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Antibacterial nano-photosensitizers in Photodynamic therapy: an update

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Short Summary: Nanotechnology has been introduced in Antibacterial photodynamic therapy (PDT) to enhance the clinical performance of this promising therapeutic tool in Medicine and Dentistry. In this paper we summarize the potential use of nano-photosensitizers in PDT systems.

Abstract

Bacterial infections constitute a major challenge of clinical medicine, specifically in specialties like Dermatology and Dental Medicine. Antiseptics and antibiotics have been until now the main adjunctive therapies to anti-infective procedures in the latter specialties. Antibacterial Photodynamic Therapy (PDT) was introduced as a novel and promising alternative to conventional antibacterial approaches. PDT relies on the formation of reactive oxygen species by a photosensitizer (PS), after activation by a specific light source. Nanotechnology was later introduced to enhance the antibacterial efficacy of PS during PDT. In this review, we will describe the different nanoparticles used in PDT and their properties. The recent *in vivo* data of nanoparticles in antibacterial PDT in Dermatology and Dental medicine will also be reviewed.

Introduction

Bacterial infections are one of the major challenges of many medical specialties such as dental medicine and dermatology etc. Antibiotic discovery was a game changer in the fight against pathogenic bacterial strains, but with time, the emergence of bacterial resistance to antibiotics, has become a major concern to the medical community. Hence, research effort has been put to develop novel antibacterial tools that can bypass this adaptation capacity of bacteria ¹. Furthermore, we discovered that bacterial colonies form in a biofilm configuration. The latter confers protection against antibacterial molecules in contact with the surface of these biofilms as they cannot penetrate to the deepest layers. In this context, photodynamic therapy (PDT), initially investigated for cancer treatment, has been introduced and tested as a local antimicrobial tool ², that does not generate any bacterial resistance ³. PDT is a technique by which an inert photosensitizer (PS) called also dye, capable of passively entering/binding bacterial cells is activated by a specific light source to form and release reactive oxygen species (ROS). ROS will have a local bactericidal effect, proportionate to the light activation timing (Figure 1). Many molecules have been used with variable but promising efficacy ⁴, and PDT was considered to have great potential in many medical fields such as dermatology or dental medicine where fighting bacterial biofilms is crucial. On the other hand, one of the limitation of PS molecules is their accessibility to the bacterial biofilms forming in the deep tissues or complex anatomical regions. Nanotechnology has been introduced to PDT ⁵ as a potential solution to overcome the tissues penetration limitations of PS molecules, the hydrophobic nature of certain PS and to enhance the final antibacterial effect ^{6,7}. Thus, nanoparticles with PS carrier properties were developed, as well as Nano-PS (n-PS) molecules with an ROS proper capability. In this paper, we will describe the different families of n-PS, and we will review the recent preclinical data about their antibacterial effect, specifically in accessible, surface biofilms such as in skin and oral infectious models. Substantial published data has shown an *in vitro* bactericidal effect of n-PS on different bacterial cultures in planktonic states as well as in biofilm configuration ⁸⁻¹¹. Research efforts were in major part oriented towards periodontal ¹², endodontic ¹³ or skin infections-related bacteria¹⁴. To the best of our knowledge, there is still no published clinical studies testing a n-PS use in bacterial infections. Hence, this review focuses on the recent *in vivo* data in oral and skin infections' models, as well as the safety and regulatory

concerns raised by this novel technology as this could give a clearer idea about the future clinical potential of these n-PS.

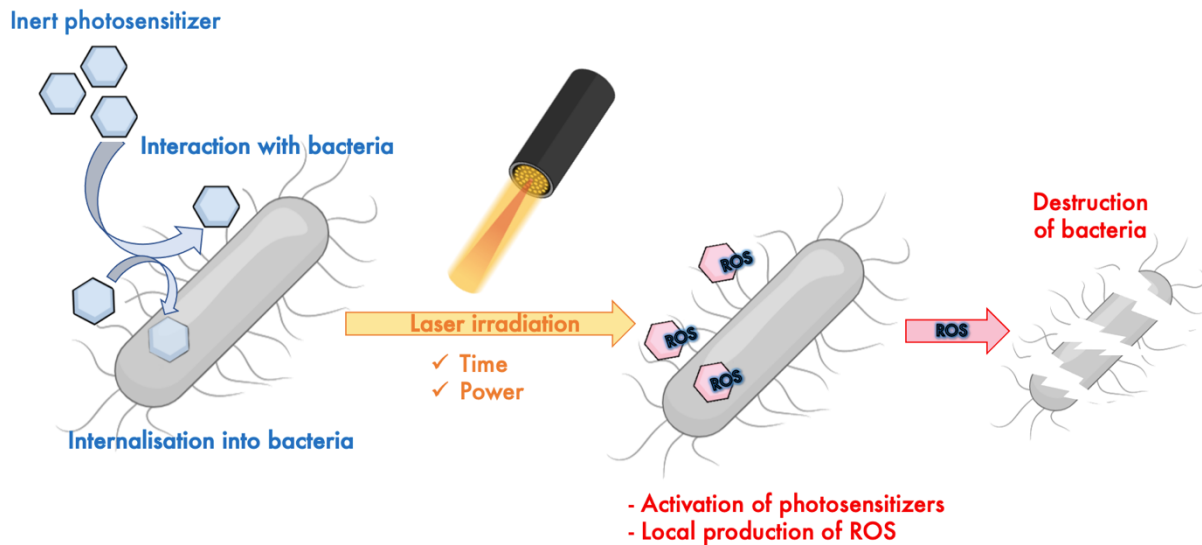


Figure 1

Principle of antibacterial photodynamic therapy (PDT). An inert photosensitizer (PS) can bind to the bacterial wall or enter the bacterium. Once excited with a specific wavelength light source, reactive oxygen species (ROS) are released from the PS and exert a bactericidal effect.

Nano-Photosensitizers

Nano-Photosensitizers (n-PS) can be divided into two categories, nanoparticles as carriers/delivery systems of dyes or nanoparticles with intrinsic ROS production capacities. (Figure 2, Table 1). Their use as carriers has been the most frequent.

Nanoparticle as delivery systems of PS

Different nanocarriers have been used to synthesize stable dispersion of pharmaceutical formulations in aqueous medium for protecting and delivering PS¹⁵.

Thanks to their physico-chemical properties such as their porosity and their high surface to volume ratio, they provide a great encapsulation capacity. In addition, it is quite easy to functionalize their surface to target bacteria. Interestingly, strategies have been explored to control the release of PS to reduce side effects/toxicity due to an inappropriate leakage. For example, a pH-responsive PS release approach has been developed by Nakayama et al (ref).

Briefly, PS (i.e methylene blue) was released once the carrier entering the acidic tumor microenvironment and generate ROS upon light irradiation ¹⁶.

Considering the acidic environment also observed in infected tissues, this approach could offer potential perspectives for tackling bacterial infections.

Liposomes

Liposomes are vesicles formed by concentric lipid layers combining phospholipids and cholesterol. It contains a hydrophilic inner phase that could harbor hydrophilic dyes. As for the lipophilic dyes, they can be carried in the interior lipid layer. This characteristic makes it a suitable carrier for different types of PS ¹⁷. Liposomes have also shown adequate biocompatibility and biodegradability.

After the fusion with the bacteria wall, liposomes can release PS directly into the bacteria. Liposomes loaded with PS can also bind to bacteria resulting in migration of PS from the lipid bilayer to the bacteria wall. In absence of direct interaction between liposome and bacteria, PS can be released from the liposome into the surrounding medium and diffuse through the bacteria wall ¹⁸.

Unfortunately, liposomes showed short half-life circulation. Initially, a water-soluble polymer (i.e PEG) has been used to extend the half-life of liposomes with success (around 45 hours as compared to unmodified liposomes) ¹⁹.

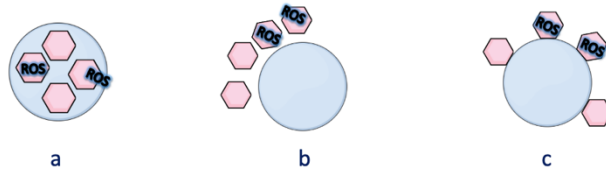
In addition, enhancements to increase the half-life of liposomes or to transform them to cationic state have been made to increase their antibacterial effects. In fact, an overall cationic charge optimizes interactions with the negative bacterial wall. For example, cetyltrimethyl ammonium bromide is a cationic compound displaying strong antimicrobial activity. Cationic liposomes embedded with this compound have been synthesized to enhance the activity of the loaded PS (i.e Ce6) ²⁰. The resulted liposomes showed efficacy on drug-resistant strains of *C. albicans*.

Recently, Mai et al. used a modified cationic liposome to carry sinoporphyrin sodium as PS against Gram-negative *P. aeruginosa* strains ²¹. The modified liposomes permitted to increase the intrabacterial PS uptake and thus the bactericidal and antibiofilm effects were enhanced in bacterial culture models.

Although antimicrobial efficacy has been demonstrated, these formulations raised some concerns due to their high affinity to biological membranes resulting in a binding

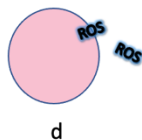
competition between the host cells and the bacteria. This will cause damages of the host tissues upon illumination.

❖ **Nanoparticle as delivery systems of PS**



- Liposomes
- Silica Nanoparticles
- Polymers
- Metallic Nanoparticles
- Quantum dots
- Carbon nanotubes

❖ **Nanoparticle as PS**



- Fullerenes
- Metal oxides NPs
- Quantum dots, Semiconductors (TiO₂, ZnO)
- Carbon dots
- Black phosphorus nanosheets

Figure 2

Two main categories of nano-photosensitizers (n-PSs) used in photodynamic therapy (PDT). PS can be delivered by nanoparticles (NPs) by being embedded in NPs (a), bound to their surface (b) or by accompanying them (c). Given their intrinsic chemical reactivity, some NPs have the capacity to produce and release reactive oxygen species (ROS) after being excited by laser-emitted light (d).

Silica Nanoparticles

Silica matrices have high biocompatibility and hydrophilic properties. They can be loaded with PS and protect them from interaction with bacterial degradation mechanisms, such as pH modification. They are also permissive to ROS and can be functionalized by surface modification to enhance the antibacterial activity and stability of the PS molecules. Thus, a photoexcited chlorin e6 has been attached to the surface of silica NPs to enhance antimicrobial activity towards *S. aureus* and MRSA²². Photostability of chlorin e6 was significantly increased.

Furthermore, Lin et al. tuned the hydrophobicity of mesoporous organo-silica nanocarriers of protoporphyrin IX (green light activation, 532 nm) using a hydroxylation process, hence increasing their interaction with lipidic membranes of Gram-negative *E. coli* in antibacterial photodynamic therapy²³.

Polymers

Polymers have been widely used as carriers of hydrophobic PS. Thus, they are biocompatible with high permeability through cell membranes. They have different forms such as nanospheres or micelles and can carry more than one agent and in higher quantities in comparison to other carriers.

In a recent *in vitro* study, indocyanine green loaded in nanospheres coated with chitosan, was used for PDT against *E. faecalis* biofilms in extracted porcine teeth²⁴. After laser activation (810 nm) of the infected root canal, almost 98% bacterial reduction could be observed, with no deleterious temperature rise of the tooth structures.

Metallic Nanoparticles

Metallic nanoparticles such as zinc oxide (ZnO), silver nanoparticles (AgNP) or gold nanoparticles (AuNP) have been known to have an antibacterial effect by different direct or indirect bactericidal mechanisms. They have been conjugated to PS to enhance the aPDT effect. For instance, AuNP have the capacity to enhance the excitation phenomena of PS and the subsequent ROS generation. AuNPs were able to increase the methylene blue (MB) excitation rate and so the production of ROS²⁵. MB-conjugated to AuNPs inactivated a 4-day biofilm of MRSA. As compared to MB alone, MB-AuNPs decreased biofilm by over 5 log₁₀ (vs 1 log₁₀).

By contrast, methylene blue conjugated with AuNP, failed to yield significant antibacterial/antibiofilm superiority in comparison to conventional PDT when used against *S. Mutans* strains cultured *in vitro*²⁶.

Qiu et al. used antibacterial peptides conjugated with Ce6 PS and combined them with AuNP. The latter addition increased the antibacterial effect of PDT against *S. aureus* and *E. coli*, and reduced the biofilm formation in comparison to the PS alone²⁷.

Silver nanoparticles has also been combined to PS to increase the overall antibacterial effect²⁸, as silver has intrinsic antibacterial properties due to silver ions release and there destructive effect on bacterial membranes and proteins. Titanium di-oxide (Ti O₂) has also been successfully functionalized to different PS to enhance the bacterial inhibition properties. TiO₂, like silver has antibacterial properties related to the oxidative attack on

bacterial cells²⁹. ZnO and TiO₂ can also be used as PS as these semiconductors can be excited in the Ultraviolet band to form ROS.

Quantum dots

Quantum dots (QD) can absorb the light and reemit it to the surrounding loaded PS. The reemission can be at higher wavelengths than the ones used for the excitation light. Therefore, they have been considered interesting carriers of PS. Though, the initial QD showed potential cytotoxicity³⁰. Graphene QD have been found to have ROS emitting properties and can be used as a PS³¹. They can be modified by surface functionalization to enhance their biocompatibility and their dispersion in aqueous environment³². Kuo et al. functionalized nitrogen doped graphene QD with amino groups³³. They used the composite n-PS to successfully eliminate methicillin-resistant *S. aureus in vitro*, after PDT with a 670 nm laser. Antibacterial activity of the test PS was higher in comparison to non-modified graphene QD.

Carbon nanotubes

Carbon nanotubes (CNTs) possessed high capabilities to develop ultrathin films with high surface exchange facilitating immobilization of PSs. Protoporphyrin IX has been conjugated to multi-walled CNTs by Banerjee et al³⁴.

Upon 1 hours of irradiation with visible light, colonies of *S. aureus* were reduced by 15-20% as compared to the untreated control. Obviously, a continuous and controllable production of ROS with high selectivity would provide a better antimicrobial efficiency.

Nanoparticle as PS

Fullerenes

Fullerenes (C₆₀, C₇₀, C₈₄ etc.) are cage shaped molecules that can produce ROS after illumination and attaining a triplet state. They have a high ultraviolet absorption spectrum, and manifest high hydrophobic and insolubility behavior. Their neutral charge is usually modified with cationic components including quaternary ammonium groups to enhance their properties³⁵.

Fullerenes and their derivatives including the cationic-functionalized ones possessed broad-spectrum antimicrobial photodynamic activities against bacteria and fungi by producing superoxide anions and free radicals³⁶.

To sum up, fullerenes display advantages over classical PSs such as their resistance to photobleaching, their enhancement of the overall ROS quantum yield and their ability to form self-assembled NPs. Note that surface modifications must be considered to improve their aqueous solubility³⁷.

Metal oxides NPs

Metal oxides nanocomposites (ZnO/TiO₂) displayed greater inactivation of *E. coli* as compared to ZnO or TiO₂ NPs alone³⁸. It appears that the anatase form exhibited a better antimicrobial activity as compared to other crystalline forms such as rutile and brookite³⁹. Coating nanostructured anatase TiO₂ on an implant surface provided antibacterial effects towards periodontal pathogens including *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum*⁴⁰.

Quantum dots, Semiconductors (TiO₂, ZnO)

Described previously

Carbon dots

Carbon dots (CD) have been shown to be particularly promising quantum dots as nPS⁴¹. They have shown bacterial membrane penetration capacities due to their positive charge⁴². Furthermore, they can produce ROS by a triplet energy transfer phenomena⁴³ if excited with a visible or near infrared light. They present the advantage of high photostability and biocompatibility⁴¹ and can be combined to other antibacterial molecules to enhance their antibacterial effect.

Black phosphorus nanosheets

Recently, black phosphorus (BP), one of the most stable allotropes of phosphorus, has been used as a PS when it is peeling off into ultra-thin 2-dimensional nanosheets⁴⁴. Even if these nanosheets have been mostly tested in vitro on a model of growth tumor, further experiments are expected to also demonstrate their antimicrobial activity.

***In vivo* antimicrobial application of n-PS**

Promising preliminary results can be found from animal models of skin infections⁴⁵. In a subcutaneous abscess model in mice (*S. Aureus*), antibacterial effect of manganese dioxide nanoparticles (MnO₂) modified with photosensitizer chlorine e6 (Ce6) polyethylene glycol (PEG) (Ce6@MnO₂-PEG), was tested after 30 min irradiation with a diode light-emitting device (661 nm, 5 mW cm²)⁴⁶. The treated animals with Ce6@MnO₂-PEG showed faster healing and less residual bacterial counts 5 days after treatments, in comparison to MnO₂-PEG, Ce6 or saline. Furthermore, the authors found that treated abscesses with PDT developed an immune reaction superior to other group most interestingly kept an immunological memory that was shown when inoculating again *S. Aureus* at the same abscess site at day 28. A similar study using mice skin infection model, tested a n-PS system formed by mesoporous silica nanoparticles, loaded by graphic quantum dots and erythromycin⁴⁷. The healing after 4 days of treatment, as determined by wound closure was superior for the test group in comparison to controls. After 14 days histomorphometrical analyses from test group showed an almost complete granulation tissue resorption and epidermis reformation. This correlates with the reduced monocyte chemoattractant protein-1 (MCP-1) and Interleukins-6 levels found in the test group serum. The same efficacy to treat *S. aureus* induced skin infection in mice models was reported in the study of Qiu et al.²⁷.

In another study on induced diabetic foot ulcer on Wistar rats⁴⁸, toluidine blue conjugated chitosan coated gold-silver core-shell nanoparticles (TBO-chit-Au-AgN-PS) were used as a PS complex and activated with diode laser emitting at 630 nm for 12min 50s. The tested groups were induced infections by *S. Aeruginosa*, *S. Aureus* or polymicrobial with the latter two strains. PDT applied daily for 7 days showed a significant reduction of bacterial strains and healing of the skin lesions. The test group also showed a decrease of IL-1b and TNF-a, thus an immunoregulatory effect of the therapy participates in the accelerated healing pattern of diabetic foot ulcer. Xu et al⁴⁹ tested the efficiency of a n-PS formed by polylactic-co-glycolic acid coated Toluidine blue on a mice model of methicillin-resistant *S. aureus* induced skin infection. The activation device was a 660 nm laser (5 min). The test group showed significant wound healing in comparison to controls starting from day one after treatment. Interestingly, the authors evaluated the *in vivo* biocompatibility of the tested n-PS by

assessing its effect on distant organs (heart, spleen, lungs, liver, and kidneys) 14 days after intravenous application. Histopathological evaluation of tissues of the different organs failed to detect any inflammatory reaction or tissue damage, thus showing an absence of systemic toxicity. Similar results to Xu et al. study were found in *P. aeruginosa*-infected skin burn mice model, treated with cationic liposomes carrying sinoporphyrin sodium PS²¹.

On the other hand, n-PS for periodontal treatment have been developed. Indocyanine green assembled to polycationic brush poly(2-(dimethylamino) ethyl methacrylate (PDMA) was successfully used as a n-PS to treat experimental periodontitis alveolar bone resorption in rats⁵⁰. Three laser activation sessions at 808 nm were carried for 5 min at one week interval. This yielded a significant reduction for the test group in comparison to the controls, indocyanine green or PDMA when used alone as PS. Also, the test group exhibited at the histological epithelial healing and resolution of the connective tissue infiltrate. This has been confirmed by the reduced TNF-a and IL-1b levels found in the periodontal tissue, in comparisons to all other groups.

Furthermore, functionalized chitosan nanoparticles with rose Bengal were used as n-PS in an animal model of intraosseous implantation of dentin cylinders, inoculated with *P. aeruginosa* LPS⁵¹. A 60 sec photoactivation with a light source of 540 ±15 nm was applied to the test groups. After 4 weeks, histological analysis showed the PDT permitted a superior tissue ingrowth with no inflammation markers, thus showing an efficient LPS activation. In the latter study, the antibacterial efficacy was not evaluated.

Safety concerns relative to nanotechnology applied to PDT?

While great advances have been reported in the antibacterial efficacy of smart nanomaterials used in PDT systems, specific safety issues are pertinently raised. Furthermore, we could regret the lack of studies evaluating the biocompatibility of the latter systems while safety issues related to the nanometer scale are well documented^{52,53}. Briefly, toxicity starts with molecular interactions between nanoparticles and macromolecules from organism such as proteins, lipids and nucleic acids. Nanoparticles display a large surface exchange enhancing adsorption of biomolecules. For instance, interaction with proteins leads to the formation of a dynamic layer called “corona” which is in constant interaction with the surrounding environment. The composition of this envelop

depends on environmental parameters (i.e. nature and concentration of proteins at the site), and on nanoparticles characteristics (shape, structure, size, charge, and hydrophobicity). As expected, hydrophobic nanoparticles adsorb more plasma proteins than hydrophilic ones, thus resulting in fast opsonization processes (i.e. capture by the immune system for phagocytosis). Nanoparticles avidly adsorb albumin, complement proteins, immunoglobulins, fibrinogen and apolipoproteins. The Corona structure acts as a “Trojan-horse” facilitating internalization of nanoparticles into cells. Following cellular uptake, nanoparticles are then exposed to acidic environment and degrading enzymes, such as cathepsin. The latter can process almost all bio-conjugated nanoparticles⁵⁴.

Furthermore, nanoparticles can interact with mitochondria, thus resulting in the production of ROS such as superoxide anions, singlet oxygen, hydrogen peroxide and hydroxyl ions⁵⁵. By disrupting electron transport in the inner mitochondria membrane, nanoparticles interrupt energy production and induce the formation of ROS⁵⁶. Excessive level of ROS can cause a deleterious oxidative stress. Beside these inflammatory responses due to ROS hyperproduction, low levels of ROS can also activate transcription factors which, in turn, increase the expression of inflammation-associated genes⁵⁷.

It has also been demonstrated that ROS can induce the formation of protein-based radicals from ROS interactions⁵⁵. The denaturation results in a loss of protein structure and/or function and induces protein fibrillation/aggregation to form amyloid fibrils, which may lead to unexpected blood clot formation consecutively to fibrinogen binding. Moreover, unfolded proteins may expose new antigenic determinants, which can lead to hypersensitivity reactions or inflammation in vulnerable patients⁵².

In addition, ROS can cause lipids lipoperoxidation, which alters considerably cells or organites membrane permeability⁵².

Lastly, ROS can diffuse easily via nuclear pores and translocate into the nucleus where they oxidate nucleic acids, thus causing genetic material damages. All these modifications can result in genotoxicity, and in long-term carcinogenesis⁵⁸.

Note that these toxic mechanisms depend mainly on the conditions of exposition to nanoparticles⁵³. In the case of antibacterial PDT, application of nanoparticles is mainly topical (i.e oral sphere, skin). However, passage in blood circulation can't be excluded according to the duration of exposition and the concentration of nanoparticles administered at a particular site. Consequently, conducting a toxicokinetic study is highly recommended

firstly to quantify the potential systemic passage of nanoparticles by collecting blood samples at different time points after treatment. In addition, biodistribution and the potential tissue accumulation of nanoparticles can be followed through *in vivo* fluorescence imaging or *ex-vivo*, after sacrificing animals and collecting major organs (ex: liver, spleen, kidney, heart, lung).

Moreover, severity of toxicity depends on other conditions of exposition to nanoparticles including the frequency of applications and the duration of treatment (acute vs chronic). In the case of antibacterial PDT, no long-term treatment is required. This reduces the risk of biopersistence of nanoparticles in body.

To sum up, considering the local application of nano-photosensitizers, the low systemic circulation of nanoparticles expected, the short-term treatment, the toxic risk related to the use of nanoparticles can be considered low but must be assessed. In this attempt, the regulation relative to the safety of nanomaterials has been recently updated⁵³. Their safety assessment is discussed in the context of the general framework for biological evaluation of medical devices, as described in the ISO/TR 13014:2012 and ISO 10993-1:2009 standards and in ISO 14971 standard (“Medical devices – Application of risk management to medical devices”). Note that a complete characterization is highly recommended (see ISO 10993-1 standard) given the impact on toxicity of nanomaterials physicochemical properties⁵². It includes size distribution, chemical composition, particle and mass concentration, charge (ζ -potential), surface properties, pH and viscosity. Recently, ISO 10993-22 standard has been published to provide a general framework for the evaluation of nanoparticles or nanoparticles-containing medical devices, and to define all nanomaterials aspects that require specific investigations. For example, table 1 of ISO 10993-22 standard reports considerations that should be addressed when assessing different types of nanomaterials (i.e. surface nanostructures, nano-objects bound or incorporated within a medical device, with or without intentional/expected release, ...). And table 2 of ISO 10993-22 standard recapitulates fundamental parameters useful for characterizing nanomaterials and gives adequate measurement methods.

Conclusion

PDT has crossed a long way from oncological use to antimicrobial indications. Due to the multiple advantages in comparison to antibiotics, they gained more and more interest in the medical community. Nanotechnology was a novelty in PDT as it permitted to significantly enhance the bactericidal effect directly or indirectly by increasing the penetration of PS. Current data from animal studies shows very promising results regarding the use of nanotechnology in anti-infectious treatments specifically in Dermatology and dentistry, as surface biofilms are the major etiological factor. Hence, the accessibility of excitation lights and PS to the infection areas is relatively easy in comparison to other anatomical sites. Interestingly, preliminary results are showing an immunomodulation effect of PDT with n-PS, which can prevent infection recurrence and accelerate the healing pattern of treated sites.

On the other hand, the antibacterial effect of n-PS increases with the more active molecules are conjugated to the nano-system. In fact, complex n-PS systems need to be developed to achieve the optimal results related to the target tissues and biofilms. This would require extensive research effort to find the best molecules combinations. In our literature search, we found clinical studies using n-PS related to plantar warts⁵⁹ (viral infection) and a case report in an external root resorption case⁶⁰. Thus, in the future, clinical research is needed to assess the clinical performance of n-PS in bacterial infections as well as their supposed safety.

Conflict of interest: The authors declare no conflict of interest

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Nanoparticles as carrier of PS	Nanoparticles as PS
<p>Liposomes¹⁹</p> <p>Delivery of hydrophobic agents</p>	<p>Fullerenes^{35,61}</p> <p>Hydrophobic</p> <p>UV absorption spectrum</p> <p>Neutral charge</p>
<p>Silica nanoparticles^{17,22}</p> <p>Biocompatibility+</p> <p>Hydrophilic</p> <p>Biodegradable</p> <p>Permissive to ROS</p>	<p>Graphene^{31,43}</p> <p>Biocompatibility</p> <p>Carbon nanotubes functionalized to enhance ROS emission</p>
<p>Polymers^{2,45,62}</p> <p>Biocompatibility</p> <p>Delivery of hydrophobic agents</p> <p>Loading of multi agents</p>	<p>Semiconductors metal oxide (ZnO , TiO₂)^{5,43}</p> <p>UV absorption spectrum</p> <p>Biocompatibility</p> <p>Possible functionalization to enhance ROS emission</p>
<p>Gold NP (Aurum)^{26,63}</p> <p>Amplification of the PS excitation</p> <p>Surface modification to bind to PS</p>	
<p>Quantum dots (CdSe or ZnS)^{30,32,43}</p> <p>Light Reemitting properties</p> <p>High PS loading capacity (for Carbon Nanotubes)</p>	

Table 1

Author-year	Animal model	Methods for PDT	Results	Conclusion
Wang et al. 2019	Subcutaneous abscess in 6–8 weeks female BALB/c mice : S. Aureus induced infection	Control: saline Test : nPS : manganese dioxide nanoparticles modified with photosensitizer chlorine e6 and coated with polyethylene glycol (Ce6@MnO2-PEG NPs) : After 30 min of injection : activation with a 661 nm LED light (5 mW cm ² for 30 min	Ce6@MnO2-PEG NPs showed better healing and less residual bacterial load at day 5 after treatment	PDT with Ce6@MnO2-PEG NPs photosensitizer is effective in treating the skin abscess and reducing its recurrence
Wang et al. 2020	6 weeks mice	Ctrl group: sterile saline, PS: mesoporous silica nanoparticles, loaded by graphic quantum dots and erythromycin. 15 min of activation with led lamp (5W)	PDT improved the healing at day 4 and at day 14 test group showed almost complete healing with a decreased cellular inflammatory component	NPs photosensitizer is effective in treating the skin abscess and modulating the inflammatory response
Qiu et al. 2021	E. coli and S. aureus induced skin infection on female BALB/c Mice	Ctrl : PBS Test : PS : self-assembled nanocomposites of an antibacterial photodynamic peptide and poly(ethylene glycol) stabilized gold nanoparticles. 660 nm diode laser irradiation (0.8 W/cm ²) for 10 min	Almost complete wound healing at day 10 for the nPDT group, and histological normal skin structure	NPs photosensitizer is effective in treating the skin abscess and regenerating the damaged skin cell layers
Akhtar et al. 2021	Diabetic male wistar rats model : Induction of foot ulcers by scalpel- induced ulceration on a footpad	Ctrl : untreated foot ulcer group Test : PDT : nPS : toluidine blue conjugated chitosan coated gold–silver core–shell nanoparticles Daily irradiation for 7 days with a 630 nm diode laser light : 12 min and 50 s (100 J/cm ²)	Significant reduction of S. aureus and P. aeruginosa colonies in 7 days, increased neo-angiogenesis and collagen formation in the healed ulcer sites	NPs photosensitizer is effective in treating diabetic foot ulcer infections, and promotes skin regeneration

Mai et al. 2021	Skin burn infection model (<i>P. aeruginosa</i>) on BALB/c mice	Test: PDT: liposomes carrying sinoporphyrin sodium PS activated with laser at 100 J/cm ²	Superior wound healing for the test group and reduction in inflammatory cytokines and bacterial count at day 14	NPs photosensitizer is effective in treating burn-associated infections and reduce bacterial counts
Shi et al. 2021	ligature-induced periodontitis model was established in Sprague–Dawley rats	nPSself-assembled nanoparticles containing ICG and polycationic brush, PDT : Diode Laser 808 nm at 2 W/cm ² for 5 min, once/week for 3 weeks	Reduction of alveolar bone loss for the test group, and reduction of inflammatory cytokines	NPs photosensitizer is effective in treating periodontitis

Table 2