Immobilized Photosensitisers for antimicrobial applications

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Abstract

Photodynamic antimicrobial chemotherapy (PACT) is a very promising alternative to conventional antibiotics for the efficient inactivation of pathogenic microorganisms; this is due to the fact that it is virtually impossible for resistant strains to develop due to the mode of action employed. PACT employs a photosensitizer, which preferentially associates with the microorganism, and is then activated with non-thermal visible light of appropriate wavelength(s) to generate high localized concentrations of reactive oxygen species (ROS), inactivating the microorganism.

The concept of using photosensitizers immobilized on a surface for this purpose is intended to address a range of economic, ecological and public health issues.

Photosensitising molecules that have been immobilized on solid support for PACT applications are described herein. Different supports have been analyzed as well as the target microorganism and the effectiveness of particular combinations of support and photosensitiser.

Keywords: Photodynamic antimicrobial chemotherapy, Pathogen inactivation, Photosensitizer immobilized, disinfection, Reactive Oxygen Species

Contents:

1. Introduction

Nosocomial infections or "Healthcare Associated Infections" (HAI) can cause disability and emotional stress for the patient and may, in some cases, lead to disabling conditions or even death. In addition, since infected patients remain in hospital on average 2.5 times longer than uninfected patients the economic cost derived by the increased length of stay for infected patients is considerable [1,2]. In Europe every year healthcare associated infections cause 25 million extra-days of hospital stay, 37,000 attributable deaths, and contribute to an additional 135,000 deaths every year with a corresponding economic burden of €13–24 billion [3], while in the United States it is estimated that about two million patients develop HAI with a total number of deaths of 99,000, and cost of $33 billion each year [1].

The Centre for Disease Control and Prevention has recognized that contaminated environmental surfaces provide an important potential source for indirect transmission of many healthcare-associated pathogens and contribute to the spreading of infections, thus indicating the need for new and sustainable strategies [4,5,6,7].

Another major challenge is associated with the large number of water-borne diseases which arise from contaminated water [8].

Worldwide 884 million people lack access to clean potable water: developing countries lack access to clean water (1.8 million children die every year from diarrhea) [9], while developed countries face an urgent need to provide efficient waste water treatment, as populations grow. The increasing prevalence of bacterial resistance is another problem for which an urgent solution is needed.

In fact, the traditional methods for water disinfection currently used are effective against bacteria and viruses but have their drawbacks: chlorine disinfection can produce carcinogenic by-products when organic compounds are present in the water [10], whilst use of ozone is expensive and requires *in situ* generation due to its unstable nature [11,12,13]. Thermal [14] and UV-based [15] disinfections require excessive amounts of energy, and thus are expensive and non eco-friendly.

Photodynamic antimicrobial chemotherapy (PACT) offers an alternative, and radically different, strategy for the inactivation of pathogenic micro-organisms [16,17,18,19]

PACT is based on the "photodynamic effect" where a photosensitizer, preferentially associated with a microorganism, is activated with non-thermal visible light of appropriate wavelength(s) to generate toxic species that inactivate the microorganism.

Upon absorption of a photon, the photosensitizer (Ps) is promoted from a lower-energy 'ground state' to a higher-energy singlet state (S) and then, by intersystem crossing, it can convert to an

excited triplet state (T). From the, relatively, long lived triplet state it can then follow two photochemical pathways, named Type I and Type II reactions (Fig. 1).

In the Type I mechanism, Ps molecules react with bio-organic molecules such as the cell membrane constituents and transfer a proton or an electron to form free radicals and radical ions. In a Type II reaction, the excited Ps can transfer its energy directly to molecular oxygen resulting in production of reactive oxygen species (ROS) that are able to kill microbial cells and viruses [20,21].

Finally, the ability to inactivate microorganisms without inducing resistance makes PACT an appealing and useful alternative in treating infections [22,23].

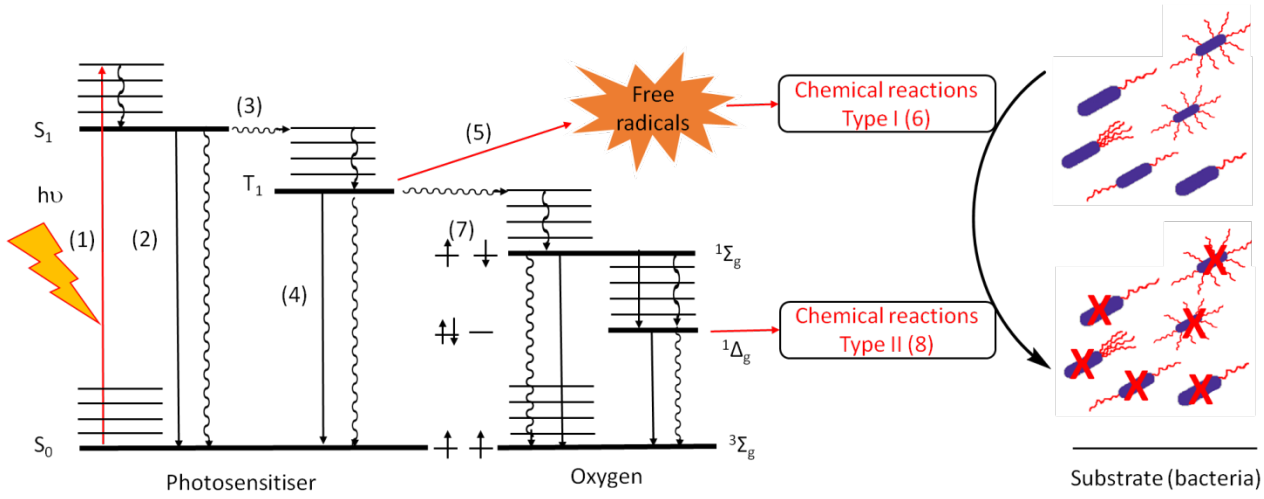

Fig. 1: Jablonski diagram showing the various modes of excitation and relaxation in a chromophore. Light of an appropriate wavelength is absorbed by the photosensitizer molecule. Thereby the photosensitizer changes from its initial ground state (S_0) into an energetically exited state (S_1). From this state the molecule can return to its ground state through (2) = fluorescence emission (3) = intersystem crossing.

Provided that the triplet T_1 state is long-lived in comparison to S_1, it can return to its ground state by (4) = phosphorescence emission or it can (5) = react with surrounding molecules to produce (6) = Type I reactions with free radicals. Otherwise it can react with oxygen to produce (7) = spin exchange and (8) = Type II reactions ($^1O_2$). Singlet oxygen is highly reactive and plays a major role in photodynamic inactivation of pathogens. Curved arrows describe internal conversion and in general loss of energy.

In most cases, Gram (+) and Gram (–) bacteria are susceptible to the photosensitizing action of a variety of sensitizers under appropriate conditions. Examples of photodynamic inactivation of various Gram (+) and Gram (–) bacteria [24], such as *E. coli* [25,26], *S. aureus* [26,27], *S. mutans* [28], *P. gingivalis* [29], and *P. aeruginosa* [27,30] have been documented in the literature.

Various studies have shown that there is a fundamental difference in susceptibility to PACT between Gram (+) and Gram (–) bacteria.

Gram (+) species are more susceptible towards PACT inactivation because their outer wall, located outside the cytoplasmic membrane, is a relatively porous structure that is permeable to nutrients, glycopeptides and polysaccharides with a molecular weight in the 30,000–60,000 Da range and in the same way it allows photosensitisers to cross [31].

Gram (–) bacteria are characterized by the presence of an additional 10-15 nm thick and highly organized outer membrane, which inhibits the penetration of some photosensitisers and photogenerated reactive species [32].

Only relatively hydrophilic compounds with a molecular weight lower than 600–700Da can diffuse through the porin channels that are located in the outer membrane [33].

Since the Gram (–) outer membrane is more negatively charged [34], cationic hydrophilic photosensitizers are attracted to it, while anionic photosensitizers are repelled, and thus are generally only active against Gram (+) bacteria (Fig. 2).

Cationic photosensitizers or anionic photosensitizers co-administrated with an outer membrane disrupting agent can, however, inactivate both Gram (+) and Gram (–) microorganisms.

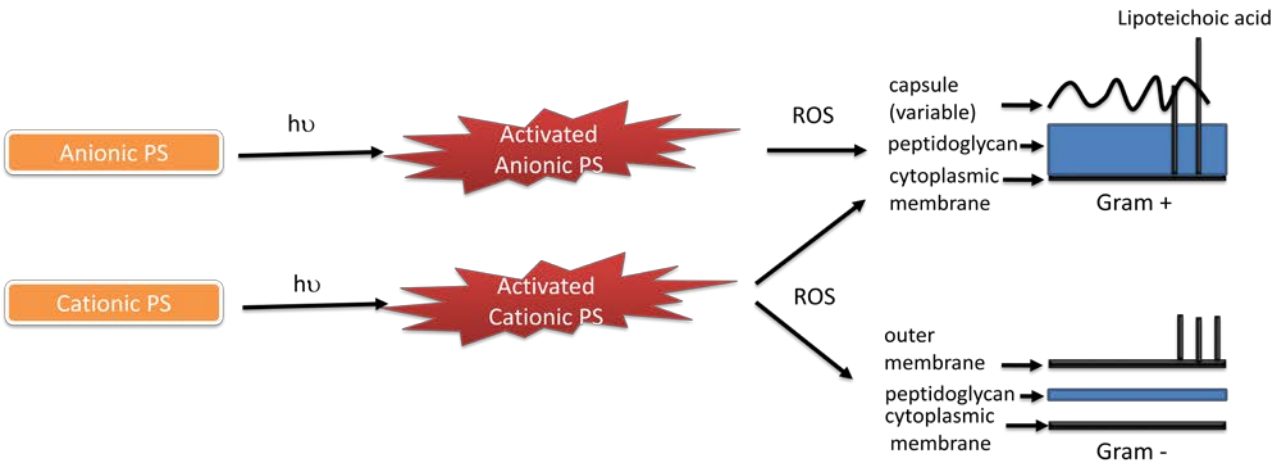

Fig. 2. Schematic representation of antimicrobial PDT. The photosensitizer (Ps) in the presence of light becomes excited and produces toxic oxygen species which damage DNA and/or membrane sites. Anionic photosensitizers are generally active only against Gram (+) bacteria because they cannot permeate the more negatively charged Gram (−) outer membrane [35].

A wide variety of cationic and anionic photosensitizers, such as Rose Bengal (RB), porphyrins, phthalocyanines (Pc), methylene blue (MB), toluidine blue O (TBO), anthraquinones and ruthenium complexes have been utilised for PACT in solution/suspension [36,37,38,39,40].

Ideally, since the Ps do not have to penetrate the bacterium or even come into a contact with the cell in order to be effective [41], immobilization of the photosensitiser aims to allow both the

efficient elimination of microorganisms, possibly during several cycles of use, and also the complete photosensitiser removal from the treated medium.

Other possible benefits include, reuse of the Ps and the possibility of water recycling after disinfection, and the gradual photobleaching of the dyes by solar light, which prevents their accumulation in the environment.

Many patents [42] and publications describe the immobilization of photosensitisers to combat bacterial infections. The aim of this review is to present the photosensitising molecules that have been immobilized on a support, the different supports utilized, and the bacteria that can be inactivated using particular combinations of support and photosensitiser (Table 1) (Fig 3).

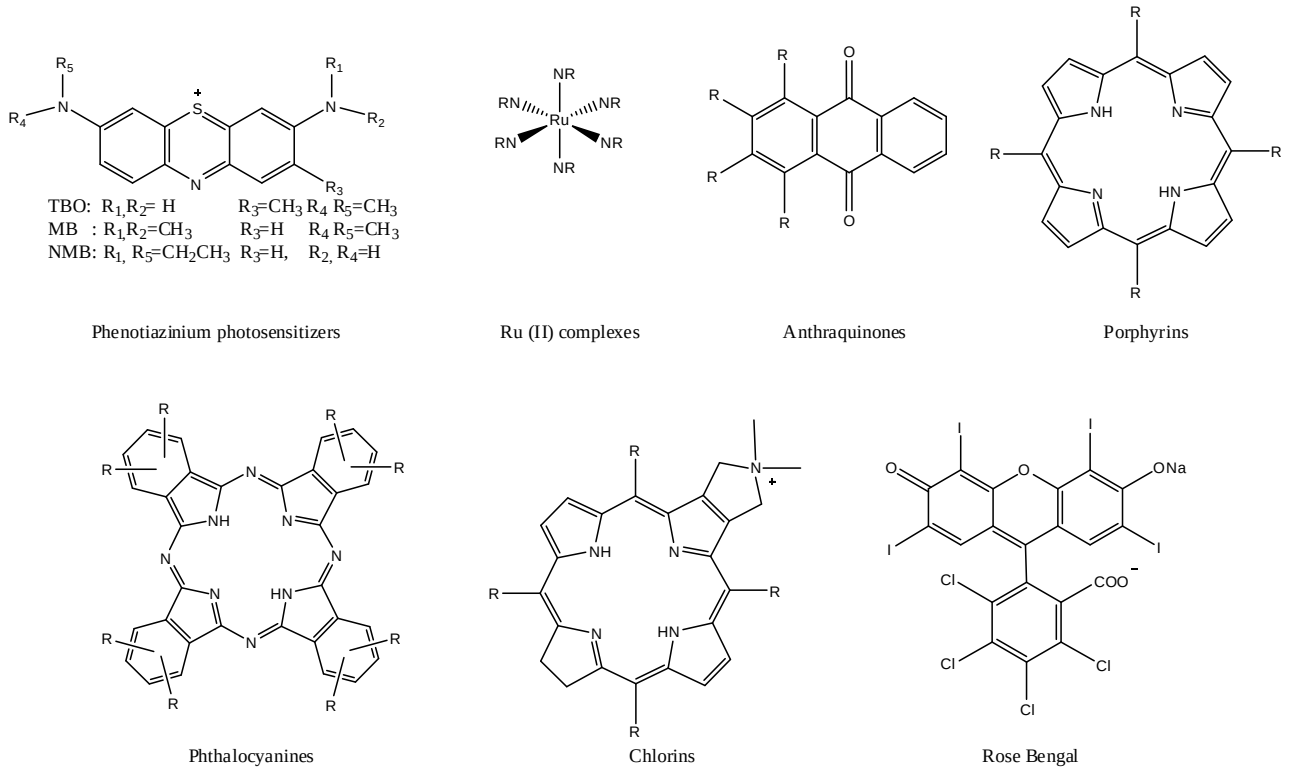

Fig 3. Natural and synthetic photosensitizing unit described in this review used in PACT.

2. Phenothiazinium based photobactericidal materials

Methylene Blue (MB) and Toluidine Blue O (TBO) have great potential applications in PACT due to their low toxicity, the presence of a positive charge that makes them active against both Gram (+) and Gram (–) bacteria, and their favorable photochemical and photophysical characteristics such as light absorption at 650 nm. Both of these photosensitising molecules can be used as PACT agents [43,44,45] for inactivation of viruses and bacteria in blood fractions, and for plasma sterilization [46].

MB and TBO have been incorporated into silicone [47,48,49,50], polyurethane [51,52], polyethylene [53], cellulose acetate [54,55,56], plastics commonly used to fabricate devices used in hospitals such as catheters, and the photoantimicrobial ability of the resulting materials evaluated.

Cahan et al. [53] developed an inexpensive and simple method for preparing antibacterial surfaces by spreading a mixed powder of poly (vinylidene fluoride) nanobeads and three photosensitizers (RB, MB or TBO, 1:10 wt/wt each and previously immobilized on the same type of nanobeads) on the surface of a thermoplastic, low-density polyethylene film (thickness 100 μm). The sandwich layers were covered with a crimpled stamp and exposed to a hot pressing device for 1 h at 95° C. The polyethylene layer was softened under the heat pressing and it trapped the nanobeads with and without the Ps, which remained solid under the pressing temperature. Goniometrical measurements confirmed the hydrophobicity of all the surfaces and energy dispersive X-ray spectroscopy (EDS) analysis was used to determine the concentration of the photosensitisers on the surface, that were 4.59 % and 1.68 % wt/wt, respectively for the MB and TBO, while the concentration of RB on the surface was undetectable, probably because it was below the 1 %, detection limit for this mode of analysis. Significant reactive oxygen species were generated after illumination of the immobilized photosensitizers with a light fluence rate of 1.46 mW cm^{-2} for 30 minutes. Photodynamic inactivation assays performed in nutrient broths under similar conditions for 24 h demonstrated an increase in the antibacterial activity of the photoactive materials as a function of the initial bacterial cell concentration (10^3, 10^5 and 10^7 CFU mL^{-1} for *E. coli*) increasing to more than 4 $\log_{10}$ reduction of the attached *E. coli* after illumination (1.46 mW cm^{-2}) for 24 h when the inoculum was 10^3 CFU mL^{-1}. However, with the same inoculum, more than 4 log reduction of *S. aureus* was observed when the cultures were illuminated for 6 h, showing that *Gram* (+) cells are significantly more sensitive to the antibacterial effect of the surfaces than Gram (–).

Dyes were also incorporated together with nanogold into medical grade polymers commonly used in urinary catheter devices i.e. silicone and polyurethane using the “swell-encapsulation-shrink” method. An appropriate mixed solvent system allowed the polymer to swell thus enabling both dye and the nanoparticles (if used) to enter into the polymer matrix. After drying in air to allow

evaporation of the solvent mixture, the polymer contracted to its original size, resulting in strongly colored dye-encapsulated polymer [48,49,50,51,52,57]. These antimicrobial polymers show significant antimicrobial activity against *S. aureus* and *S. aureus* (MRSA) when exposed to white light for 24 hours [50,51] or for 1 to 10 min against *S. aureus* (MRSA), *E. coli*, *S. epidermidis* when exposed to light from a low power 660 nm laser [48,49,52,57].

Interestingly, the material properties, with regard to both the surface roughness and elasticity were investigated before and after the exposure to radical species [47]. It is known that the radical species produced during gas plasma sterilization result in a decrease of elasticity of the polyurethane and an increase in brittleness, both undesired effects as they would cause problems during catheter removal [58,59].

The result demonstrated that exposure to laser light did not modify the elasticity (Young's modulus), the friction coefficient [52] or breaking point of the silicone containing photosensitizer. The surface roughness of the material and other surface topography parameters, such as the asperity density and the asperity height showed instead a continuous decrease with energy dose, thus making the material less prone to microbial adhesion [47]. The authors also demonstrated that a laser irradiation performed for 10 mins every 60 mins for 6 hours can inhibit biofilm formation and can reduce the extent of surface colonization.

Furthermore, since the irradiated material didn't become more brittle, this makes the light-activated material still suitable for catheter production since a reduction in elasticity would make the material more brittle causing problems during catheter insertion/removal [47].

TBO was incorporated into cellulose acetate polymer, which could be applied as a coating (either permanently or on a renewable basis) to hospital surfaces for surface disinfection [54,55,56]. The ability of TBO to kill a range of microbes under lighting conditions similar to those present in hospitals was evaluated.

The incorporation of TBO into cellulose acetate resulted in an antimicrobial material that can kill effectively both a methicillin-resistant strain of *S. aureus* (EMRSA) and *P. aeruginosa* in 24 hours eradicating in the order of 10^5 CFU/cm^2 of both bacteria over a 24 hour period using white light illumination (60 W domestic lamp bulb), a level adequate to potentially reduce the bacterial population found on common surfaces in hospitals [54].

The antimicrobial TBO cellulose acetate polymer demonstrated a potent photoinactivation of a range of microorganisms such as *S. aureus*, *E. coli*, *C. albicans*, *C. difficile*, and *bacteriophage X174* (host organism, E. coli ATCC 13706) upon illumination with a white light source (28 W fluorescent lamp) for periods ranging from 2 h to 16 h [55]. *C. albicans* was found to be the least

sensitive to photosensitization using this system, with an 88% reduction in the viable count of *C. albicans* after 16 h irradiation. [55]

Furthermore, Decraene evaluated the effectiveness of the coatings against microbes deposited onto surface from aerosols, as this is closer to the true situation found in hospitals [56].

Interesting, for *E. coli* the efficacy of bacterial photoinactivation was found to be dependent on the fluid the bacteria was suspended in with greater values for PBS than for human saliva, or horse serum (99.8 %, 97.6 % and 78.9 % respectively).

TBO was also conjugated to chitosan and this resulted in an improved efficacy against biofilm cells of *S. aureus* (MRSA) and planktonic cells of *P. aeruginosa*, and *A. baumannii*. Chitosan alone and without illumination had no antimicrobial activity, suggesting that the potentiated effect of chitosan worked after the bacterial damage induced by PACT [60].

TBO was also incorporated into a mucoadhesive patch as a potential delivery system for use in PACT of oropharyngeal candidiasis [61].

The authors also investigated the effect on *C. albicans* biofilms using TBO and illumination at 635 nm. With biofilms, higher concentrations of TBO and longer incubation times were required to achieve a total inactivation of' biofilms than for planktonic cells. Therefore, the authors suggested that short application times of TBO-containing mucoadhesive patches should allow treatment of recently acquired oropharyngeal candidiasis, whereas longer times are required for persistent disease where biofilms are already formed [61].

Wainwright et al. [62] dispersed new methylene blue, a methylene blue analogue, in urethane-acrylate and styrene-butadiene copolymers (40% w/w) and the antimicrobial activity of the resultant copolymer films was tested against both *S. epidermis* and *E. coli* bacteria.

When compared to polyacrylic ester films, the MB-containing styrene-butadiene films exhibited a greater antibacterial activity. This might be related to the different hydrophobicities of the two polymer types. Overall, the antimicrobial activity was more evident against the Gram (+) bacteria *S. epidermidis*, than the Gram (–) bacteria *E. coli*. Furthermore, for both bacterial strains, photodynamic inactivation assays gave the best results at both highest photosensitizer concentration (1000 μM) and highest light dose (11.5 J cm^{-2}).

Piccirillo et al. [49] reported the first example of TBO covalently bound at the surface of an activated silicone polymer. The antibacterial efficiency was tested against *E. coli* and *S. epidermidis* by exposure to 634 nm laser light. The polymer possessed significant activity even when the dye was present at a relatively low concentration, probably because the dye was held at the surface and the generated ROS were in the best position to interact with bacteria, owing to their short diffusion distances.

It was found that the presence of 2 nm in diameter gold nanoparticles synergistically enhanced the killing of *E. coli* and *S. epidermidis* when encapsulated in silicone with MB even though the mechanism of action is still poorly understood [48].

In another study, a polysiloxane polymer embedded with MB and 2 nm nanogold particles showed up to a 3.5 $\log_{10}$ reduction of *S. aureus* (MRSA) and *E. coli* when exposed for 5 min to a low power 600 nm laser. [57]

Naik et al. [51] incorporated MB and TBO with gold nanoparticles into polyurethane. When irradiated with white light for 24 hours, MB and TBO impregnated polyurethane polymers showed a 2.8 log and 4.3 log reduction in *S. aureus* respectively. An additional 1 $\log_{10}$ reduction in bacteria in the case of MB and 0.5 log in the case of TBO was observed when the gold nanoparticles were incorporated with the two photosensitizers.

Interestingly, in both cases the incorporation of 2 nm nanogold particles significantly enhanced the ability of MB to kill bacteria even though the mechanism of action is still poorly understood. It has been hypothesized that the gold nanoparticles might enhance the hydrophobic properties of the polymer or they might increase the kinetics of the reactions between the ROS generated by the photosensitizers and the microorganisms.

Since it is known that optical and electronic properties of gold nanoparticles are affected by their size, Perni et al [48] studied the effect of the size of the gold nanoparticles on the antimicrobial properties of MB silicone polymer demonstrating an enhanced light-activated antimicrobial activity against Gram (+) and Gram (–) bacteria for nanoparticles of 2 nm.

Another study by Perni [52] however, indicated that the presence of nanogold did not improve the antimicrobial activity of TBO embedded in polyurethane, even if the uptake of TBO in polyurethane was higher than that reported for silicone. This might be due to the inaccessibility of the dye entrapped in the polyurethane. In fact, a study of suspended TBO-tiopronin-gold nanoparticle in aqueous solution demonstrated a four-fold decrease in minimum bactericidal concentration under white light or 632 nm laser illumination when compared with the free TBO. [63].

In an earlier paper, Savino [64] reported a MB conjugate, where the photosensitizer was covalently immobilized on 2% poly(styrene) copolymer by nitration, reduction and diazotization. That conjugate was found able to disinfect contaminated tap water with *E. coli* to levels acceptable for drinking.

MB and TBO are active against a wide range of bacteria and viruses and they have been successfully immobilized in a wide range of polymers, showing the ability to inactivate bacteria and viruses even under light conditions similar to those commonly used in hospitals. Those materials in

the future may play an important role in decreasing the incidence and the spreading of nosocomial infection. They can find other key applications, such as the development of devices to disinfect water.

The field still faces with the key challenge of having a photobactericidal material with significant activity and with the dye present at as the lowest concentration as possible, avoiding the leaching of the dye from the material, a problem that has been observed sometimes. A covalent attachment of the phenothiazinum dyes to the surface of the inert support may minimize the leaching of the biocidal agent into the surrounding environment and prevent aggregation.

3. Ruthenium complexes

Ru(II) metal complexes with ligands, such as 2, 2′-bipyridine (bpy) [65,66] and 1,10-phenanthroline-5,6-dione (phendione) [67] recently showed remarkable photo-killing ability and therefore potential PACT applications. In fact those compounds appear particularly appealing due to the intrinsic positive charges and the consequent potent binding capacity to the negatively charged outer membrane of Gram (–) bacteria i.e. *E. coli* [68], the production of ROS [69,70], and the possibility of assembling peripheral ligands around the central metal to design a transition-metal complex with favorable functions such as, water solubility and biological compatibility.

Bourdelande et al. [71] demonstrated that aqueous suspensions of a Ru(II) complex, $[Ru(bpac)_3]^{2+}$ where bpac = 4,4'-dicarboxy-2,2'-bipyridine, both free in solution and covalently immobilized on Sephadex G-25 (a hydrophilic resin formed by copolymerization of dextran and epichlorohydrine) forming an insoluble hydrophilic polymer, are able to effectively generate singlet oxygen.

With the aim of carrying out a laboratory-to-pilot-installation study on water disinfection by polymer-supported Ru(II) complexes, porous silicone hollow cylinders, cationic derivatives of nylon, poly(vinylidene difluoride) (PVDF) membranes and cellulose membranes were selected to immobilized different Ru(II) complexes, such as [tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II)] dichloride, tris(1,10-phenanthrolinyl-4,7-bis(benzenesulfonate) ruthenate(II)) and tris(4,4'-dinonyl-1,10 phenanthroline)ruthenium(II) from concentrated hydroalcoholic or aqueous solutions (typically in the mM range) until saturation of the support was achieved [72].

Among all the different couples investigated, [tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II)] dichloride (abbreviated RDP^{2+}) embedded in porous silicone hollow cylinders yielded the best combination of O_2 quenching efficiency and singlet oxygen lifetime, efficient singlet oxygen generation and bactericidal action against *E. coli* and *E. faecalis* under sunlight with no photosensitizer leaching into the water.

On the contrary, significant leaching was observed with tris(1,10-phenanthrolinyl-4,7-bis(benzenesulfonate) ruthenate(II)) embedded in cationic nylon and cellulose membrane.

Manjon [73,74,75] and Villien [76] (Fig. 4.) recently evaluated the efficiency of different $^1O_2$ photosensitizing Ru(II) tris–chelate complexes immobilized on anionic and cationic porous silicone in solar reactor prototypes for the disinfection of water contaminated with *E. coli* or *E. faecalis*. Anionic and cationic porous silicone were selected as support due to their optical transparency in the visible region, excellent oxygen permeability, the durability and the porosity, that increases the accessibility of the lethal ROS to the target microorganisms (Fig. 4).

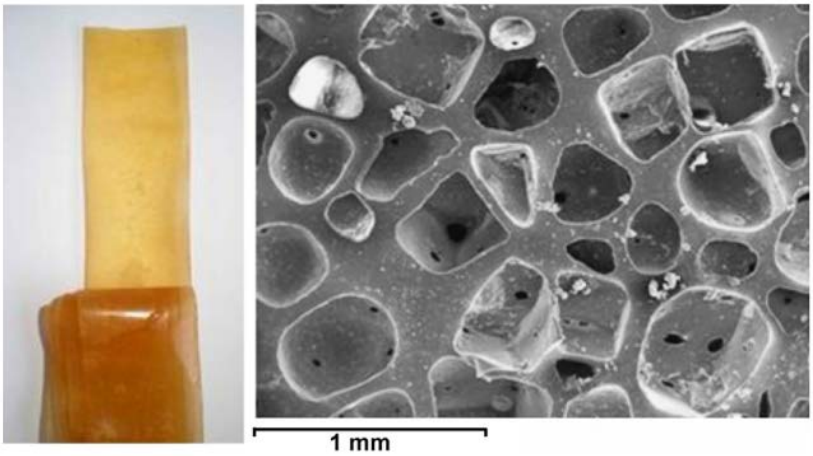

Fig. 4. Photograph of a 35 mm wide porous silicone (pSil) stripe with RDP^{2+} photosensitizer dye (left) and a scanning electron micrograph of an undyed porous silicone strip (right) [76].

All provided significant inactivation using both artificial light or exposing the silicone strips to sunlight. Furthermore, the solid support proved to be reusable after reloading the sunlight-bleached substrate with new photosensitising material [75].

The recovery and reuse of immobilized photosensitizer opens the possibility to apply the photodynamic process in a real waste treatment system, avoiding the photosensitizer release and consequent contamination of water effluents.

Manjon et al. [73] synthesized a new photosentising material where C 60–fullerene and tris(4,7-diphenyl-1,10-phenanthroline)ruthenium(II) dichloride were embedded into porous silicone using the swell-encapsulation-shrink method. That material had favorable photophysical properties, but exhibited poor inactivation of waterborne bacteria due to aggregation.

Ru(II) based photokilling materials only recently have showed photobactericidal properties, thus offering the possibility of new developments for PACT applications. The possibility to turn the properties of the desired complex by changing the peripheral ligands is a key property that can be used to develop highly efficient photosensitizers to combat antibiotic resistant pathogenic bacteria and to create new photobactericidal materials that may have potential key applications in domestic and healthcare settings.

4. Rose Bengal

Rose Bengal (RB) is a commercially available, highly water soluble anionic photosensitizer with high singlet oxygen quantum yield, low rate of photodegradation and with a remarkable antibacterial activity against Gram (+) bacteria [77] when irradiated with simulated sunlight [78]. The cellular envelope has been identified as a probable target [79].

To increase the killing efficiency against both Gram (+) and Gram (–) bacteria at lower concentrations, RB has been incorporated into natural polymers, such as cellulose acetate [55,56] and chitosan [80,81,82,83,84,85].

Chitosan (CS), the N-deacetylated derivative of chitin, is a natural linear biopolyaminosaccharide consisting of 1,4-linked N-acetyl-D-glucosamine (GlcNAc) and D-glucosamine (GlcN).

It is inexpensive, biodegradable, and nontoxic for mammals. It has an antimicrobial activity itself [86,87] and it possess free amino groups, which makes it attractive for the development of new chemical bonds. Due to these favorable characteristics, it has received significant interest in a broad range of scientific areas such as the food industry [88], cosmetics [89], pharmaceutical and biomedical sciences [90] such as dentistry [91].

Moczek et al. proved that RB attached to the chitosan did not decrease the photosensitizing activity of the chromophore when attached through dehydration or covalent linkage to form two conjugates with different degrees of substitution with RB [80].

The content of RB attached to the polymer was found to be 0.013 mol % for the conjugate obtained through dehydration and 0.35 mol % for the conjugate obtained through covalent linkage with respect to the glucosamine unit of the chitosan, respectively.

Results indicated that the shape of the absorption spectrum and the ratio of the absorbance at the maxima were not dependent on polymer concentration in the range studied (1–0.1 g/L), and the quantum yield of singlet oxygen formed by RB after conjugation with chitosan was very similar to free RB in water.

Since chitosan has mucoadhesive properties, the possibility to use CS nanoparticles functionalized with photosensitizer RB (CSRBnp) was explored in dentistry to improve root canal disinfection [85] even in the presence of tissue inhibitors within root canals [82].

E. faecalis, a Gram (+) facultative microbe, was selected as a model since it plays an important role in the biofilm formation on biomedical devices and it is frequently the only surviving bacterium in recurrent root canal infection.

Chitosan nanoparticles were functionalized with RB to provide a single-step treatment in a synergistic approach combining the antibacterial properties of the conjugate and the chitosan reinforcing ability on dentin-matrix [81].

RB was immobilized onto chitosan nanoparticles *via* amide bonds using N-ethyl-N ′ -(3-dimethyl aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) as coupling agents.

Based on the absorption spectra, the amount of RB bound in the conjugated CSRBnp was calculated to be 14 μM per 0.1 mg/mL. Photocytotoxicity studies revealed higher fibroblast cell survival, where compared to RB alone highlighting the biocompatibility of the conjugate [81]. When tested on planktonic cultures and biofilms of *E. faecalis*, a similar CSRBnp conjugate, but with a lower concentration of RB bound in the conjugate (3 μM in 0.3 mg/ml of CSRB), demonstrated better PACT efficacy than RB alone [85].

When dentin collagen was crosslinked to CSRBnp, the CSRBnp-cross-linked dentin collagen showed higher resistance to collagenase degradation and superior mechanical properties [81].

The antibacterial and antibiofilm efficacy of a polycationic chitosan-conjugated Rose Bengal photosensitizer (CSRB) were also tested on *P. aeruginosa* (Gram –) [84] since Pseudomonas species are frequently associated with chronic infections and they have been detected in persistent root canal infections. Concentration of CSRB uptaken by the bacterial biofilms was significantly higher than that of RB alone, especially in the biofilms. Photoactivation studies resulted in significantly higher elimination of bacterial biofilms with CSRB than RB alone, highlighting the advantage of using polycationic CSRB over anionic RB to achieve improved antibiofilm efficacy.

Rose Bengal and chitosan were covalently attached to the surface of polydimethylsiloxane (PDMS) through a two-step argon plasma treatment. First acrylic acid was grafted onto PDMS to form PDMS-pAAc films that were further conjugated to CH.RB through chitosan amino groups, via EDC/NHS mediated coupling [83]. The amount of RB present in the CH.RB conjugate was found to be 0.1 mol% by UV–vis spectroscopy at 575 nm and the grafted CH.RB was estimated to be 10.4 ± 0.1 mg/cm^2 on PDMS-pAAc films.

Preliminary antibacterial testing against *S. aureus* and *E. coli* revealed that the system might be potentially applicable towards Gram (+) bacteria.

Decraene et al. [55] investigated the photokilling ability of RB immobilized together with TBO at the same concentrations on cellulose acetate by evaporation of acetone. They showed that the leakage of photosensitizer was extremely small and produced a microbiocidal surface active under visible (white) light conditions. The coating was shown to be effective against *S. aureus*, *S. aureus* (MRSA), *C. difficile*, *E. coli, bacteriophage X174*, and *C. albicans*, but exhibited a greater photo killing ability for Gram (+) bacteria.

Rose Bengal has been linked to synthetic polymeric supports such as silica [92], polystyrene (PS) [64,93,94,95,96,97,98], Merrifield Resin [99] and polyethylene films with poly(vinylidene fluoride) PVDF nanobeads [53].

The concept of inclusion of RB into a solid phase was raised in the 1970s [99, 96].

The first report of RB immobilized on a surface was by Shaap [99] in 1975 who linked RB by covalent bonds to Merrifield Resin, a co-polymer consisting of styrene and divinylbenzene.

Studies of singlet oxygen production found that the immobilized photosensitizer had a lower rate of singlet oxygen generation, due most likely to diffusion problems.

Bezman et al. in 1978 [96] first showed the photokilling ability of RB-PS nanoparticles towards *E. coli*, which was reported to be effective in killing 99.99% of *E. coli* in a contaminated water sample after 1-2 h exposure to white light.

Since polystyrene is considered to be a commonly available and low-cost material, RB was immobilized on polystyrene porous films of cationically functionalized 2% DVB-crosslinked polystyrene beads [95].

Nakonechny et al. [94] immobilized MB and RB on polystyrene by casting in chloroform and subsequent air evaporation. The films were shown to have a porous structure with pores ranging from 1 to 3 μm. Bacterial cells grew well on the surface of polystyrene and some of them even starting to develop biofilms for a stronger attachment to the polystyrene surface. After 3 h under illumination with white light in the presence of the immobilized RB the concentrations of *S. aureus* and of *E. coli* dropped by 3 log and by 2.5 log respectively when using bacterial cells at a concentration of 10^4 cells mL^{-1}. Under the same experimental conditions, immobilized MB demonstrated lower efficiency than RB for both *S. aureus* and *E. coli.*

Recently, RB was immobilized onto a honeycomb film [93] made of poly(styrene-4-vinylbenzyl chloride) (*ca*. 20 000 g mol^{-1} molar mass, with a low 1.2 dispersity) formed by nitroxide-mediated radical polymerization. Rose Bengal was introduced subsequently by grafting through nucleophilic substitution.

The porous polymer film, with a 2 − 2.5 μm diameter and a well-organized hexagonal patterned surface, was more efficient for oxidation of organic molecules *via* singlet oxygen production at a liquid/solid interface when compared with the corresponding non-porous flat films, revealing promising PACT potential.

Nanoparticles surfaces modified with photosensitizer have also been proposed to enhance antimicrobial activity of free RB [92].

Rose Bengal was used in silica nanoparticles to inactivate the Gram (+) bacteria, *S. epidermis* and *S. aureus* (MRSA) [92].

The transparent silica nanoparticles functionalized with amine groups (SiO_2-NH_2), were prepared by hydrolysis of TEOS in a reverse micro-emulsion method, functionalized with amino groups then covalently attached to RB using EDC in MES buffer (pH = 6).

SiO_2 –NH_2 –RB were shown to be more potent than free RB at inactivating Gram (+) bacteria. The same conjugate was reported to have a singlet oxygen quantum yield lower than free RB (0.60 vs 0.75). Nevertheless, this value is higher than 0.43 previously obtained for RB bound to micron-size polymer beads [99]. This suggest that nanoparticles increase the surface area making easier the access of RB to the molecular oxygen present in the solution, thus increasing the damage to the bacterial cells.

Overall, it appears that RB is a good candidate for PACT applications because it's commercially available at high purity. Furthermore its carboxylate function, through a nucleophilic substitution, allows the formations of covalent bonds between the inert support and the dye, resulting in antimicrobial materials that may show more stability. On the other hand, the anionic character might decrease the antibacterial activity spectrum. The conjugation of the anionic dye to polycationic polymers such as chitosan seems to be an interesting approach to improve antibacterial efficacy. Also the use of porous materials might improve increases the accessibility of the lethal ROS to the target microorganisms.

5. Phthalocyanines

Phthalocyanines (Pc) are extended macrocyclic systems that have provoked significant interest in PACT. Cationic water soluble phthalocyanines were shown to be active against Gram (–) *E. coli* [100], *P. aeruginosa* [100,101,102] Gram (+) *S. aureus* (MRSA) [101,102], *E. faecalis* [102] and the fungi *C. albicans* [101,102,103]. Chen et al. [104] demonstrated that poly-cationic lysine moieties used as support for zinc phthalocyanine were active against Gram (+) and Gram (–) bacteria, both *in vitro* and *in vivo*. The presence of a positive charge appears to promote a tight electrostatic interaction with negative charges on lipopolysaccharides at the outer surface of Gram (–) bacteria.

Recently, polymeric fibers doped with phthalocyanines were applied for the fabrication of photoantimicrobial surfaces using the electrospinning technique [105,106,107,108,109].

Electrospinning has proven to be a relatively simple and versatile method for forming non-woven fibrous mats with a defined porosity and water permeability with a very high fraction of surface available to interact with cells. The possibility to host a variety of molecules to fine-tune their properties for specific applications, together with the possibility to modify the structure, the chemical and mechanical stability, and the functionality, makes this method appealing for antimicrobial applications [110]

Following the photodegradation of Orange-G, Modisha et al. [111] confirmed that electrospun conjugates of (2,3,9,10,16,17,23,24-octacarboxyphthalocyaninato)zinc(II) with magnetic nanoparticles in polyamide-6 (PA-6) fibers were able to generate singlet oxygen after the electrospinning process and Tombe [107] reported singlet oxygen quantum yields of 0.28 and 0.13 for a (4,11,18,25-tetrabenzylphthalocyaninato)zinc(II)-gold nanoparticle conjugate immobilized on electrospun polystyrene fibers with and without gold atoms, respectively. The immobilized conjugate was active as a photocatalyst for oxidizing organic pollutants, such as 4-chlorophenol and Orange G using oxygen as an oxidant. Interestingly, Goethals et al. [112] reported that PA-6 membranes functionalized with [2,9,16,23-tetra(2-thioquinoline)phthalocyaninato]zinc(II)) after the electrospinning deposition were capable of photobleaching significantly more DPBF than membranes that were non functionalized.

Polystyrene electrospun fibers were also employed as they have extensive π–π electronic interactions between the aromatic systems of the phthalocyanine and the polymer [105,106,107,108].

Masilela et al. [106] first reported the antimicrobial photo-inhibitory activity of a series of Zn(II) phthalocyanines incorporated into electro-spun polystyrene fibers. The biocidal effect of asymmetrical versus symmetrical substitution on the phthalocyanines was investigated using *S.*

aureus. All the unsymmetrically substituted complexes showed antimicrobial activity towards *S. aureus* under illumination with visible light. The symmetrical (phthalocyaninato)zinc(II) (ZnPc) and its symmetrical tetracarboxy derivative [2,9,16,23-tetra(4-carboxyphenoxy)phthalocyaninato]zinc(II) showed no activity under illumination with light in the fiber matrix due to low levels of singlet oxygen production.

Since heavy metals are expected to increase singlet oxygen quantum yield through enhanced intersystem crossing, as a result of the heavy atom effect, Osifeko [105] incorporated into polystyrene electrospun fibers low symmetry Pcs (i.e. mono substituted), including [2,9,16,23-tetra(4-pyridyloxy)phthalocyaninato]lead(II) (PbTpyPc) and its tetracationic derivative [2,9,16,23-tetra(4-*N*-methylpyridyloxy)phthalocyaninato]lead(II).

The tetracationic electrospun photosensitizer exhibited better singlet oxygen quantum yield and improved inactivation response against *E. coli*, compared to the neutral precursor. Similarly, when the tetracationic conjugate was tested, it was found to be more active than the non-ionic precursor as no colony was observed on the agar plates after 30 minutes of irradiation with white light. Since leaching studies revealed that the phthalocyanines are not released from the fibers, the authors concluded that Pb doesn't result in additional toxicity.

Alternatively, Mosinger used polyurethane (PUR) electrospun nanofibers as the polymeric support for incorporation of unsubstituted ZnPc [108].

Zinc phthalocyanine was revealed to be an efficient photooxidizing substrate. When exposed to white light for 30 minutes, electrospun PUR-ZnPc doped nanofibers where able to kill *E. coli*, however better results were obtained with polyurethane nanofibers doped with 5,10,15,20-tetraphenylphorphyrin (TPP).

Artarsky et al. also investigated the use of zinc phthalocyanines for PACT [113]. In this particular example two different phthalocyanine compounds, [2,9,16,23-tetra(4-terbutyl)phthalocyaninato]zinc(II) (TBZnPc) and (2,9,16,23-tetrasuphoxyphthalocyaninato)zinc(II) (ZnPcTS) were entrapped into a silicate matrix prepared from tetraethylorthosilicate (TEOS) by the sol–gel method.

The tetracationic ZnPcTS conjugate demonstrated more effective singlet oxygen production than the neutral TBZnPc conjugate.

The photobactericidal results confirmed that the tetracationic ZnPcTS was more effective than the neutral TBZnPc in killing *E. coli* in microbially polluted waters (*E. coli* reductions of about 1 log after 120 minutes of exposure)

The authors hypothesized that ZnPcTS, being the dye with the more pronounced hydrophilic character is likely to be preferentially deposited near the sol–gel surface, where the hydrophilic

506 character is prevailing and thus not evenly distributed throughout the whole bulk, while the tertiary
507 butyl derivative (TBZnPc) is mainly present in the internal parts of the matrix as a result of which it
508 is less accessible and therefore less active.
509 Phthalocyanines have been immobilized on a polymeric cellulose diacetate film [114] by co-
510 dissolution and casting or covalent attachment to a membrane [115] of chitosan, and used in a
511 circulating water photoreactor system as a model for a large-scale water-flow system [115].
512 For this purpose chitosan membranes were found to be very brittle, but their flexibility was
513 improved by casting the polymer into a nylon support, which offered flexibility without altering the
514 final transparency or translucency of the membranes.
515 The concentration of the (2,9,16,23-tetrasuphoxyphthalocyaninato)zinc(II) as tetrasodium salt
516 (ZnPcS) covalently attached to the membrane was roughly estimated as 9 μg cm^{-2} based on solution
517 molar absorbance.
518 Photoantimicrobicidal activity was observed for the reinforced zinc phthalocyanine/chitosan
519 membrane after 35 mins with a 0.90 $x10^{-5}$cfu ml^{-1} cell count (initial cell count was 1.99 $x10^{-5}$cfu ml^{-1}) that dropped to $8.3x10^{2}$ cfu ml^{-1}, showing a bacterial kill of >2 log in 160 min. Interestingly, the
521 same membrane, kept in the dark, after 9 months still showed a detectable activity with a reduction
522 of approximatively 1 log of bacteria after 160 min, reflecting the thermodynamic stability of the
523 phthalocyanine system.
524 Pcs have shown to be promising candidate for the development of new antibacterial materials. The
525 possibility to make them positively charged with an appropriate choice of the substituent is an
526 interesting feature that is making them suitable starting materials for the development of new
527 photokilling surfaces. The efficient immobilization of Pc onto solid support and the stability that
528 those materials seem to have help to reduce the cost through an efficient recycling.

6. Porphyrins immobilized on Natural Polymers

Porphyrins have been linked to natural polymers for multiple purposes, for example they have been bound to cellotriose moieties for optoelectronics purposes [116] and they have been conjugated to chitosan to enhance gene transfection using PDT [117].

Commercially available protoporphyrin IX (PPIX) was successfully attached to nanoparticles composed of an iron oxide core coated with dextran by an esterification reaction using 1,1'-carbonyldiimidazole (CDI: 2 eq./porphyrin) as the electrophilic activator. These particles were incorporated into cultured cancer cell lines showing a potential application in PDT [118].

Organized, multilayer organic–inorganic films of sulfonated C60, 5,10,15,20-tetra(4-*N*-methylpyridyl)porphyrin ($TMePyP^{4+}$) and chitosan were formed using electrostatic layer-by-layer (LBL) assembly technology, which has proved to be a facile method for generating a wide range of organized and stable thin films [119].

Porphyrin-based photobactericidal materials have been developed by grafting porphyrin-based compounds onto natural polymers, such as chitosan [114,115] cellulose [120,121,122, 123,124,125,126,127,128,129] and dextran [118].

Porphyrins have been immobilized on polymeric cellulose diacetate films [114] or incorporated into translucent reinforced nylon chitosan membranes by adsorption using 5,10,15,20-tetra(4-hydroxyphenyl)porphyrin, (p-THPP) or by dissolution and casting with 5,10,15,20-tetra(4-aminophenyl)porphyrin, (p-TAPP) and used in a circulating water photoreactor system as a model for a large-scale water-flow system [115]. The concentration of the adsorbed porphyrin was estimated to be about 5.7 μg cm^{-2} while the concentration of the porphyrin immobilized by casting was found to be 7.5 μg cm^{-2} based on solution molar absorbances or on the weight of porphyrin added, respectively.

When tested on *E. coli*, both p-THPP/chitosan and p-TAPP/chitosan membranes displayed a photokilling ability after 40 mins of white light irradiation.

Neutral, anionic, and cationic porphyrins were covalently linked to cotton fabric [120,121,122] as well as to cellulose esters [123,124,125] (Fig. 5) and were able to confer photobactericidal activity on the cellulosic materials.

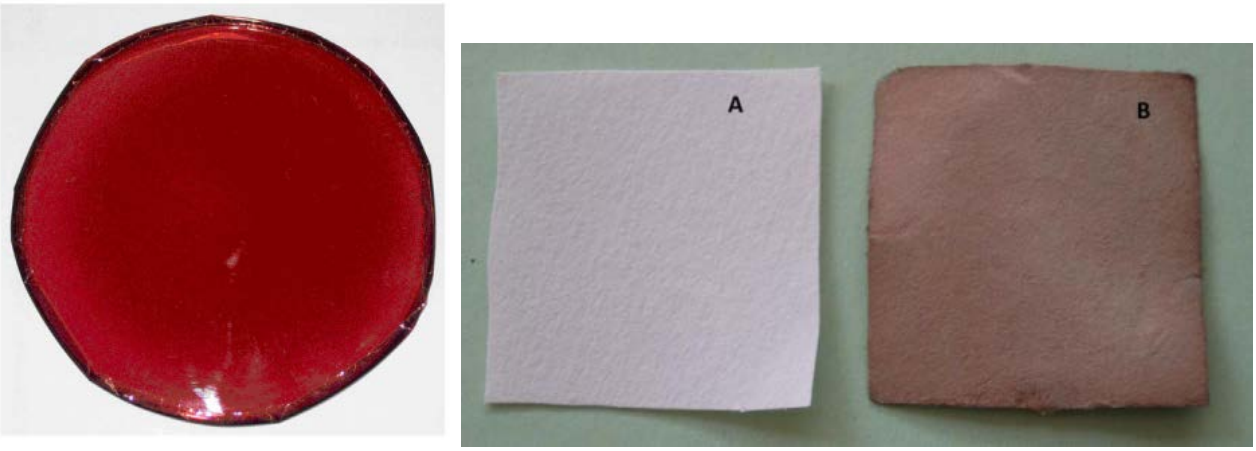

Fig. 5. (Left) Porphyrinated cellulose laurate plastic film of 0.52% PPIX content [123]. (Right) Photographs of (A) filter paper and (B) filter paper after reaction with aminoporphyrin and cyanuric chloride [129].

Protoporphyrin IX (PPIX) [123], 5,10,15-tri(4-methylphenyl)-20-(4-methylpyrydyl)porphyrin [124] and porphyrins with a spacer arms comprising 4- or 11-carbons such as 5-[4-(3-carboxypropyloxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin) and 5-[4-(10-carboxydecanoxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin [125] were covalently attached to cellulose laurate esters films by a ‘‘one-pot, two-step” esterification reaction starting from cellulose and porphyrin.

PPIX [123] was covalently bound to the cellulose using Tosyl chloride and pyridine in dimethylacetamide/lithium chloride (DMA/LiCl) as binary solvent followed by esterification of the remaining carboxylic groups of cellulose by lauric acid in the same binary solvent system. This synthetic procedure allowed the dissolution and chemical modification of cellulose, a natural polymer having stiff, shape-stable structure into a plastic material allowing also the incorporation of the PPIX through a covalent bond. Seven porphyrinated films with different PPIX contents from 0.19% to 1.1% were obtained by casting in a glass Petri dish (Fig. 5.)

No surviving colonies, for both *S. aureus* and *E. coli*, were seen for films with a porphyrin content of 0.52 or higher for PPIX [123], 0.35 for the cationic porphyrin 5,10,15-tri(4-methylphenyl),20-mono(4-*N*-methylpyrydyl)porphyrin [124] and 0.18 and 0.30 for the porphyrins with the 4- or 11-carbons spacer arms, respectively [125].

Ringot et al. [120,122] used the Cu(I) catalyzed Huisgen 1, 3-dipolar cycloaddition or “click reaction” to covalently graft anionic, neutral, and cationic amino porphyrins on cotton fabric, without previous chemical modification of the cellulosic support.

Previously, the same group reported a direct cellulose azidation, followed by a “click” reaction in THF and water with acetylenic porphyrins [122].

5-(4-aminophenyl)-10,15,20-triphenylporphyrin (TPP-NH_2), anionic 5-(4-aminophenyl)-10,15,20-tri(4-sulphonatophenyl)porphyrin (TPPS-NH_2) and cationic 5-(4-methylpyrydyl)-10,20-di(2,4,6-trimethylphenyl)-15-(4-aminophenyl)porphyrin (trans-MePy-NH_2) were grafted to cotton fabric (3.5 x 3.5 cm, 0.27 g) using cyanuric chloride [120,121].

The highly reactive porphyrin with triazine link was characterized after the complete substitution of the chlorine atoms with the use of piperidine (for neutral and cationic products) and sodium sulfanilate (for the anionic compound) [121].

The modified fabric was non-toxic towards either bacterial species in the dark. After 24 h exposure to white light irradiation, all modified surfaces caused a photobactericidal effect in Gram (+) bacteria *S. aureus*. Cationic cotton gave the best result in terms of bacterial growth inhibition, followed by neutral cotton and anionic cotton, percentages of bacterial growth inhibition were 100% for cationic cotton, 93.7% for neutral cotton and 37% for anionic cotton, respectively [120].

Following a similar approach, Mbakidi et al. [129] developed a novel antimicrobial paper by grafting the tricationic porphyrin 5-(4-aminophenyl)-10,15,20-tri(4-*N*-methylpyridyl)porphyrin using the same 1,3,5– triazine derivative described before (Fig. 5).

The porphyrin grafted paper was characterized using diffuse reflectance UV-Vis.

Results have showed a grafting yield of 55% (0.03 µmol/mg of paper simple), which was similar with grafting yields of different porphyrins on cotton fabrics [120].

The photobiocidal activity of the photoantimicrobial filter paper was tested against *E. coli* and *S. aureus.* After 24 hours exposure to white light at a fluence of 9.5 J/cm^2, no surviving bacteria were detected on the grafted filter paper.

While there are several studies of porphyrins immobilized onto cellulosic materials (cotton fabrics, microcrystalline cellulose) or cellulose strands, there have been few studies that describe the covalent bonding of a porphyrin derivative onto a nanocrystalline cellulose (CNC) scaffold and its use as photokilling surface [126,127].

Carpenter [127] and Feese [126] both used nanocrystalline cellulose (CNC) as the support for a photobactericidal material formed from the covalent attachment of a [5,10,15-tri(4-*N*-methylpyridyl)-20-(4-alkylphenyl)porphyrinato]zinc(II) to the surface of an azide-modified cellulose nanocrystals through a “click” reaction.

Nanocrystalline cellulose (CNC) is obtained from cotton fiber through the acid hydrolytic disruption of the amorphous domains of cellulose and the consequent conversion of native cellulose fibers into a colloidal dispersion. Due to its good properties such as large surface area, good mechanical strength and biodegradability, as well as availability and biodegradability, it is currently being investigated as a component of transparent flexible films.

The photobactericidal activity of porphyrin–cellulose nanocrystals films was investigated against a wide variety of bacteria, such as *A. baumannii*, multidrug-resistant *A. baumannii* (MDRAB), methicillin-resistant *S. aureus* (MRSA), *P. aeruginosa* [127], *E. coli*, *S. aureus* and *M. smegmatis* (mycobacterium) [126].

Gram (+) positive *S. aureus*, *S. aureus* (MRSA), Gram (–) *A. baumannii* and *A. baumannii* (MDRAB) gave a reduction in colony forming units (CFUs) even after 15 min illumination with white light.

P. aeruginosa appeared to be susceptible to photodynamic inactivation with no statistically significant inactivation observed when incubated for less than 30 min. For all the bacterial strains examined a 30 min light dose achieved a reduction in viable cells greater than a 15 min light dose, attributable to the higher amount of cytotoxic reactive oxygen species formed, in particular $^1O_2$.

Since confocal laser scanning microscopy after incubation with *S. aureus* suggested a lack of internalization of the Ps, this study also suggested that reactive oxygen species produced extracellularly by photodynamic therapy can be effective without internalization of the photosensitizer.

It has been shown that porphyrins keep their antimicrobial properties when grafted to natural polymers, such as chitosan or cellulose or dextran. These modified polymers have been casted into photobactericidal membranes or films or used as cotton textiles to form eco-friendly materials with potential industrial, medical, and household applications.

The field still faces with the key challenge of having a photobactericidal material with significant activity and with the dye present at the lowest concentration as possible, minimizing the leaching and with an improved durability of the material.

7. Porphyrins linked to synthetic polymers

Porphyrins, due to their versatile nature, have been linked to a great variety of synthetic polymers.

Water-soluble, [5,10,15,20-tetra(4-sulphophenyl)porphyrinato]iron(III) was effectively immobilized into anionic Dowex resin for catalytic purposes. By having a suspension of the Dowex resin in distilled water with 4 mg of the iron(III) porphyrin and stirred for 2-3 hours at room temperature, the attachment of the porphyrin was followed due to the surface changing color and becoming green [130]. This system was stable, in fact even after filtration and washing with distilled water, the solid was found to retain completely the adsorbed iron porphyrin, and it was easily recovered after the reaction and reused without loss of activity.

Similar results were obtained with Mn(II) porphyrins supported on commercially available resins [131].

Ribeiro et al. [132] studied the photocatalytic behavior of porphyrins covalently linked to a Merrifield polymer previously modified with an excess of α, ω-diamines to obtain the aminoalkylated polymers, making them suitable for reaction with chlorosulfonated porphyrins. The authors also reacted the chlorosulfonyl porphyrin with commercially available aminomethylated polystyrene divinylbenzene co-polymer to obtain a porphyrin covalently linked to the polymer but close in proximity to the polymer backbone due to the absence of a spacer molecule. This conjugate was found to have the highest value of porphyrin incorporated. All of the supported photosensitizers were able to generate singlet oxygen with an efficiency dependent on the structure of the spacer between porphyrin and polymer. The catalyst was filtered, washed and dried and could be recycled with a new substrate batch, with one of the catalysts being reused for three catalytic cycles.

Water-soluble Pd(II), Pt(II) and Rh(III) complexes with 5,10,15,20-tetra(4-*N*-methylpyridyl) porphyrin ($TMPyP^{4+}$) and 5,10,15,20-tetra-(4-*N*,*N*,*N*-trimethylaminophenyl)porphyrin were immobilized in per-fluorinated ion-exchange membranes (e.g. Nafion®) after boiling in concentrated nitric acid for 30 min and in double distilled water for 30 min to clean them and to make them optically transparent above 240 nm [133]. The membranes revealed a good photostability and high oxygen permeability.

[5-(4-hydroxyphenyl)-10,15,20-tris(4-sulfonatophenyl)porphyrinato]zinc(II) and [5-(4-hydroxyphenyl)-10,15,20-tris(4-*N*-methylpyrydyl)porphyrinato]zinc(II) were transesterified on transparent poly (methylmethacrylate) polymer films in toluene in the presence of p-toluenesulfonic acid [134]. Related to the number of methyl esters present in the PMMA, the concentration of the porphyrins in the polymer was found to only be 1%.

Xing et al. [135] described the complex formed by electrostatic interactions of water-soluble anionic polythiophene with tetracationic 5,10,15,20-tetra[4-(6-*N*,*N*,*N*-trimethylammoniumhexyloxy)phenyl]porphyrin bromide (TPPN). This electrostatic complex adsorbed Gram (–) and Gram (+) bacteria and generated singlet oxygen effectively to kill the bacteria under white light. In this case, the photokilling ability of the system was tested against *E. coli* and *B. subtilis*, for which ca. 70% and 90% of bacterial viability reduction, respectively, was observed after only 5 min of irradiation with white light at a fluence rate of 90 mW cm^{-2}.

Doped polysilsesquioxane films were synthesized adding the anionic 5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin at different concentrations in THF/water using an appropriate amount of formic acid as catalyst [136]. The final concentrations were 2.6 $x10^{-4}$ w / w and 5.2 $x10^{-4}$ w / w respectively.

Bridged silsesquioxanes allowed the creation of a moldable, versatile and flexible material at room temperature, which could be used for the dispersion of dyes.

In vitro investigations showed that they were able to kill *C. albicans* upon irradiation with visible light. The doped films produced a ~2.5 log decrease in *C. albicans* (99.7 % cellular inactivation) after 60 min irradiation, but 96% cellular inactivation was observed after 30 minutes irradiation.

When tested under conditions of microbial growth, yeast cells exposed to the film and illuminated, showed growth delay compared with controls. The free form of photosensitizer was evaluated as well and it was found to have a small photoinactivation effect of 0.5 log decrease after 60 mins.

5,10,15,20-tetra(4-*N*,*N*-diphenylaminophenyl)porphyrin and its Pd(II) complex immobilized on optically transparent indium tin oxide (ITO) electrodes have been proposed to inactivate *C. albicans* cells for possible applications in the control and disinfection of the aqueous suspension of microorganisms [137].

These films exhibited a photosensitizing activity causing a ~3 log decrease (99.9 % cellular inactivation) of *E. coli* after 30 minutes and ~2.0 log decrease (99.7%) of *C. albicans* survival after 60 minutes, suggesting that eukaryotic cells are more difficult to inactivate than bacteria.

As before [136] yeast cells showed growth delay compared with controls when tested under condition of microbial growth.

Banerjee et al. [138] described the covalent functionalization of carbon nanotubes with porphyrins for antiviral purposes. PPIX was immobilized onto nanomaterial scaffolds such as multi-walled carbon nanotubes (MWNTs) to develop antimicrobial nanocomposite films by combining the biocidal ability of porphyrins with the mechanical strength of the nanotubes.

A treatment with 1000 μg mL^{-1} of the porphyrin-nanotube conjugate caused more than a 250-fold reduction in the effective infectious Influenza A viral dose after a 30 min exposure to visible light. Both the conjugated and the unconjugated MWNTs were incubated in the dark and in both cases there was no observable photokilling effect.

Since carbon nanotubes can be easily recovered by filtration making them appealing for possible reuse of the material, the authors showed that the conjugate can indeed be recovered and reused and it still effectively causes a 50-fold reduction in the infectious viral dose, even after five uses.

In previous work [139], the same porphyrin-nanotube conjugate showed a high bactericidal activity against *S. aureus* cells upon irradiation with visible light. In fact the MWNT - PPIX conjugate, after coating on nitrocellulose filter membranes, was able to inactivate more than 80% of the bacterial colonies after 1 h exposure to visible light.

Gao et al. investigated the use of cross-linked polystyrene (CPS) microspheres (0.32 - 0.45 mm in diameter), with a cross-linking degree of 4% for the direct synthesis of a porphyrin-polystyrene conjugate through modification of the polystyrene microspheres themselves [140].

CPS are readily available, cheap, mechanically robust and chemically inert and they can undergo facile functionalization.

Pyridylporphyrin (PyP) was synthesized directly on the surface of chloromethylated crosslinked polystyrene microspheres (CMCPS microspheres).

Pyridinecarboxaldehyde groups were introduced through a quaternization reaction to form the modified microspheres (PyAL-CPS). Finally, PyAL-CPS microspheres were condensed with pyrrole and free 4-pyridinecarboxaldehyde using the Alder reaction to form the porphyrin *in situ*.

PyP-CPS microspheres were reacted with CH_3I as the quaternization reagent, to obtain the cationic analogue. In other papers [141,142] 4-hydroxybenzaldehyde (HBA)-bound CPS microspheres, pyrrole, and benzaldehyde were condensed similarly, again using the Adler reaction.

Two different methods of analysis allowed confirmation of the attachment of the porphyrin to the microsphere. Through IR spectroscopy it was possible to confirm attachment of the porphyrin to the microsphere, while UV-visible spectroscopy confirmed the presence of the Soret and Q-bands of the porphyrin molecules.

Interestingly, in previous papers [141,142], the amount of porphyrin immobilized on the microsphere surface was determined through complexation of the immobilized porphyrin with zinc ($ZnCl_2$ solution) followed by analysis of Zn ion content in the final solution using EDTA through a complexometric reaction.

Griesbeck et al. [143] more recently reported polymer-bound sensitizer systems using TPP or 5,10,15,20-tetra(4-methylphenyl)porphyrin (TTP). Commercially available polystyrene beads (approx. 60 μm) cross-linked with divinylbenzene were utilized as the polymeric support.

The PS beads were loaded with the sensitizing molecule by swelling with a solution of catalytic amounts of TPP and TTP in ethylacetate followed by evaporation of excess solvent from the solution. Following this photooxidation of β-pinene and ethyl tiglate was used to quantify photoactivity of the porphyrin loaded beads. The authors were able to show that singlet oxygen is produced in a solvent-free photooxygenation process.

Recently Griesbeck et al. designed a solventless reaction which has been the subject of considerable interest as an eco friendly synthetic approach, reducing the amount of environmentally problematic and expensive solvents and retarding the production of side products as a result of the enhanced selectivity [144].

Commercially available PPIX and 5,10,15,20-tetra(4-vinylphenyl)phorphyrin (TSP) were attached to polystyrene beads cross-linked with divinylbenzene. The process was carried out using emulsifier free polymerization of styrene with divinyl benzene for the formation of nanosized polystyrene-divinylbenzene particles. The method was a one-pot synthetic method with the porphyrin embedded

751 in the backbone of the polymer. This technique allowed the syntheses of the translucent particles in
752 a simple and reproducible way.
753 In particular, the production of singlet oxygen under irradiation conditions was of interest from the
754 viewpoint of PACT.
755 Inbaraj et al. [145] reported the functionalization of cationic N-alkylpyridinium polystyrene
756 supports with 5,10,15,20-tetra(4-sodiumsulphonatophenyl)porphyrin (TPPS) and its metallo
757 complexes [5,10,15,20-tetra(4-sodiumsulphonatophenyl)porphyrinato]cadmium(II) (CdTPPS) and
758 [5,10,15,20-tetra(4-sodiumsulphonatophenyl)porphyrinato]zinc(II) (ZnTPPS). Since the polymeric
759 support used was 2% cross-linked divinylbenzene with styrene, the porphyrin molecule was
760 attached by ionic interactions from a pyridine to the sulfonate group on the porphyrin. *N,N*-
761 dimethyl-4-nitrosoaniline (RNO) was used as an indicator for photo-induced singlet oxygen with
762 imidazole as a chemical trap for singlet oxygen. Quantum yields were reported as 0.29, 0.27, and
763 0.16 for PS–H_2TPPS, PS–ZnTPPS, and PS–CdTPPS, respectively, whilst the unbound porphyrins
764 had singlet oxygen quantum yields of 0.62 and 0.81 for H_2TPPS and ZnTPPS, respectively. The
765 binding of the porphyrin to the polymer was found to decrease the quantum yield. The authors
766 attributed this to structural deformation of the appended porphyrins on the spherical shape of
767 polymer bead surface, and the resulting decrease in exposition to light.
768 TTPS and their metalloderivatives [MTPPS; M=Cu(II), Zn(II), Ag(II), and Cd(II)] immobilized on
769 a support made of poly(4-vinylpyridine) (PVP), crosslinked and linear polystyrenes partially
770 chloromethylated and quaternized, and polyethylene glycol (PEG) have demonstrated their ability
771 to carry out enzyme mimetic reactions efficiently [146].
772 Recently, the commercial hydrophilic [5,10,15,20-tetra(4-
773 sodiumsulphonatophenyl)porphyrinato]manganese(III) chloride (MnTPPS) was mixed with
774 dimethyldioctadecyl-ammonium bromide (DODMABr) to form a hydrophobic complex that was
775 used to construct microporous honeycomb films (MHFs) on glass substrates *via* casting an organic
776 solution of MnTPPS-DODMA at relative humidities higher than 80% [147].
777 PS was used to increase the strength of the film but also to modulate the pore sizes.
778 The porous polymer film, 800 nm in diameter and a well-organized hexagonal patterned surface,
779 was more efficient for oxidation of organic molecules *via* singlet oxygen production when
780 compared with the corresponding non-porous thin films. This is in agreement with results by
781 Pessoni et al. [93].
782 These microporous honeycomb films of MnTPPS-DODMA were shown to have more efficient
783 antibacterial activity when compared with MnTPPS-DODMA non-porous thin films (83%
784 reduction versus 43% respectively) upon irradiation with visible light for 1 h. Bacterial reduction in

the dark was 7%, showing a direct correlation between irradiation with light and photokilling ability of the substrate.

Magaraggia et al.[148] encapsulated an hydrophilic porphyrin into silica microparticles prepared by the Stöber method through the ammonia-catalyzed hydrolysis of TEOS to form a conjugate with a mean particle diameter of ca. 0.9 μm.

Limited photobleaching of the encapsulated porphyrin was carried out when the porphyrin was exposed to visible light. The microparticles exhibited a photosensitizing activity causing a decrease in survival by a 4 log reduction after a 20 min irradiation of the Gram (+) bacterium *S. aureus* (MRSA), and a 30 min irradiation of the Gram (–) *E. coli* in the presence of 10 μM of the porphyrin silica microparticles.

Silica based nanomagneto-porphyrin hybrids were described by Alves [149] and Carvalho [150], these materials were particularly appealing due to the possibility to easily isolate and purify them using a magnetic field.

Carvalho et al. investigated the use of magnetic nanoparticles (Fe_2O_3 in this case) surrounded by a silica shell for the attachment of porphyrins for use as antimicrobial agents. Characterization of the nanoparticles was carried out using pXRD and UV-visible spectroscopy. The attachment was monitored by UV-visible spectroscopy and showed that the relative amount of porphyrin attached was 4 – 5% (w / w).

These new multicharged nanomagneto-porphyrin hybrids were very stable in water. The cationic hybrids induced a total photoinactivation of *E. faecalis*, *E. coli*, and T4-like phage, even when used at 20 μM, upon irradiation with white light of 21.6, 43.2, and 14.4 J cm^{-2}, respectively.

5-(2,3,4,5,6-pentafluorophenyl)-10,15,20-tripyridylporphyrin and the corresponding cationic 5,10,15-tri(4-*N*-methylpyridyl)-20-(2,3,4,5,6-pentafluorophenyl)porphyrins as tri-iodide salt were grafted to cationized silica-coated magnetic nanoparticles of Fe_3O_4 and $CoFe_2O_4$ [149]. Their use in PACT against the Gram (–) bacteria *A. fischeri* was investigated by monitoring the decrease in its natural bioluminescence during the photosensitization process using a luminometer and monitoring the photo-inactivation kinetics in real-time.

The cationic nanomagneto-porphyrin hybrids were found to be highly efficient at bacterial inactivation and they also showed sustained photoinactivation over six cycles. It was also shown that 2.5 h (150 min) was required to inactivate 7 log of bacteria (first cycle), but they could cumulatively inactivate 42 log of bacteria in 21.5 h.

Porphyrins have been covalently linked to aminoalkylated silica particles by initial activation of the porphyrin nucleus using chlorosulphonation [151].

Rychtarikova et al. [152] entrapped $TMePyP^{4+}$ in microporous silica gels prepared by the sol–gel method using tetrakis(2-hydroxyethoxy)silane (THES) and tetra methoxysilane (TMOS).

All the composites containing THES showed good adhesion to glass, and the THES composite showed no shrinkage in three months, as well as being shape and volume stable in air for three months. The main disadvantage of the composite is low mechanical and chemical stability.

All of the composities were particularly active against *E. coli* but, in general, THES composites with lower specific surface areas were more effective than TMOS analogs, probably because the PEG 600 improved the flexibility, and thus oxygen diffusion, in the gel.

Recently, nanofibre materials were developed with encapsulated porphyrinoid photosensitizers that generate $^1O_2$ in high quantum yields upon irradiation with visible light. The small diameter of the nanofibres allowed the efficient diffusion of $^1O_2$ outside the nanofibres to kill *E. coli*, *S. aureus* and *P. aeruginosa*. bacteria [108,153,154,155].

Mosinger et al. utilized Polyurethane (PUR), TPP and its Zn(II) derivative (5,10,15,20-tetraphenylphorphyrinato)zinc(II) (ZnTPP) [108] to form nanofiburous layers 0.03 mm thick and with 0.12 % TPP and 0.10 % ZnTPP content, respectively [108,153]. When exposed to light the nanofabrics produce enough singlet oxygen to kill the bacteria cells. PUR, used as control without the incorporated porphyrin sensitizers, either exposed to light or kept in the dark had no effect on the bacterial growth.

The PUR nanofabrics have bactericidal effects at their surfaces, however free-base porphyrin TPP showed better efficiency and photostability.

Electrospun nanofibres were prepared by doping polyurethane Larithane™ (PUR), polycaprolactone (PCL), polystyrene (PS) and polyamide 6 (PA6) with TPP with a final 1 wt % TPP each [156,157].

The doped nanofibre textiles efficiently photo-generate $^1O_2$. When tested against *E. coli*, after 60 minutes irradiation with white light, the PUR, PS, and PCL nanofibre materials exhibited antibacterial activity and completely inhibited bacterial growth upon irradiation with visible light. The PA6 nanofiber showed lower antibacterial activity probably due to lower production of $^1O_2$.

Since metal nanoparticles have been reported to have antibacterial and antifungal properties, Managa et al. [154] tested the antibacterial properties of the conjugate formed between [5,10,15,20-tetra-(4-carboxyphenyl) porphyrinato]gallium(III)chloride (ClGaTCPP) and platinum nanoparticles PtNPs in solution, and after immobilization onto electrospun styrene nanofibers. Gallium was chosen as the central metal in this case because it enhances the intersystem crossing to the triplet state thus improving singlet oxygen, this happens due to the size of gallium, in relation to the heavy atom effect.

When tested in solution, the conjugate (ClGaTCPP)-PtNPs had an improved antibacterial activity when compared to the nanoparticles alone, due to synergistic effects.

The doped nanofabrics, when irradiated with light, showed positive growth inhibition against *S. aureus* when compared to those that were kept in the dark; there was also an enhanced effect for ClGaTCPP–PtNPs, compared with ClGaTCPP.

Recently, Henke et al. [155] studied the influence of the wettability of the surfaces of TPP-PS electrospun nanofibres on the antibacterial activity of *E. coli* on the surface of the electrospun fibres.

Sulfonation, oxygen plasma treatment, and even the application of a thin polydopamine coating on the surface of the polystyrene electrospun nanofibres strongly increased the wettability/hydrophilicity of the hydrophobic polystyrene nanofibers, without causing damage to the nanofibers, leakage of the photosensitizer, or any change in the spectral characteristics of TPP. The increase in surface wettability resulted in acceleration of the photo oxidation of external substrates, and an increase in the antibacterial activity of the nanofibres.

Sherrill et al. investigated the use of nylon films as supports for immobilization of PPIX and zinc PPIX to create an antimicrobial material [158]. Nylon 6,6 was grafted *via* poly (acrylic acid) (PAA) resulting in a surface coverage of 57%.

Grafting the two different porphyrin derivatives (PPIX and Zn-PPIX) resulted in nearly identical values of surface coverage, approximately 36%, for both sample types, however, no biological studies have been carried out on these surfaces to date.

Bozja et al. also investigated the use of nylon fibers as a support for porphyrin molecules [159]. The samples were prepared in a similar way to that reported by Sherrill et al.

The PPIX-nylon conjugate efficiently killed more than 95% of *S. aureus* bacteria after a 30 minutes exposure at a fluence of 60,000 lux, while no effect was observed with Gram (–) *E. coli*. The ZnPPIX-nylon conjugate was revealed to be slightly more efficient against *E. coli*, with a 30% cell killing using 60,000 lux after 30 min exposure. For the Gram (+) bacteria *S. aureus* 94% of bacteria was killed using 40,000 lux. Overall the ZnPPIX was found to be more effective against both Gram (+) *S. aureus* and Gram (–) *E. coli* bacteria.

The attachment of porphyrins to synthetic polymers have been extensively investigated. Since a lot of polymers precursors are cheap and commercially available, this approach presents the advantage of being cheaper than others previously analyzed. Some new photobactericidal materials offered the possibility to be recovered and reused, making the materials very interesting for an eco friendly approach.

8. Others Photosensitiers

Benabbou et al. [160] grafted or incorporated into inert solid supports an anthraquinone derivative, 9,10-anthraquinone 2-carboxylic acid (ANT) and a benzo-[b]triphenylene-9,14-dicarbonitrile (DBTP), as they are known to be good singlet oxygen generators.

The ANT was converted to its triethoxysilyl derivative by condensation with APTES and grafted to commercial silica beads (3–5 mm diameter, pore diameter ca. 9 nm), by reflux in toluene and was shown to be more effective than the DBTP derivatives grafted on a commercial amino functionalized silica powder (Si-NH_2 40–63 μm particles) when tested against *E. coli*. This may have been due to the higher photo-oxidation efficiency of ANT [161]. Both derivatives displayed a good stability in aqueous suspension, with no leakage of the sensitizing molecule into the water. Commercially available silica powders or beads were chosen because 9, 10-anthraquinone immobilized on silica gel was found to be transparent [162].

Chen et al. studied the ability of chitosan to potentiate the activity of erythrosine (ER) against bacteria and yeast through the preparation of nanoparticles by the ionic gelation method.

Comparing the PACT effect against erythrosine alone and chitosan alone, the combination of ER／CS nanoparticles showed an enhanced antimicrobial effect against *S. mutans, P. aeruginosa* and *C. albicans* [163].

Neutral and cationic pyrrolidine fused chlorins have also been investigated recently as potential PACT agents when immobilized on 3-bromopropyl-functionalized silica and Merrifield resin [164]. Since it had been observed from the same research group that the efficiency in the photoinactivation of *E. coli* was influenced by the number of charges on the final immobilized conjugate [165] further treatments with 1-methylimidazole or pyridine were performed on silica gel and on Merrifield resin to increase the number of positive charges on the surface of the material.

Overall, this study showed that the increased number of positive charges and their dispersion on the surface of the materials strongly influences the photodynamic efficiency of the conjugate.

Silica with chlorin and Merrifield resin/chlorin in combination with pyridine showed the best activity against *E. coli*.

9. Conclusions

PACT is a field of ongoing and active research to meet the urgent need to find alternative options for microbial killing.

This technique is particularly appealing due to the possibility of using visible light (and possibly sunlight) to inactivate microorganisms, the possibility to recycle and reuse the photosensitizers in an eco friendly approach, and the lack of bacterial resistance induced in microorganisms.

Since the initial investigations in the 1970s many photosensitizers, including Methylene Blue, Toluidine Bue, Rose Bengal, Ruthenium Complexes, Phthalocyanines and Porphyrins have been immobilized onto a huge variety of supports, mostly natural or synthetic polymers, such as chitosan, cellulose, cotton, polystyrene, polyurethane, nylon, but also silica beads, nanotubes.

Since currently available materials suffer of loss of antimicrobial activity by leaching of the biocide with the potential risk of releasing hazardous agents in the media, PACT community should invest in the development of new supports, and, most important, in the development of new ways to immobilize photosensitizes on solid support to create new photokilling materials with the potential capability of rapid efficient, and low-cost sterilization of a range of bacteria.

A stable and uniform surface coating would allow an high availability of the dye at the surface and the most favorable conditions for the interaction with bacteria and with the oxygen naturally present in the environment. Nevertheless this approach might be difficult due to the fact that sometimes ìs difficult to have an uniform coating of the surface, leading with reproducibility problems. Embed the dye in a porous support might be a promising alternative, proving that the oxygen must be able to and interact with sensitizer and bacteria.

The leaching of the photosensitizer is a general problem that emerged frequently. Thus the development of new ways to immobilize photosensitizes on solid support seems to be a key challenge towards the practical application of solid-supported PACT devices.

The choice of the sensitizer (cationic/anionic) beside being a key factor going to influence the bacterial strains activity of the solid-supported PACT device, need to be done taking into considerations also other desiderables characteristics, such as an economic and easily scale up synthesis.

The progress and the possible applications of those photosensitizing surfaces demonstrate that this is a promising approach for the killing of bacteria, viruses and fungi.

Acknowledgements

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10. Appendix: Table and Abbreviations

Table 1. Reports of photoinactivation of Gram (+) and Gram (–) bacteria and *in vitro*.

Ref	Ps	Support	Light type	Target organism	Fluence rate or Light Dose or lux	Period of illumination
[47]	MB	silicone	laser light (660 nm)	*S. epidermidis*	58 J cm^{-2} 117 J cm^{-2}	5 min every 30 min, 10 min every 60 min or 20 min every 120 min (6 h total)
[48]	MB	silicone elastomers	laser light (660 nm)	*E. coli* *S. epidermis*	19.5 J cm^{-2}	10 min
[49]	MB TBO	silicone polymer	laser light (634 nm)	*S. epidermis* *E. coli*	0.19 W cm^{-2} (TBO) 0.0325 W cm^{-2} (MB)	4 min (TBO) 21 min (MB)
[50]	MB	Silicone polymers	28-W BIAX 2D T5 compact fluorescent lamp, white light	*S. aureus* (MRSA)	2,305 lux	24 h
[51]	MB TBO	PUR	white light	*S. aureus*	2000 lux	24 h
[52]	TBO	PUR silicone polymers	laser light (634 nm)	*E. coli* *S. aureus* (MRSA)		2 min 3 min
[53]	RB, MB TBO	PVDF nanobeads on polyethylene film	luminescent lamp, white light	*S. aureus* *E. coli*	1.46 mW cm^{-1}	3 h 24 h
[54]	TBO	cellulose acetate layer	white light	*S. aureeus* *P. aeruginosa*	778 ± 12 lux	8 h 16 h 24 h
[55]	TBO RB	cellulose acetate	General Electric 28W Biax 2D compact fluorescent lamp, white light	*S. aureus* (MRSA) *E. coli* *C. albicans* *C. difficile* *bacteriophage X174*	3700 ± 20 lux	2 h 4 h 6 h 16 h
[56]	TBO RB	cellulose acetate	General Electric 28W Biax 2D compact fluorescent lamp white light	*S. aureus*	3700 ± 20 lux	6 h
[57]	MB	Silicone elastomers	laser light (660 nm)	*S. aureus* *E. coli*		5 min
[60]	TBO	CS	high Power LED 635±5 nm	*S aureus* *P. aeruginosa* *A. baumannii*	60 mW cm^{-2}	30 min
[61]	TBO	PMVE/MA copolymer	635 nm Paterson Lamp	*C. albicans*	100 mW cm^{-2} 100 J cm^{-2} 200 J cm^{-2}	
[62]	NMB	Urethane-acrylate Styrene-butadiene copolymers	Paterson lamp with filter at 615-645 nm	*S. epidermis* *E. coli*	11.5 J cm^{-2} 5.8 J cm^{-2} 2.9 J cm^{-2} 1.5 J cm^{-2}	0.5 min 1 min 2 min 4 min
[64]	MB RB Eosin	PS resin Silicagel Activated carbon	four cold white TLE 22 W23 Philips lamps	*E. coli*		10 min 20 min 30 min 40 min 60 min
[72]	Ru(II) phenantroline complexes Ru(II) bipyridyl complex	pSil	150 W Xe lamp or sunlight	*E. coli* *E. faecalis*	0.6 M J m^{-2} 0.8 M J m^{-2}	9 h
[73]	Ru(II) phenantroline complex-C60 fullerene	pSil	laboratorary solar simulator-white light	*E. faecalis*	20 W m^{-2}	9 h
[74]	Ru(II) phenantroline complex	pSil	150 W Xe lamp and sunlight	*E. faecalis*	20 W m^{-2} 400 W m^{-2}	4h (Xe lamp) 60 min (sunlight)
[75]	Ru(II) complexes	pSil	150 W Xe lamp and sunlight	*E. faecalis*	400 W m^{-2}	9 h
[76]	Ru(II) phenantroline complex	pSil	sunlight	*E. coli*	0.6 M J m^{-2}	5 h

				E. faecalis	0.8 M J m^{-2}	
[81]	RB	CS membrane	broad-spectrum Lumacare lamp with a 540 ± 15 nm filter (grren light)	*E. faecalis*	5 J cm^{-2} 20 J cm^{-2} 40 J cm^{-2} 60 J cm^{-2}	
[82]	RB	chitosan	broad-spectrum Lumacare lamp with a 540 ± 15 nm filter (grren light)	*E. faecalis*	5 J cm^{-2} 10 J cm^{-2}	1.66 min 3.33 min
[83]	RB	PDMS with chitosan on the surface	120 W incandescent lamp	*E. coli* *S. aureus*		60 min
[84]	RB	CS	broad-spectrum Lumacare lamp with a 540 ± 15 nm filter (grren light)	*E. faecalis* *P. aeurigosa*	20 J cm^{-2} 40 J cm^{-2} 60 J cm^{-2}	15 min 30 min 60 min
[85]	RB	CS	broad-spectrum Lumacare lamp with a 540 ± 15 nm filter (grren light)	*E. faecalis*	20 J cm^{-2} 40 J cm^{-2} 60 J cm^{-2}	
[92]	RB	silica nanoparticles	Lumacare lamp with a 525 nm bandpass filter	*S. aureus* (MRSA) *S. epidermis*	33 J cm^{-2}	40 min
[94]	RB MB	PS films	white luminescent lamp emitting in the range of 400 – 700 nm (visible light)	*S. aureus* *E. coli*	1-3 mW cm^{-2}	30 min 3 h
[96]	RB	PS beads	four 15 W Silvana Fluorescent Bulb	*E. coli*		
[105]	PbTpyPc and its tetracationic derivative [2,9,16,23-tetra(4-*N*-methylpyridyloxy)phthalocyaninato]lead(II)	Electrospun PS fibers	300 W lamp with 600 nm glass and water filters	*E. coli*		30 min
[106]	ZnPc	Electrospun PS fibers	300 W lamp with 600 nm glass and water filters visible light	*S. aureus*		90 min
[108]	ZnPc	Electrospun PUR fibers	150 W cold white light	*E. coli*		30 min
[113]	TBZnPc ZnPcTS	silicate matrix	Bonnett-Pell lamp with a maximum emission at 660 nm	*E. coli*	0.60 mW cm^{-2}	120 min
[115]	ZnPcS p-THPP p-TAPP	CS membrane	500 W halogen lamp	*E. coli*		90 min
[120]	TPP-NH_2 TPPS-NH_2 Trans(Me-Py+) NH_2	cotton fabric	LED model Luxeon Star white Lambertian LXHL-MW1D 5500K) 400-800 nm (white light)	*S. aureus* *E. coli*	9.5 J cm^{-2}	24 h
[121]	TPP-NH_2 TPPS-NH_2 Trans(Me-Py+) NH_2	cotton fabric	white light	*S. aureus*	9.5 J cm^{-2}	24 h
[122]	[5,10,15-tri(4-*N*-methylpyridyl)-20-(4-alkylphenyl)porphyrinato]zinc(II)	cotton fabric	white light	*S. aureus* *E. coli*	1000 lux	24 h
[123]	PPIX	cellulose	four 150 W tungsten bulbs (visible light)	*S. aureus* *E. coli*	1.7 mW cm^{-2}	24 h
[124]	5,10,15-tri(4-methylphenyl)-20-(4-*N*-methylpyrydyl)porphyrin	cellulose	four 150 W tungsten bulbs (visible light)	*S. aureus* *E. coli*	1.7 mW cm^{-2}	24 h
[125]	5-[4-(3-carboxypropyloxy)phenyl]-	cellulose	four 150 W tungsten bulbs	*S. aureus* *E. coli*	1.7 mW cm^{-2}	24 h

	10,15,20-tri(4-methylphenyl) porphyrin) and 5-[4-(10-carboxydecanoxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin		(visible light)			
[126]	[5,10,15-tri(4-*N*-methylpyridyl)-20-(4-alkylphenyl)porphyrinato]zinc(II)	CNC	white light (400 700 nm)	*E. coli* *S. aureus* *M. smegmatis*	54 J cm^{-2} 108 J cm^{-2}	15 min 30 min
[127]	[5,10,15-tri(4-*N*-methylpyridyl)-20-(4-alkylphenyl)porphyrinato]zinc(II)	CNC	visible light (400–700 nm)	*A. baumannii* *A. baumannii (MDRAB)* *S. aureus* (MRSA) *P. aeruginosa*	59 J cm^{-2} 118 J cm^{-2}	15 min 30 min
[128]	PPIX 5-(4-hydroxy phenyl)-10,15,20-tritolylporphyrin , 5-[4-(3-propargyloxy) phenyl]-10,15,20-tritolylporphyrin, [5-[4-(3-propargyloxy) phenyl]-10,15,20- tritolyl porphyrinato]zinc(II)	cellulose	ten 23 W bulbs (visible light)	*E. coli* *S. aureus* *P. aeruginosa*	1.7 mW cm^{-2}	24 h
[129]	5-(4-aminophenyl)-10,15,20-tri(4-*N*-methylpyridyl)porphyrin	cellulose paper	LED model white Lambertian LXHL-MW1D 5500 K (white light)	*S. aureus* *E. coli*	9.5 J cm^{-2}	24 h
[135]	TPPN	polythiophene	white light	*E. coli* *B. subtilis*	90 mW cm^{-2}	5 min
[136]	5-(4-carboxyphenyl)-10,15,20-tris(4-methylphenyl)porphyrin	PDMS film	slide projector equipped with a 150 W lamp (350–800 nm)	*C. albicans*	90 mW cm^{-2}	60 min
[137]	5,10,15,20-tetra(4-*N*,*N*-diphenylaminophenyl)porphyrin and its Pd(II) complex	ITO films	Novamat 130 AF slide projector with a 150 W lamp (350–800 nm)	*E. coli* *C. albicans*	90 mW cm^{-2}	60 min
[138]	PPIX	MWNTs	compact fluorescence lamp 350 W, Sunlite (visible light)	*Influenza A virus*		5 min 10 min 15 min 30 min 45 min 60 min 90 min
[139]	PPIX	MWNTs	compact fluorescence lamp 350 W, Sunlite (visible light)	*S. aureus*		1 h
[147]	MnTPPS	porous honeycomb films immobilizer onto glass surface	100 W halogen bulb	*E. coli*		1 h
[148]	5,10,15-tri(*N*-methylpyridyl)-20-(*N*-tetradecylpyridyl) porphyrin	Silica microparticles	400 – 800 nm light	*E. coli* *S. aureus* (MRSA)	100 mW cm^{-2}	30 min
[149]	5-(2,3,4,5,6-pentafluorophenyl)-10,15,20-tripyridylporphyrin and the corresponding cationic 5,10,15-tri(4-*N*-methylpyridyl)-20-(2,3,4,5,6-pentafluorophenyl)porphyrins as tri-iodide salt	silica magnetic nanoparticles	13 parallel placed OSRAM lamps of 18 W each emitting in the 380e700 nm range	*A. fischeri*	40 W m^{-2}	24 h
[150]	5-(2,3,4,5,6-pentafluorophenyl)-10,15,20-tris(4-pyridyl)porphyrin, 5-(2,3,4,5,6-pentafluorophenyl)-10,15,20-tris(4-*N*-methylpyridyl) porphyrin tri iodide, 5-(2,3,4,5,6-	silica magnetic nanoparticles	13 parallel placed OSRAM lamps of 18 W each emitting in the 380e700 nm range	*E. coli* *E. faecalis*	40 W m^{-2}	90 min 180 min 270 min

	pentafluorophenyl)-10,15,20-triphenyl porphyrin					
[152]	$TMePyP^{4+}$	THES TMOS	white visible light	*E. coli*	7.9 J cm^{-2} 15.8 J cm^{-2}	1.5 h 3h
[153]	TPP ZnTPP	Electrospun PUR fibers	cold white light of a 150 W halogen bulb	*E. coli*		60 min
[154]	ClGaTCPP ClGaTCPP–PtNPs	PS	General electric Quartz line lamp (300 W) with a water filter	*S. aureus*	0.05 W cm^{-2}	30 min 60 min 90 min
[155]	TPP	Electrospun polystyrene nanofoibers	400W solar simulator equipped with water filter	*E. coli*		5 min 10 min 15 min 20 min
[157]	TPP	PUR PS PCL PA-6	150 W halogen bulb (white light)	*E. coli*		5 min 10 min 15 min 20 min 25 min 30 min
[159]	PPIX ZnPP IX	Nylon fibers	Incandescent light	*E. coli* *S. aureus*	10000 lux 40000 lux 60000 lux	30 min
[160]	ANT DBTP	silica powder or beads	125 W lamp, emitting in the 200–600 nm range with UVA filter	*E. coli*	3.9 mW cm^{-2}	6 h
[163]	ER	CS	led source 540±5 nm	*S. mutans* *P. aeruginosa* *C. albicans*	25 J cm^{-2} 50 J cm^{-2}	
[164]	Cationic chlorin	3-bromopropyl-functionalized silica Merrifield resin	13 OSRAM lamps 18 W white light	*E. coli*	40 W m^2	3h

950

951 **Abbreviations**

ANT	9,10-anthraquinone 2-carboxylic acid
APTES	(3-aminopropyl) triethoxysilane
Bpac	4,4'-dicarboxy-2,2'-bipyridine
CDI	1,1'-carbonyldiimidazole
ClGaTCPP	[5,10,15,20-tetra-(4-carboxyphenyl) porphyrinato]gallium(III)chloride
ClGaTCPP–PtNPs	[5,10,15,20-tetra-(4-carboxyphenyl) porphyrinato]gallium(III)chloride conjugated with platinum nanoparticles
CNC	Nanocrystalline cellulose
CS	Chitosan
CSRB	Chitosan Rose Bengal conjugate
CMCPS	Chloromethylated crosslinked polystyrene microspheres
CSRBnp	Chtosan nanoparticles functionalized with Rose Bengal
CPS	Cross-linked polystyrene
DBTP	Benzo-[b]triphenylene-9,14-dicarbonitrile
DODMAB	Dimethyldioctadecyl-ammonium bromide
DMA	Dimethylacetamide
DPBF	1,2-diphenylisobenzofuran
DVB	Divinylbenzene
EDC	N-ethyl-N ′-(3-dimethyl aminopropyl) carbodiimide

EMRSA	Epidemic strain of Methycillin Resistant *Staphylococcus aureus*
ER	Erythrosine
GlcNAc	1,4-linked N-acetyl-D-glucosamine
GlcN	D-glucosamine
Gram (+)	Gram positive
Gram (–)	Gram negative
HAI	Healthcare Associated Infections
HBA	Hydroxybenzaldehyde
ITO	Indium tin oxide
LBL	Layer by layer
LiCl	Lithium chloride
NHS	N-Hydroxysuccinimide
NMP	Nitroxide-mediated radical polymerization
MA	Maleic anhydride
MB	Methylene Blue
MES	2-(N-morpholino)ethanesulfonic acid
MDRAB	Multidrug-resistant *A. baumannii*
Min	Minutes
MnTPPS	[5,10,15,20-tetra(4-sodiumsulphonatophenyl)porphyrinato]manganese(III) chloride
MRSA	Methycillin Resistant *Staphylococcus aureus*
MWNTs	Multi-walled carbon nanotubes
$^1O_2$	Singlet oxygen
RB	Rose Bengal
RDP $^{2+}$	[tris(4,7-diphenyl-1,10-phenanthroline)-ruthenium(II)] dichloride
ROS	Reactive oxygen species
PACT	Photodynamic antimicrobial chemotherapy
PA-6	Polyamide 6
Pc	Phtalocyanine
PCL	Polycaprolactone
PDMS	Polydimethylsiloxane
PDMS-pAAc	Polydimethylsiloxane grafted acrylic acid
PMVE	Perfluorinated methyl vinyl ether
PbTpyPc	[2,9,16,23-tetra(4-pyridyloxy)phthalocyaninato]lead(II)
Pc	Phthalocyanine
PDMS	Polydimethylsiloxane
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PMMA	Poly(methylmethacrylate)
PPIX	Protoporphyrin IX
Ps	Photosensitizer
PS	Polystyrene
PSil	Porous silicone
pXRD	Powder x-ray diffraction
PUR	Polyurethane
PVP	Poly(4-vinylpyridine)
p-THPP	5,10,15,20-tetra(4-hydroxyphenyl)porphyrin
p-TAPP	5,10,15,20-tetra(4-aminophenyl)porphyrin

PtNPs	Platinum nanoparticles
RNO	N,N-dimethyl-4-nitrosoaniline
TBO	Toluidine Blue O
TEOS	Tetraethylorthosilicate
TBZnPc	[2,9,16,23-tetra(4-terbutyl)phthalocyaninato]zinc(II)
THES	Tetrakis(2-hydroxyethoxy)silane
$TMePyP^{4+}$	5,10,15,20-tetra(4-*N*-methylpyridyl)porphyrin
TMOS	Tetramethoxysilane
TBZnPc	[2,9,16,23-tetra(4-terbutyl)phthalocyaninato]zinc(II)
TPP	5,10,15,20-tetraphenylphorphyrin
TPP-NH_2	5-(4-aminophenyl)-10,15,20-triphenylporphyrin
TPPN	5,10,15,20-tetra[4-(6-*N*,*N*,*N*-trimethylammoniumhexyloxy)phenyl]porphyrin bromide
TPPS-NH_2	5-(4-aminophenyl)-10,15,20-tri(4-sulphonatophenyl)porphyrin
Trans(Me-Py^+) NH_2	5-(4-methylpyrydyl)-10,20-di(2,4,6-trimethylphenyl)-15-(4-aminophenyl)porphyrin
TSP	5,10,15,20-tetra(4-vinylphenyl)phorphyrin
TTP	5,10,15,20-tetra(4-methylphenyl)porphyrin
ZnPcTs	(2,9,16,23-tetrasuphoxyphthalocyaninato)zinc(II)
ZnPc	(phthalocyaninato)zinc(II)
ZnPcS	(2,9,16,23-tetrasuphoxyphthalocyaninato)zinc(II) as tetrasodium salt
ZnTPP	(5,10,15,20-tetraphenylphorphyrinato)zinc(II)

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