compared to bare Ptl. Thus, authors conclude that the new system could be successfully used to photodynamic treatment of solid tumors.

For topical PDT, NP-based delivery systems are also reported. MB-loaded PLGA nanoparticles of positive charge and with a diameter of about 200 nm showed higher photodynamic effect compared to anionic NP and free MB in suspensions of bacteria isolated from human dental plaque. In biofilms, cationic NP, anionic NP, and free MB showed similar photodynamic action. Authors conclude that cationic PLGA nanoparticles have potential to be used as carriers to diffuse and release MB conducting to photodestruction of bacteria and oral biofilms; however, preclinical assays should be performed to guarantee microbial killing without damage to host cells [24]. Posteriorly, Fontana and collaborators showed that MB-loaded PLGA cationic NP may target oral biofilm safely and fast in rats without injuries to normal tissue [25].

14.3.2 PS Bound to the NP Surface

PS has been bound to the NP surface to prepare new PSs with improved characteristics compared to the former. In the Eshghi's work, authors hypothesized that protoporphyrin IX (PpIX)-conjugated gold NP could improve PS solubility and oxygen singlet quantum yield [26]. PpIX-conjugated gold NP was synthesized, characterized, and used for the delivery of a hydrophobic PS to a cervical cancer cell line. They reported that the PpIX-gold NP conjugate was an excellent carrier for the delivery of surface bound PpIX into HeLa cells. Cellular viability reduction was dependent on conjugate concentration and irradiation time.

Antimicrobial PDT using functionalized NP has also been explored. Tomás et al. reported the functionalization in aqueous media of tiopronin (a thiolate to protect the NP)-gold NPs and ortho-toluidine blue (TBO) to augment the PDT effect on *S. aureus* [27]. TBO was covalently coupled to tiopronin-gold NPs and showed that the minimum bactericidal concentration was at least four times lower than that of free TBO.

14.3.3 PS Alongside NP

When PS accompanies the NP frequently is to enhance the photodynamic action through physical/chemical interactions between PS and NP in the target surroundings. Here, we report localized surface plasmon resonance (LSPR) and Förster (or fluorescence, when both molecules are fluorescent) resonance energy transfer (FRET) to improve PDT.

LSPR is an optical phenomena produced by light when it interacts with noble metal NPs (e.g., gold and silver) that are smaller than the incident wavelength. The electric field of incident light excites the electrons of the conduction band generating localized plasmon oscillations with a resonant frequency that depends on the composition, size, geometry, dielectric environment, and particle-particle separation distance of NPs [28].

Light interaction with PS can be improved by LSPR when LSPR frequency and the PS absorption spectrum overlap. Thus, the field density can be perceived by PS that is placed close to the metallic NP inducing luminescence enhancement to a better excitation of the PS. In fact, gold NP was tested to enhance the antimicrobial effectiveness on *S. aureus* of the PS ortho-toluidine blue (TBO) when irradiated with broad-spectrum light by Narband and collaborators [29]. Bacterial suspension was exposed to white light in the presence of either TBO or a combination of TBO and gold NP (2 nm and 15 nm). Authors observed an increase in bacterial kills concluding that 15 nm gold NPs augment the light-capturing ability of the TBO.

FRET comprises the energy transfer between two photosensitive molecules in close proximity. The donor molecule may transfer energy to the acceptor molecule through nonradiative dipole–dipole coupling (e.g., Coulomb interactions; see Chap. 2), i.e., the donor does not emit a photon that is then absorbed by the acceptor, but instead, the energy is coupled through the molecule dipoles that emit energy in the same manner as a radio antenna [30]. For efficient FRET to occur, the distance and donor and receptor must be too small (<10 nm) since the efficiency of this energy transfer is inversely proportional to the sixth power of the distance between donor and acceptor.

Narband's group also explored FRET to optimize PDT [21]. The authors investigated if CdSe/ZnS quantum dots (QD) (emission maximum at $\lambda=627$ nm) could enhance the antibacterial activity of TBO (absorption maximum at $\lambda=630$ nm)-mediated PDT on *S. aureus* and *Streptococcus pyogenes* exposed to white light. Bacterial killing depended on type of MO and TBO/QD ratio. However, authors suggested that enhanced killing seemed to be not attributable to a FRET since QD converted a part of the incident light to the absorption maximum for TBO, which in turn absorbed more light to produce bactericidal radicals.

By the other hand, sulfonated aluminum phthalocyanines (AIPcS) were conjugated with amine-dihydrolipoic acid-coated QD by electrostatic binding [31]. The AIPcS–QD conjugates easily penetrated into human nasopharyngeal carcinoma cells and carried out the FRET in cells, with efficiency around 80%. Authors used a green laser emitting at $\lambda=532$ nm, which excited the QD but not the AIPcS, and the cellular AIPcS–QD conjugates damaged most cancer cells via FRET-mediated PDT.

14.3.4 NP as PS

Mostly NP acting as PS are inorganic that strongly absorb ultraviolet (UV) light. However, the use of UV lamps in biomedical sciences could bring safety and health risks. Some strategies to their use as PS encompass the use of sunlight or doped them with other elements (e.g., Er^{3+} and Yb^{3+}) to shift their absorbance toward visible light.

Zinc oxide (ZnO) NP displays an excellent photooxidation activity but with low photocatalytic decomposition. Metal ions (e.g., Ag) can be incorporated to ZnO NP to improve its photocatalytic activity. Thus, Arooj et al. investigated the effects of ZnO/Ag nanocomposites on human malignant melanoma (HT144) and normal (HCEC) cells [32]. The ZnO/Ag nanocomposites killed cancer cells more efficiently than normal cells under daylight exposure. Cytotoxicity was dependent on Ag concentration. Besides the incorporation of Ag into ZnO NP significantly improved their photooxidation capabilities.

Functionalized fullerenes, i.e., fullerenes with attached side chains, are also explored in PDT. The fullerenes are known for their photostability and experience less photobleaching than other PS. As they are insoluble, they can be modified to have a certain degree of lipophilicity. Other modifications can also be carried out to make fullerenes suitable for PDT [33].

Grinholc and colleagues studied the effects in vitro of a C60 fullerene functionalized with one methylpyrrolidinium group (fulleropyrrolidine) on Gram-negative and Gram-positive bacteria, as well as fungal cells under white light exposure. Due to the high antimicrobial activity, authors tested its potential in vivo on *S. aureus*-infected wounds in mice [34]. Fullerene-mediated PDT was efficient to eradicate bacteria, and wounds remained clear up to the third day post-PDT. Incubation of human dermal keratinocytes with fullerene up to 1 μM under illumination did not significantly influence cell viability.

As reported in Chap. 7, light sources used in cancer PDT usually emit between 600 and 700 nm. Thus, cancer PDT still faces some limitation due to poor tissue penetration of these wavelengths compared to near-infrared (NIR) wavelengths (800–1000 nm) to activate PS molecules. Thus, NIR-excited upconversion nanoparticles (UCNPs) emerge as a new strategy that could be used to activate PS molecules in much deeper tissues. UCNPs are usually lanthanide-doped nanocrystals, which emit high-energy photons (e.g., blue light) under excitation by low-energy photons (NIR light). Loading of PS molecules on to UCNP can be by encapsulation, non-covalently physical adsorption, or covalent conjugation [35].

Park et al. were the first to describe effective in vivo PDT through the systemic administration of UCNP-chlorine6 (Ce6) followed by 980-nm irradiation [36]. UCNP-Ce6 was injected in nude mice bearing U87MG tumors through the tail. Accumulated UCNP-Ce6 in tumor was visualized by luminescence and magnetic resonance imaging. Following irradiation, tumor growth of mice was significantly inhibited compared with other control groups. Authors concluded that UCNP-Ce6 presents great potential for multimodal imaging-guided PDT.

Human trials using NP-based PDT are still scarce in literature. In fact, there is one study which reported that MB-loaded poly(lactic-co-glycolic) (PLGA) nanoparticles may be a promising adjunct to treat chronic periodontitis under 660 nm light [37]. However, animal models as reported above demonstrate a promising future to this emerging therapeutic platform.

References

1. Shen J, Chui C, Tao X. Luminous fabric devices for wearable low-level light therapy. *Biomed Opt Express*. 2013;4(12):2925–37.
2. Lucroy MD. Photodynamic therapy for companion animals with cancer. *Vet Clin North Am Small Anim Pract*. 2002;32(3):693–702.
3. Truschnegg A, Pichelmayer M, Acham S, Jakse N. Nonsurgical treatment of an epulis by photodynamic therapy. *Photodiagnosis Photodyn Ther*. 2016;14:1–3.
4. Ribeiro MG, Rodrigues de Farias M, Roesler U, Roth K, Rodigheri SM, Ostrowsky MA, et al. Phenotypic and genotypic characterization of *Prototheca zopfii* in a dog with enteric signs. *Res Vet Sci*. 2009;87(3):479–81.
5. Sellera FP, Sabino CP, Ribeiro MS, Gargano RG, Benites NR, Melville PA, et al. In vitro photoinactivation of bovine mastitis related pathogens. *Photodiagnosis Photodyn Ther*. 2016;13:276–81.
6. Tardivo JP, Adami F, Correa JA, Pinhal MA, Baptista MS. A clinical trial testing the efficacy of PDT in preventing amputation in diabetic patients. *Photodiagnosis Photodyn Ther*. 2014;11(3):342–50.
7. Tardivo JP, Baptista MS. Treatment of osteomyelitis in the feet of diabetic patients by photodynamic antimicrobial chemotherapy. *Photomed Laser Surg*. 2009;27(1):145–50.
8. Siqueira EG, Rahal SC, Ribeiro MG, Paes AC, Listoni FP, Vassalo FG. Exogenous bacterial osteomyelitis in 500 dogs: a retrospective study of etiology and in vitro antimicrobial susceptibility profile (2002–2013). *Vet Q*. 2014;34(4):201–4.
9. Traxler HV, Maguire PJ, Fischetti AJ, Lesser AS. What is your diagnosis? Osteomyelitis of the patella. *J Am Vet Med Assoc*. 2013;243(11):1529–31.
10. Sherman AB, Clode AB, Gilger BC. Impact of fungal species cultured on outcome in horses with fungal keratitis. *Vet Ophthalmol*. 2016. doi: [10.1111/vop.12381](https://doi.org/10.1111/vop.12381). [Epub ahead of print].
11. Lahav M, Dueker D, Bhatt PN, Albert DM. Photodynamic inactivation in experimental herpetic keratitis. *Arch Ophthalmol*. 1975;93(3):207–14.
12. Shih MH, Huang FC. Effects of photodynamic therapy on rapidly growing nontuberculous mycobacteria keratitis. *Invest Ophthalmol Vis Sci*. 2011;52(1):223–9.
13. Wentink G, Frankena K, Bosch J, Vandehoek J, van den Berg T. Prevention of disease transmission by semen in cattle. *Livest Prod Sci*. 2000;62(3):207–20.
14. Givens M, Waldrop J. Bovine viral diarrhea virus in embryo and semen production systems. *Vet Clin North Am Food Anim Pract*. 2004;20(1):21–38.
15. Givens M, Marley M. Pathogens that cause infertility of bulls or transmission via semen. *Theriogenology*. 2008;70(3):504–7.
16. Suttmoller P, Wrathall A. The risks of disease transmission by embryo transfer in cattle. *Rev Sci Technol OIE*. 1997;16(1):226–39.
17. Wrathall A, Simmons H, Van Soom A. Evaluation of risks of viral transmission to recipients of bovine embryos arising from fertilisation with virus-infected semen. *Theriogenology*. 2006;65(2):247–74.
18. Van Soom A, Wrathall A, Herrler A, Nauwynck H. Is the zona pellucida an efficient barrier to viral infection? *Reprod Fertil Dev*. 2010;22(1):21–31.

19. Jha RK, Jha PK, Chaudhury K, Rana SV, Guha SK. An emerging interface between life science and nanotechnology: present status and prospects of reproductive healthcare aided by nano-biotechnology. *Nano Rev.* 2014;5:22762. doi: [10.3402/nano.v5.22762](https://doi.org/10.3402/nano.v5.22762).
20. Perni S, Prokopovich P, Pratten J, Parkin IP, Wilson M. Nanoparticles: their potential use in antibacterial photodynamic therapy. *Photochem Photobiol Sci.* 2011;10(5):712–20.
21. Narband N, Mubarak M, Ready D, Parkin IP, Nair SP, Green MA, et al. Quantum dots as enhancers of the efficacy of bacterial lethal photosensitization. *Nanotechnology.* 2008;19(44):445102.
22. El-Hussein A, Mfouo-Tynga I, Abdel-Harith M, Abrahamse H. Comparative study between the photodynamic ability of gold and silver nanoparticles in mediating cell death in breast and lung cancer cell lines. *J Photochem Photobiol B.* 2015;153:67–75.
23. Duchi S, Ramos-Romero S, Dozza B, Guerra-Rebollo M, Cattini L, Ballestri M, et al. Development of near-infrared photoactivable phthalocyanine-loaded nanoparticles to kill tumor cells: an improved tool for photodynamic therapy of solid cancers. *Nanomedicine.* 2016;12(16):1885–97.
24. Klepac-Ceraj V, Patel N, Song X, Holewa C, Patel C, Kent R, et al. Photodynamic effects of methylene blue-loaded polymeric nanoparticles on dental plaque bacteria. *Lasers Surg Med.* 2011;43(7):600–6.
25. Fontana CR, Lerman MA, Patel N, Grecco C, Costa CA, Amiji MM, et al. Safety assessment of oral photodynamic therapy in rats. *Lasers Med Sci.* 2013;28(2):479–86.
26. Eshghi H, Sazgarnia A, Rahimizadeh M, Attaran N, Bakavoli M, Soudmand S. Protoporphyrin IX-gold nanoparticle conjugates as an efficient photosensitizer in cervical cancer therapy. *Photodiagnosis Photodyn Ther.* 2013;10(3):304–12.
27. Gil-Tomas J, Tubby S, Parkin I, Narband N, Dekker L, Nair S, et al. Lethal photosensitisation of *Staphylococcus aureus* using a toluidine blue O-tiopronin-gold nanoparticle conjugate. *J Mater Chem.* 2007;17(35):3739–46.
28. Petryayeva E, Krull U. Localized surface Plasmon resonance: nanostructures, bioassays and biosensing—a review. *Anal Chim Acta.* 2011;706(1):8–24.
29. Narband N, Tubby S, Parkin I, Gil-Tomas J, Ready D, Nair S, et al. Gold nanoparticles enhance the toluidine blue-induced lethal photosensitisation of *staphylococcus aureus*. *Curr Nanosci.* 2008;4(4):409–14.
30. Piston D, Kremers G. Fluorescent protein FRET: the good, the bad and the ugly. *Trends Biochem Sci.* 2007;32(9):407–14.
31. Li L, Zhao JF, Won N, Jin H, Kim S, Chen JY. Quantum dot-aluminum phthalocyanine conjugates perform photodynamic reactions to kill cancer cells via fluorescence resonance energy transfer. *Nanoscale Res Lett.* 2012;7(1):386.
32. Arooj S, Nazir S, Nadhman A, Ahmad N, Muhammad B, Ahmad I, et al. Novel ZnO:Ag nanocomposites induce significant oxidative stress in human fibroblast malignant melanoma (Ht144) cells. *Beilstein J Nanotechnol.* 2015;6:570–82.
33. Huang YY, Sharma SK, Yin R, Agrawal T, Chiang LY, Hamblin MR. Functionalized fullerenes in photodynamic therapy. *J Biomed Nanotechnol.* 2014;10(9):1918–36.
34. Grinholc M, Nakonieczna J, Fila G, Taraszkievicz A, Kawiak A, Szewczyk G, et al. Antimicrobial photodynamic therapy with fulleropyrrolidine: photoinactivation mechanism of *Staphylococcus aureus*, in vitro and in vivo studies. *Appl Microbiol Biotechnol.* 2015;99(9):4031–43.
35. Wang C, Cheng L, Liu Z. Upconversion nanoparticles for photodynamic therapy and other cancer therapeutics. *Theranostics.* 2013;3(5):317–30.
36. Park YI, Kim HM, Kim JH, Moon KC, Yoo B, Lee KT, et al. Theranostic probe based on lanthanide-doped nanoparticles for simultaneous in vivo dual-modal imaging and photodynamic therapy. *Adv Mater.* 2012;24(42):5755–61.
37. de Freitas LM, Calixto GM, Chorilli M, Giusti JS, Bagnato VS, Soukos NS, et al. Polymeric nanoparticle-based photodynamic therapy for chronic periodontitis in vivo. *Int J Mol Sci.* 2016;17(5):769. doi: [10.3390/ijms17050769](https://doi.org/10.3390/ijms17050769).