Light-Activated Conjugated Polymers for Antibacterial Photodynamic and Photothermal Therapy

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With the growing crisis of the availability of effective antimicrobial treatments, the use of phototherapy has gained increasing interest as an alternative to traditional antibiotic therapy. Even though phototherapy is already used in the clinic, there is an emerging interest in new materials with enhanced antimicrobial activity triggered by light. Among the different light-responsive materials, conjugated polymers are emerging candidates with successful application in the field of antimicrobial photodynamic and photothermal therapy. Due to their organic composition, easy processability, tailorbility of physicochemical properties, and capability of reactive oxygen species (ROS) and heat generation, their use as photoresponsive agents against bacteria has greatly expanded. Conjugated polymers have been designed to interact with specific bacterial targets, and their chemical composition has been optimized to enhance ROS and/or heat generation and new cationic polymers have been developed for augmented water solubility and increased interaction with negatively charged bacteria. Herein, the use of conjugated polymers in the field of phototherapy is discussed, focusing on the different approaches that have improved their performance against bacteria and their current preclinical safety assessment.

1. Introduction

Bacteria are unicellular microorganisms that lack a distinctive membrane involving the nuclei and, accordingly, are classified as prokaryotes.[1] The origin of these primordial organisms may date back about 4 billion years,[2] therefore, they have virtually adapted to all different environments found in the world. Their remarkable resilience and adaptability can be illustrated by their presence in polar regions and thermal springs, thriving under extreme temperature ranges (−20 to >100 °C).[2,3] They have also developed symbiotic relationships with many organisms, with one of the earliest proposed events named “endosymbiosis,” in which the incorporation of bacteria within eukaryotic cells led to the modern energy-producing organelles, such as the mitochondria and chloroplasts.[1] Through the development of harmonious relationships, colonies of different bacteria play a crucial role in diverse ecosystems and are now indispensable for the survival of countless species. Although animals have evolved with an essential natural flora of bacteria on their skin and gut, some microorganisms cause deleterious effects and due to the increasing difficulties in treating such diseases, pathogenic bacteria are now a public health concern.[4,5]

Pathogenic bacteria, in particular strains that are resistant to different antibiotics, pose a threat to human lives and generate significant economic impact with augmented expenditure in hospitalization and increased demand on health-care systems.[5] They also compromise the undertaking of surgical procedures, which heavily rely on antibiotic efficacy for patient safety and recovery.[6] It is estimated that bacterial resistance accounts for 700 000 deaths annually, with a forecast of 10 million yearly deaths with an associated cost of 10 trillion USD by 2050.[6] The crisis of effective antimicrobial treatments is a growing threat and the need for taking immediate action has been repeatedly emphasized by leading institutions, such as the World Health Organization (WHO).[4,5,7]

With the growing urgency to tackle pathogenic bacteria and their resilient strains, new antibiotics and alternative treatments are at the forefront of scientific investigation.[5,7] Amongst different approaches, there has been increasing interest in the study of materials with antibacterial activity triggered by light.[8] Under illumination, such materials can induce cell damage by producing reactive oxygen species (ROS), which is the basis of photodynamic therapy (PDT), and/or by generating heat, which is related to photothermal therapy (PTT).[9–12] Both photo-based therapies have been used for biomedical applications such as tumor ablation and bacteria eradication (referred to as antimicrobial photodynamic therapy (aPDT) and antimicrobial photothermal therapy (aPTT)).[9,11,12] Among the different photoresponsive agents explored to induce cell damage, conjugated polymers (conducting polymers) are emerging materials with promising results in the field.[11] Since their discovery, conjugated polymers have been used for a wide range of optical applications, such as light-emitting diodes (LEDs),[14] solar cells,[15] and as probes for
bioimaging. Their proposed use as aPDT agents started in 2005 in the work of Lu and collaborators. Subsequently, the research field of conjugated polymers for aPDT/aPTT has greatly expanded with the synthesis of new materials, functionalization with specific bacterial targets, dispersion within nanoparticle preparations, and incorporation into distinct matrices.

This Review brings to light the recent progress of conjugated polymers as phototherapy antibacterial agents and gives an overview of their current preclinical biocompatibility assessment. The important characteristics of bacteria and the relevant features of pathogenic strains are summarized, followed by a description of photodynamic and photothermal mechanisms. In sequence, this work rationalizes the development of effective conjugated polymer–based phototherapies for bacteria eradication and addresses the efforts into making such promising materials safe for future clinical use.

2. Bacteria

Bacteria are highly abundant microorganisms with minute size, which vary from ∼750 to 0.1 μm in diameter. Due to their small size, the observation of bacteria was only made possible with the advent of microscopy, with the first visualization reported by the Dutch scientist Antony van Leeuwenhoek around 1676. The association of microorganisms with the cause of disease was only established 200 years later following the work of the German medical doctor Robert Koch with Bacillus anthracis bacteria (anthrax disease). Also in the 1880s, the Danish scientist Hans Christian Joachim Gram developed a staining technique that discriminated two distinctive structures of the bacteria outer envelope (or cell wall). Gram used crystal violet and iodine staining followed by a decolorizing step with an ethanol solution, which revealed that some bacteria retained the staining, presenting a purple color (gram-positive), whereas other organisms lost the staining (gram-negative) (Figure 1b). These observations are now conventionally used to describe bacteria that present a 20-80 nm outer wall with a thick proteoglycan (or murein) layer which is rich in teichoic acid as gram-positive (Figure 1a). Alternatively, organisms with an ≈10 nm wall with a thin proteoglycan layer and an outer membrane containing lipopolysaccharide (LPS) are referred to as gram-negative. It is worth mentioning that some of the smallest bacteria, named mycoplasma, evolved without a cell wall, and under certain environmental conditions walled bacteria can turn into L-form strains, which are destitute of a cell wall. As opposed to the redundancy of the cell wall, all organisms present an essential 7.5 nm thick lipid bilayer that forms the cell membrane and encloses the cytoplasm. The latter is composed of a rich aqueous mixture of inorganic salts, protein, and carbohydrates in which vital components—nutrient storage granules, genetic material, and metabolism machinery (e.g., ribosomes and enzymes)—are dispersed (Figure 1d).

Figure 1. a) Depiction of the cell wall structure of gram-positive and gram-negative bacteria. Reproduced with permission. Copyright 2019, Springer Nature. b) Gram staining revealing the retained purple color of gram-positive bacteria and the pink-red color of gram-negative bacteria enhanced by the use of fuchsin or safranin counterstain. Reproduced with permission. Copyright 2015, Elsevier. c) Distinct shapes of bacteria revealed by scanning electron microscopy (SEM) microphotographs. Reproduced with permission. Copyright 2019, Springer Nature. d) Representation of the gram-negative bacteria E. coli shows the cell wall and external appendages (green), cytoplasm (magenta/blue), and nucleoid (yellow/orange). Reproduced with permission. Copyright 2009, John Wiley and Sons.
Bacteria are also commonly classified according to their shape, with the most familiar named Coccus (spherical), Bacillus (rod shaped), and Spirillum (curved to spiral shape) (Figure 1c); however, bacteria are found in a myriad of morphological features.\[^{[35]}\] Another key feature is that some bacteria can be equipped with one or many flagella (either at their extremities or randomly around their surface), which aid locomotion and contribute toward the virulence of pathogens (Figure 1d).\[^{[33,36]}\] Bacteria destitute of flagella motors are still able to move even on solid surfaces, although the mechanisms involved are still under investigation.\[^{[37]}\] Smaller hair-like structures named “pili” or “fimbriae” are related to cell adhesion and attachment to the surfaces to form biofilms.\[^{[33,34]}\] Some species present one to four elongated and thicker pili, referred to as sex pili, which enable bacteria conjugation (allowing the exchange of genetic material).\[^{[33]}\]

The outer structures and appendages of bacteria are directly related to their performance as pathogens.\[^{[38]}\] which is associated with the microorganism’s success in colonizing hosts and evading their immune system.\[^{[19]}\] The flagellum not only acts as a propeller toward favorable conditions, but also aids adhesion and invasion, as well as modulating the host immunoreactivity.\[^{[40]}\] The presence of fimbriae or pili is also linked to adhesion and colonization, while the sex pili is related to antibiotic resistance.\[^{[33,34]}\] Bacterial attachment leads to the formation of biofilms—a colony of bacteria held together in a matrix of polysaccharide fibers (glycocalyx) which are responsible for a variety of diseases (e.g., dental caries and pneumonia).\[^{[14,41]}\]

Some bacteria also produce an external capsule of high-molecular-weight polysaccharides with immunoevasion properties that preclude their clearance by immune cells.\[^{[42]}\] In addition, the death or lysis of gram-positive and gram-negative bacteria triggers the release of toxic chemical components (such as LPS or endotoxin in gram-negative and teichoic acids in gram-positive strains), causing lethal septic shock (failure of the circulatory system).\[^{[42]}\] Apart from the pathogenicity displayed by the bacteria architecture and chemical composition, bacteria also excrete toxins that inhibit cell pathways and induce cell lysis, which are manifested as symptoms of distinct severity by the host (e.g., cholera, botulism, and tetanus diseases).\[^{[42]}\]

Common bacterial diseases include the ones caused by the innate flora (e.g., dental caries),\[^{[43]}\] but also those caused by exogenous sources, such as gastroenteritis (or food poisoning).\[^{[44]}\] Although common, gastroenteritis is among the leading causes of deaths in the world, affecting mostly children who are under 5 years old.\[^{[44]}\] However, the pathogen leading the cause of deaths worldwide is Mycobacterium tuberculosis, causing life-threatening tuberculosis disease and around 1.8 million deaths in 2015.\[^{[4]}\] According to the WHO, tuberculosis is amongst the most hazardous drug-resistant bacteria, which also include other species, such as Salmonella spp., Campylobacter spp., Helicobacter pylori, Neisseria gonorrhoeae, and Enterobacteriaceae.\[^{[4]}\]

### 3. Photodynamic and Photothermal Mechanisms

Over the last century, antibiotics have been the first choice of treatment for both common and life-threatening infectious diseases and, although very effective initially, their extensive and indiscriminate use over time has led to the selection of drug-multiresistant bacteria that are causing a global crisis in treatment options.\[^{[45]}\] As a consequence, multiple efforts have been made to initiate an urgent appeal for the development of new therapies, which include the search for new antibiotics as well as alternative treatments.\[^{[4,45]}\] In this context, aPDT has emerged as a powerful tool to inactivate bacteria through oxidative stress caused by photosensitization (Figure 2).\[^{[46]}\] The technique is based on the exposure of bacteria to an agent capable of visible light absorption, called a photosensitizer, followed by light irradiation, triggering the generation of ROS.\[^{[8,12]}\] Oxygen radicals that are highly reactive, such as hydrogen peroxide (H$_2$O$_2$), nitric oxide, peroxynitrite, singlet oxygen (\(^{1}O_2\)), superoxide anions (O$_2^{-}$) and hydroxyl radicals (•OH), are necessary for normal cellular functioning; however, their uncontrolled presence is associated with cell damage and death.\[^{[46]}\] As a replacement or combined with aPDT, aPTT requires photothermal agents, which convert light absorption energy into heat, causing cell impairment by hyperthermia (Figure 2).\[^{[12,47]}\] An increase in temperature of a few degrees is able to trigger a heat shock response and incremental heat compromises protein conformation, resulting in aggregation and denaturation, which are the major cause of cell death.\[^{[12,47,48]}\] A distinguishable difference between aPDT and aPTT is that the first is typically stimulated with visible light irradiation, whereas the second usually requires the use of near infrared (NIR) illumination.\[^{[12,23]}\]

The major advantages of using phototherapy in place of traditional antibiotics include 1) the multitargeted action of photosensitive agents (unlike conventional antimicrobial drugs with specific cellular targets), killing bacteria regardless of their level of drug resistance; 2) higher localized action due to controllable exposure to areas of interest; 3) the avoidance of bacterial resistance mechanisms, by using nontoxic agents that are only activated under light exposure; and 4) efficacy against a broad spectrum of microorganisms.\[^{[8,49,50]}\] Another advantage would arguably be the reduced possibility of generating resilient strains due to action through multitargeted mechanisms and rapid lethal effect;\[^{[8,49]}\] however, the adaptation of bacteria following sublethal phototreatments has already been reported.\[^{[51]}\]

The useful characteristics of photosensitive agents have their origin in the mechanisms by which materials can release energy following excitation: The light energy absorbed by a material can be converted into photoluminescence (radiative de-excitation) and heat (nonradiative de-excitation).\[^{[52–54]}\] The light emission (fluorescence or phosphorescence) and the generation of heat following light absorption can be illustrated according to the Jablonski diagram (Figure 3). Briefly, the absorption of light with appropriate energy leads to a transition from the ground state to an excited singlet state (S$_1$), generally with one electron holding its opposite spinning configuration occupying higher vibrational levels.\[^{[55]}\] This is followed by a rapid relaxation of the electron, reaching lower vibrational levels in a process denominated “internal conversion,” which involves energy dissipation through vibrational relaxation.\[^{[55]}\] Once in the excited singlet state of lower energy (S$_0$), the remaining energy is released either by 1) further vibrational relaxation (heat dissipation\[^{[47]}\] ); 2) fluorescence, or 3) phosphorescence radiation emission.\[^{[55,56]}\] The latter requires the transition to a triplet state (T$_{n}$), named intersystem crossing, in which the electron spin is
The fact that the lifetime of the triplet state is longer than an excited singlet is important for PDT, as this long-lasting process allows interaction with molecular oxygen ($O_2$), resulting in ROS generation.\cite{47,56} ROS production can be classified as type 1 when it involves electron transfer and a chain reaction of superoxide anions ($O_2^-$), followed by hydrogen peroxide ($H_2O_2$) and hydroxyl radicals (·OH), whereas type 2 requires energy transfer resulting in singlet oxygen ($^1O_2$) formation.\cite{47,56}

Figure 2. a) Illustration of bacterial damage induced by conjugated polymer nanoparticle (CPN) photothermal and photodynamic effects. b) SEM micrographs reveal morphological alterations (yellow arrows) on ampicillin-resistant E. coli (Amp$^+$ E. coli) exposed to CPNs and light irradiation. Reproduced with permission.\cite{23} Copyright 2020, John Wiley and Sons.

Figure 3. Representation of a simplified Jablonski diagram highlighting the nonradiative (heat) and radiative (photoluminescence) de-excitation mechanisms.
Given the features of photosensitizers, a suitable candidate would be a good light absorber, but its application would depend on the preferable mechanism of energy release following excitation. Energy release in the form of light emission or that in the form of heat are complementary in a way that a material with a high fluorescence quantum yield would generate low heat.\(^{[13,34]}\) As a consequence, good fluorescence emitters find use in bioimaging,\(^{[14]}\) while lower photoluminescence emitters have been used as probes for photoacoustic imaging\(^{[16]}\) and as PTT agents capable of tumor ablation\(^{[13,57]}\) and microorganism inactivation.\(^{[9,23,58]}\) As fluorescence and phosphorescence are competing radiative de-excitation pathways, successful PDT agents would typically present a low fluorescence quantum yield and long-standing triplet states.\(^{[20,59]}\)

### 3.1. Conjugated Polymer Structure and Performance

Conjugated polymers are versatile materials with a broad range of applications in different technological fields.\(^{[16,60–64]}\) Their extensive use is related to the characteristic successive distribution of carbons with π-bonding (Figure 4), which are responsible for their inherent electronic and optical properties.\(^{[14,65]}\) By changing the organic composition of conjugated polymers, their properties can be tailored toward materials that are capable of charge transport,\(^{[14]}\) good light absorption,\(^{[13,66–68]}\) and bright and stable light emission.\(^{[69]}\) The many useful characteristics of conjugated polymers are also related to their easy processability, enabling the assembly of electromechanically active devices,\(^{[70]}\) manufacture of solar cells\(^{[71]}\) and flexible light-emitting displays,\(^{[72,73]}\) and the preparation of nanoparticles with biomedical application.\(^{[74,75]}\) Their organic composition and good biocompatibility have boosted the interest for biomedical use\(^{[54]}\) with one of the earliest biological applications exploring their excellent fluorescence for biosensing.\(^{[76,77]}\) Since then, their biomedical application has greatly expanded\(^{[61]}\) as conjugated polymer–based materials have excelled as bioimaging probes,\(^{[16]}\) theranostic agents for cancer treatments,\(^{[57,78,79]}\) drug delivery systems,\(^{[74,80]}\) and light-responsive antimicrobial agents.\(^{[9,17,18,21–26,81–84]}\) The emerging interest in these promising organic materials is evidenced by the efforts put into the preparation of biodegradable conjugated polymers.\(^{[85,86]}\)

The useful characteristics of conjugated polymers are highly dependent on their chemical composition, degree of polymerization, conformation, and side chain length.\(^{[19,20,24,65,87,88]}\) It is noteworthy that, unlike single molecules, conjugated polymers are multichromophoric; as a consequence, their electronic and optical properties rely on the physical conformation of polymer chains and their packing, which can vary in solution, films, and nanoparticle structures.\(^{[63,89–91]}\) For example, the production conditions influenced the conjugated polymer chain arrangement into nanoparticles, observed as alterations in their spectroscopic properties and fluorescence quantum yield.\(^{[92,94]}\) Notably, nanoparticle aggregates of conjugated polymers have excelled in ROS generation and photoactivated antimicrobial activity in vitro and in vivo.\(^{[24]}\)

In this context, enhanced ROS generation and inactivation of bacteria were also related to conjugated polymers of increasing degree of polymerization: A benzothiadiazole-tetraphenylthene-containing conjugated polymer (PTB) bearing 4-azidoper-fluorobenzoate (APFB) side chains (PTB-APFB) showed a higher fluorescence quantum yield and generated increased ROS and singlet oxygen \((^1\text{O}_2)\) than a lower-mass counterpart.\(^{[24]}\) In addition, alterations in the conjugated polymer backbone structure and side chain length had a direct effect on the optical properties, ROS generation, and bactericidal capability.\(^{[18–20]}\) In fact, conjugated polymers with a sequence of electron-donating and electron-accepting units (donor–acceptor structures) have shown enhanced performance as aPDT and aPTT agents.\(^{[24,54,81,95]}\) In this context, conjugated polymers with the same backbone structure but functionalized with electron-donating and withdrawing groups outperformed the ROS generation and antimicrobial activity of their counterpart functionalized with two strong electron-accepting groups.\(^{[20]}\) In addition, conjugated polymers with increasing electron-withdrawing benzothiadiazole (BT) units presented a decrease in their fluorescence emission, augmented singlet oxygen \((^1\text{O}_2)\) generation, and greater antibacterial activity under visible light irradiation.\(^{[95]}\) In effect, the benzothiadazole-containing conjugated polymer generated ROS and heat under laser illumination, which combined were responsible for the antimicrobial activity (Figure 4).\(^{[81]}\) Moreover, by substituting phenylene by BT in
fluorene-co-phenylenene-based conjugated polymers[18] and increasing the length of side chains[19] the authors observed augmented ROS generation and enhanced antimicrobial effect. Also, cationic poly(fluorene-co-phenyl-eneethynylen) (PFE) derivatives showed enhanced ROS production and antimicrobial activity compared to a conjugated polymer based on poly(fluorinephenylene) (PPF).[20] Conjugated polymers containing diketopyrrolopyrrole (DPP) with NIR absorption, low fluorescence quantum yield, and good photothermal conversion have been used for PTT (Figure 4). [9,23,58,96] Although the association of different conjugated polymers with complementary optical properties has generated nanoparticles that can achieve aPTT and aPDT against bacteria,[23,96] a single conjugated polymer with donor–acceptor structure based on thiophene (electron-rich) and benzothiazidazole and diketopyrrolopyrrole (electron-deficient) (referred to as PTDDB) was able to generate sufficient ROS and heat to inactivate bacteria through both mechanisms while requiring only one light source (808 nm laser) (Figure 4).[81]

Conjugated polymers with absorption in the NIR range have been explored for antibacterial phototherapeutic treatments due to the lower light scattering and higher penetration depth of NIR light in tissues.[9,23,27,81] Another approach to improve their applicability is functionalization with cationic groups to improve the interaction with the heavily negative charged surface of bacteria.[17,18,22,24,26,81–84] Different conjugated polymers functionalized with quaternary ammonium groups showed cytotoxicity against bacteria in the dark and the bactericidal activity was greatly enhanced following light exposure.[18,19,21,97,98] The toxicity in the dark observed for cationic systems is attributed to the interaction and disruption of the bacteria wall,[29] similarly to the effect of cationic surfactants that are commonly used as disinfectants.[100] In this context, nanoparticles of fluorene-BT based conjugated polymer (PFVBT) encapsulated in quaternary ammonium salts were bactericidal against gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria without light exposure.[83] Notably, 99% reduction of gram-positive B. subtilis viability was reported for cationic poly(p-phenylenevinylene) (PPV-1) in the dark, whereas white light illumination was required to reduce 70% of gram-negative bacteria (E. coli).[99] Similarly, positively charged P3HT-IIm promoted 98.8% viability reduction of gram-positive bacteria (S. aureus) in the dark, whereas only 18% of gram-negative bacteria (E. coli) was affected under the same condition.[98] In fact, no toxicity in the dark was observed for gram-negative bacteria (Pseudomonas aeruginosa) exposed to positively charged fluorene-phenylene-based conjugated polymer (referred to as PFPPBA).[22] Therefore, the cell wall structure of bacteria plays a crucial role in the microorganism susceptibility to designed conjugated polymer systems. It is suggested that the thicker but more porous structure of the gram-positive bacteria wall makes them more vulnerable toward cationic compounds, whereas the presence of the outer membrane with LPS in gram-negative bacteria might provide resilience to electrostatic interactions.[79]

It is noteworthy to mention that positively charged conjugated polymers could inhibit gram-positive S. aureus biofilm formation and, under light illumination, disrupt established biofilms through aPDT.[24] The ability to tackle biofilms is particularly desirable due to the lower susceptibility of bacterial aggregates compared to planktonic cells; nonetheless, there are few literature reports addressing the performance of conjugated polymers against biofilms. Cationic conjugated polymers have also shown a synergistic antibacterial effect in the dark when used in association with antibiotics at low concentrations.[101] Besides acting as antimicrobials, positively charged conjugated polymers have also been used as fluorescence sensors for bacteria, enabling rapid screening of potential photosensitizers.[102] In a similar context, a cationic poly(phenylene vinylene) derivative (PPV-NMe2) enabled the distinction among fungus and gram-positive and gram-negative bacteria by varying the ionic strength of the buffer solution.[103] In contrast, anionic nanoparticles of poly(diketopyrrolopyrrole-thieno)phenithiane (PDPPTT) and poly[2-methoxy-5-((2-ethylhexyl)oxy)-p-phenylenevinylene] (MEH-PPV) also showed good affinity binding with E. coli.[23] Negatively charged conjugated polymers were associated with cationic porphyrin to form a complex that enhanced O2 generation under light exposure, showing improved antimicrobial activity against bacteria than each photosensitizer alone.[104] Apart from the electrostatic interaction with negatively charged bacteria, targeted CPNs were functionalized with phenylboronic acid (PBA) for the specific binding of the lectin—saccharide bond on the surface of P. aeruginosa[22] and mannose adherence sites on E. coli.[21]

Amongst the useful properties explored, preparations in the nanoscale dimension have excelled in their performance, with most reports of bactericidal CPNs with dimensions below 200 nm.[9,23,24,26–28] Antimicrobial conjugated polymer particles can also be found in the micrometer diameter range,[104] and in different shapes (spheres, “worm-like” structures, and rings).[55] In addition to constituting particle preparations, conjugated polymers could also tackle bacteria while embedded in a polystyrene matrix[27] and in hydrogels.[128,96] To form CPNs, hydrophilic conjugated polymers have been synthesized[104] or they have been functionalized with cationic side chains for water solubility.[19,22,24,81,97] Alternatively, hydrophobic conjugated polymers have been encapsulated in a matrix of amphiphilic molecules, such as disodium salt 3,30-dithiodipropionicacid (SDPA).[25] poly(styrene-co-maleic anhydride) (PSMA)[23] and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)] (DSPE-PEG2000).[9,96] Cationic surfactants, such as cetyltrimethylammonium bromide (CTAB), have also been used to prepare cationic CPNs.[82] CPNs can be generated by facile production methods, which have an important effect in the CPNs’ optical properties, all of which have been recently summarized elsewhere.[16] Conjugated polymers have also been assembled into antibiotic switches with controlled antibacterial activity under light irradiation related to the assembly/disassembly of cucurbit[7]uril (CB[7]).[84]

4. Conjugated Polymers as Antimicrobial Phototherapy Agents

Although light-based treatment was used to address wounds by ancient civilizations who explored their natural resources (plants, as source of photoresponsive agents, and sunlight),[29] there has been a recent interest in exploring new light-responsive materials that might achieve improved bactericidal performance.[29] Among the different classes of materials, conjugated polymers have been
Table 1. Conjugated polymers tested for in vitro antibacterial activity through photodynamic and photothermal effects.

| Conjugated polymer | Composition and size | Optical property | Concentration and incubation time | Light source and power | Bacteria species and concentration | Growth inhibition and time | Therapy reference |
|--------------------|----------------------|------------------|-----------------------------------|------------------------|-----------------------------------|-----------------------------|-----------------------|
| Th-BT              | 100% conjugated polymer, ring-shaped nanoparticle | λ<sub>abs</sub> 400–800 nm | 1 mg mL<sup>–1</sup> 60 min | White LED (1.2 W cm<sup>–2</sup>, 120 min) | E. coli (G<sup>–</sup>) and B. subtilis (G<sup>–</sup>) | Up to 95–97% 20 min | aPDT<sup>[93]</sup> |
| PDPPTT (200 kDa) + MEH-PPV (120 kDa) | Poly (styr-co-maleic anhydride) (PSMA), ≈50 nm | λ<sub>abs</sub> 428/569 nm MEH-PPV, λ<sub>abs</sub> max. 783 nm PDPPTT | 9.6 × 10<sup>–4</sup> M 30 min | NIR light (550 mW cm<sup>–2</sup>, 5 min), white light (65 mW cm<sup>–2</sup>, 5 min) | Ampicillin-resistant E. coli (G<sup>–</sup>) OD<sub>600</sub> = 1 | Up to 93% 20 min | aPDT/ aPTT<sup>[93]</sup> |
| PDPP-DBT           | DSPE-PEG<sub>2000</sub> with cell-penetrating peptide (Tat), 100 nm | λ<sub>abs</sub> max. 750 nm, quantum yield <0.1% | Not described | 808 nm laser (2 W cm<sup>–2</sup>, 5 min) | E. coli (G<sup>–</sup>) S. aureus (G<sup>+</sup>) OD<sub>600</sub> = 1 | 99.8% and 90%, respectively | aPTT<sup>[93]</sup> |
| PTB-APFB (9.6 kDa) | 100% conjugated polymer with cationic side chains, 103 nm | λ<sub>max</sub> abs. 429/621 nm | 5 μM 20 min | White light (30 mW cm<sup>–2</sup>) and sunlight (30 mW cm<sup>–2</sup>) 10 min | S. aureus (G<sup>+</sup>) Not described | 100% | aPDT<sup>[24]</sup> |
| PTDDBD            | 100% conjugated polymer with quaternary ammonium side chains | λ<sub>abs</sub> 600–1000 nm | 40 μg mL<sup>–1</sup> 20 min | 808 nm laser (1 W cm<sup>–2</sup>, 8 min) | S. aureus (G<sup>+</sup>) E. coli (G<sup>–</sup>) OD<sub>600</sub> = 1 | 98.9% | aPDT/ aPTT<sup>[91]</sup> |
| PDPP3T            | Poly(caprolactone-co-polyurethane/urea) (PCL-PU) | λ<sub>abs</sub> max. 800 nm | 0.5 wt % in elastomer matrix Not described | 808 nm laser (2 W cm<sup>–2</sup>, 2 min) | E. coli (G<sup>–</sup>) Not described | 100% | aPTT<sup>[93]</sup> |
| Fluorene- and thiophene-substituted benzothiazole (-22 kDa) | 100% conjugated polymer with quaternary ammonium side chains | λ<sub>abs</sub> max. 500/650 nm | 5 and 15 μM 20 min | 400–1000 nm light (25 mW cm<sup>–2</sup>, 15 min) | Ampicillin-resistant E. coli (G<sup>–</sup>, S. aureus (G<sup>+</sup>) OD<sub>600</sub> = 1 | 100% and 95%, respectively | aPDT<sup>[19]</sup> |
| PFPhim            | 100% conjugated polymer with quaternary ammonium side chains | λ<sub>abs</sub> max. 384/414 nm | 16 μM Not described | White light (20 mW cm<sup>–2</sup>, 5 min) | E. coli (G<sup>–</sup>) Not described | 94.7% | aPDT<sup>[93]</sup> |
| PBF (cationic)    | Disodium salt 3,3'-dithiodipropionic acid (SDPA), 100 nm | λ<sub>abs</sub> max. 550/590 nm, 1.3% quantum yield | 20 μM 30 min | White light (400–800 nm), (90 mW cm<sup>–2</sup>, 40 min) | Ampicillin-resistant E. coli (G<sup>–</sup>) OD<sub>600</sub> = 1 | 90% | aPDT<sup>[21]</sup> |
| PTP                | Complex of water-soluble anionic conjugated polymer with cationic porphyrin (TPPN), 1–4 μm | λ<sub>max</sub> abs. 410/561 nm | 9 μM PTP + 3 μM TPPN and 1.8 μM PTP + 0.6 μM TPPN 15 min | White light (400–800 nm), (90 mW cm<sup>–2</sup>, 5 min) | E. coli (G<sup>–</sup>) B. subtilis (G<sup>+</sup>) Not described | 70% and 90%, respectively | aPDT<sup>[104]</sup> |
| PPV-1             | 100% conjugated polymer with quaternary ammonium side chains | λ<sub>abs</sub> 350–500 nm, λ<sub>max</sub> em. 514 nm | 10–50 μM 30 min | White light (400–800 nm), (75 mW cm<sup>–2</sup>, 6 min) | Ampicillin-resistant E. coli (G<sup>–</sup>, B. subtilis (G<sup>+</sup>) | 70% and 100%, respectively | aPDT<sup>[98]</sup> |
| PFPPB             | 100% conjugated polymer with quaternary ammonium side chains and phenylboronic acid (PBA) | λ<sub>max</sub> abs. 370/420 nm | 20 μM 5 min | White light (400–800 nm), (40 mW cm<sup>–2</sup>, 7 min) | P. aeruginosa (G<sup>–</sup>) OD<sub>600</sub> = 1 | 97% | aPDT<sup>[22]</sup> |
| PFPPBA            | 100% conjugated polymer with quaternary ammonium side chains and PBA | λ<sub>max</sub> abs. 370/420 nm | 20 μM 5 min | White light (400–800 nm), (30 mW cm<sup>–2</sup>, 10 min) | E. coli (G<sup>–</sup>) OD<sub>600</sub> = 1 | 97% | aPDT<sup>[21]</sup> |
explored for such a purpose due to their versatility, enabling the synthesis of cationic polyelectrolytes with good water dispersibility and functionalization with specific bacterial targets, as well as their organic composition and favored optical property with amenable features for both aPDT and aPTT.\(^{[17,21,22,81]}\) There is a recent interest in exploring their light responsiveness against bacteria, and the initial outcomes have been promising (Table 1 and 2). For example, conjugated polymers exhibited selective toxicity against S. aureus in vitro while being biocompatible with the human HeLa cell line, showing no systemic side effects in mice in vivo and outperforming cephalothin (first-generation cephalosporin antibiotic) in treating mice skin infected by S. aureus (Figures 5)\(^{[24]}\). Other research groups also reported success in treating mice S. aureus skin infection with no toxicity in vivo using conjugated polymers.\(^{[19,83]}\)

It is noteworthy that the majority of studies of conjugated polymers as agents for aPDT and aPTT reported in vitro antibacterial assays, whereas in vivo performance has not been studied to the same extent (Table 1 and 2). Despite the success of phototherapy treatments based on conjugated polymers, the administered dose, light power, and exposure times are variable in the literature (Table 1 and 2). For example, bacteria cultured in vitro were exposed to 1 mg mL\(^{-1}\) Th-BT for 60 min prior to white LED illumination (1.2 W cm\(^{-2}\)) for 120 min,\(^{[93]}\) while

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**Table 1.** Continued.

| Conjugated polymer | Composition and size | Optical property | Concentration and incubation time | Light source and power | Bacteria species and concentration | Growth inhibition | Therapy and reference |
|--------------------|----------------------|------------------|-----------------------------------|------------------------|----------------------------------|------------------|----------------------|
| Water-soluble polypenthenylene (PMNT) | Hydrogel (helical oligo(ethylene glycol)-grafted polysaccharides (PICs)) | \(\lambda_{\text{max}}\) abs. 543 and 589 nm | 10-50 \(\mu\)M (PMNT), 2 mg mL\(^{-1}\) PIC (irradiated immediately) | White light with red light filter (600/40 nm), (40 mW cm\(^{-2}\)), 20 min | E. coli (G−), R. subtilis (G+) | Not described | aPDT\(^{[28]}\) |
| Water-soluble PMNT and water-insoluble diketopyrrolopyrrole polythiophene based conjugated polymer (PDPP) | PIC/PMNT hydrogel and PDPP nanoparticles stabilized with DSPE-PEG\(_{2000}\) grafted with TAT peptide | Not described | 0.5 mg mL\(^{-1}\) PIC, 2.65 \(\mu\)g mL\(^{-1}\) PMNT, 15 mg mL\(^{-1}\) PDPP | White light (30 mW cm\(^{-2}\)), 10 min and NIR light (0.7 W cm\(^{-2}\)), 5 min | E. coli (G−), S. aureus (G+) | >99% | aPDT/aPTT\(^{[76]}\) |
| BT-substituted fluorene-co-phenylenene | 100% conjugated polymer with quaternary ammonium side chains | \(\lambda_{\text{max}}\) abs./em. 312 and 432/562 nm | 3 \(\mu\)M, 20 min | White light | Ampicillin-resistant E. coli (G−) | 97% | aPDT\(^{[14]}\) |
| PFE-CN-2 | 100% conjugated polymer with cationic side chains | \(\lambda_{\text{max}}\) abs./em. 399 and 443 nm | 0.4 \(\mu\)M, 10 min | White light | E. coli (G−) | 100% | aPDT\(^{[29]}\) |
| Polyphenylene ethinylene (PPE) | 100% cationic conjugated polymer | Not described | 0.2/0.5 \(\mu\)M, 2 h | White light | Ampicillin-resistant E. coli (G−), *B. anthracis* (G+) spores at 10\(^{4}\) | Up to 100% | aPDT\(^{[77]}\) |
| PPV-M-bearing quaternized N-methyl-imidazole | 100% cationic conjugated polymer | \(\lambda_{\text{max}}\) abs./em. 365/554 nm | 10-60 \(\mu\)M, 30 min | White light | Ampicillin-resistant E. coli (G−), S. aureus (G+) | ≥99% | aPDT\(^{[83]}\) |
| Polyfluorene-co-phenylene derivative (PFP) | Cationic conjugated polymer disassembly with cucurbit[7]uril (CB[7]) in Triton X-100 solution (0.06%) | \(\lambda_{\text{em}}\) 400–525 nm | Not described | White light | Ampicillin-resistant E. coli (G−) | 92% | aPDT\(^{[84]}\) |
| Polythiophene substituted with cationic imidazolium units (P3H7T-Im) | 100% cationic conjugated polymer | \(\lambda_{\text{em}}\) max. 611 nm | 0.1 \(\mu\)g mL\(^{-1}\) | Blue-violet light \(\lambda_{\text{max}}\) 420 nm; 2.28 ± 0.03 mW cm\(^{-2}\), 1 h | E. coli (G−), S. aureus (G+) | 97.5–99.9% | aPDT\(^{[98]}\) |
| Poly-[[9,9-bis-(6-N,N,N-trimethylammonium)hexyl]fluorenylene[1,2-b]dibromide] (PFF) | 100% cationic conjugated polymer with quaternary ammonium side chains | Not described | 20 \(\mu\)M, 15 min | White light | S. aureus (G+) biofilms | ~55–85% | aPDT\(^{[24]}\) |

\(^{[a]}\)Gram-negative bacteria; \(^{[b]}\)Gram-positive bacteria; \(^{[c]}\)Colony-forming units (CFUs); \(^{[d]}\)optical density at \(\lambda\) (OD).
20 μM of PFPFPBA was kept in the dark with bacteria solution for 5 min and then exposed to white light (30 mW cm⁻²) for 10 min. Although the harmonization of conditions might be difficult to achieve, comparison with reputable photosensitizers, such as methylene blue and rose bengal lactone used in the work of Lu and collaborators,[17] might be an alternative to allow the comparison of performance with the literature record.

### 4.1. Safety Assessment and Specificity

Photoresponsive materials with biomedical application should be safe to use, causing no harmful effects to the host.[47,59] The early safety screening of conjugated polymer preparations include the in vitro biocompatibility assessment with mammalian cells.[16] In this context, photoresponsive conjugated polymers with

| Conjugated polymer | Concentration and administration | Light source and power | Bacteria species and concentration | Animal model | Observation | Therapy and reference |
|--------------------|---------------------------------|------------------------|-----------------------------------|--------------|-------------|-----------------------|
| PTB-APFB (9.6 kDa) | 50 μM (50 μL) Subcutaneously sprayed | White light (100 mW cm⁻², 15 min) | S. aureus (G+), 1 × 10⁷ CFU mL⁻¹ (50 μL) | Female/male KM mice skin infection | Enhanced wound recovery | aPDT[24] |
| PTDBD with quaternary ammonium side chains | 120 μg/mL Abscess injection | 808 nm laser (1 W/cm², 8 min) | S. aureus (G+), OD₆₀₀ = 2 (100 μL) | Female BALB/c mice subcutaneously infected | Faster wound healing | aPDT/ aPTT[11] |
| Fluorene- and thiophene-substituted benzothiazole (~22 kDa) bearing quaternary ammonium groups | 100 μM (50 μL) wound injection | Not described | S. aureus (G+), 1 × 10⁸ CFU mL⁻¹ (50 μL) | Female BALB/c mice abscess | Faster wound healing | aPDT[11] |
| BT-substituted fluorene-phenylenene | 5 μM (20 μL) topical administration | White light (25 mW cm⁻², 15 min) | Ampicillin-resistant E. coli (G−), 1 × 10⁸ CFU/mL (10 μL) | Female BALB/c mice infected wound | Enhanced wound recovery | aPDT[11] |

Figure 5. a) S. aureus colonies in solid nutrient broth agar plate (cultured after 20 min exposure to conjugated polymer solution and light irradiation for 10 min).[21] b) S. aureus inhibition in the presence of conjugated polymer and white light and sunlight illumination. c) Images of mice skin infection after receiving different treatments. d) Measurements of wound infection size during 10 days. Reproduced with permission.[24] Copyright 2020, John Wiley and Sons.
antibacterial activity have typically shown good cytocompatibility with different cell lines in the dark and under light exposure (Table 3), with the exception of cationic PBF, which showed a significant reduction in cell viability after 40 min light irradiation. It is important to note that the incubation time of conjugated polymer preparations was variable regardless of the cell lines studied. In contrast to the changeable cytocompatibility settings, the assay based on cell metabolic activity has been largely used for the early safety screening of conjugated polymer agents for phototherapy, with tetrazolium-based assays being the prevalent choice in the literature, especially the methylthiazolyl-diphenyl-tetrazolium bromid (MTT) assay, but also others such as the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Although the tetrazolium assays are amongst the standardized techniques appointed for early in vitro biocompatibility screening, there are numerous preclinical in vitro cytotoxicity tests, which vary on their principles, giving information regarding cell viability, membrane integrity, cellular morphology, cell cycle, and apoptosis. Apart from the investigation of the cell metabolic activity by MTT assay, a few studies reported that the uptake of conjugated polymers did not alter the cell morphology of the human breast cancer cell line MCF7. The use of multiple cytocompatibility techniques that provide complementary data and a more detailed investigation of the effect of new phototherapy agents based on conjugated polymer preparations was variable regardless of the cell lines studied. In contrast to the changeable cytocompatibility settings, the assay based on cell metabolic activity has been largely used for the early safety screening of conjugated polymer agents for phototherapy, with tetrazolium-based assays being the prevalent choice in the literature, especially the methylthiazolyl-diphenyl-tetrazolium bromid (MTT) assay, but also others such as the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. Although the tetrazolium assays are amongst the standardized techniques appointed for early in vitro biocompatibility screening, there are numerous preclinical in vitro cytotoxicity tests, which vary on their principles, giving information regarding cell viability, membrane integrity, cellular morphology, cell cycle, and apoptosis. Apart from the investigation of the cell metabolic activity by MTT assay, a few studies reported that the uptake of conjugated polymers did not alter the cell morphology of the human breast cancer cell line MCF7. The use of multiple cytocompatibility techniques that provide complementary data and a more detailed investigation of the effect of new phototherapy agents based on conjugated polymers would greatly enhance their future applicability. To improve the future clinical/commercial application of conjugated polymer–based products, it is important that their biocompatibility is investigated through standardized in vitro and/or in vivo assays that are compatible with the proposed administration route. In the case of conjugated polymers developed for antimicrobial phototherapy, the in vivo antibacterial effect is often studied in an animal model with skin infections and conjugated polymer solutions are administered topically or injected into the dermis (Table 2). Therefore, it is sensible that conjugated polymer preparations intended for aPTD/aPTT have their early safety screening with relevant skin cells, such as fibroblasts, but different cell lines have been explored instead (Table 3). This is particularly important considering that phototherapy is already used in the clinic, especially for the treatment of conditions that allow easy access for local administration of photosensitive agents and illumination, such as skin diseases. In addition to the in vitro characterization of the effect of conjugated polymers toward cell lines, the evaluation of their compatibility with relevant blood components (hemocompatibility) is especially relevant for preparations intended to have parenteral administration, which are subjected to achieve blood circulation. Another relevant characterization, which should be broadly used for the early screening of new biomaterials, is the study of their interaction with phagocytic cells, which are involved in the clearance of exogenous materials, in particular with macrophages, as they are resident in different tissues including in organs where such materials might accumulate (e.g., spleen and liver). As for the biocompatibility in vivo, no organ toxicity (heart, lung, liver, kidney, and spleen) was observed in mice 5 days and 12 days following phototherapy treatment with conjugated polymers functionalized with quaternary ammonium groups. In addition, mice receiving PTB-APFB did not show alterations in body weight or renal and liver functions and had no signs of organ damage 10 days after treatment. Therefore, conjugated polymer solutions were harmless to mice bearing skin infection models while aiding their recovery through phototreatments. The preferential interaction with bacteria than with the host cell is a desirable property of antimicrobials and the selectivity has been tested by exposing CPNs to a coculture of bacteria and mammalian cells. Cationic PTB-APFB presented preferential interaction with bacteria than with HeLa cells cultured simultaneously in vitro. PPV bearing quaternary ammonium side chains presented selective staining of B. subtilis (gram-positive) and E. coli Amp (gram-negative) but not of Jurkat cells in coculture. Similarly, positively charged P3HT-lm showed selective interaction with E. coli when incubated simultaneously with MCF7 for 30 min and HeLa cells for 1 h. For conjugated polymers functionalized with quaternary ammonium side chains, the interaction with microorganisms or mammalian cells is linked with the ratio of the cationic groups—a lower content favored the interaction with bacteria and fungi and a higher ratio with mammalian cells. In fact, the

| Conjugated polymer | Concentration | Incubation time | Light exposure condition | Cell line | Reference |
|--------------------|---------------|-----------------|--------------------------|-----------|-----------|
| PTB-APFB           | 0.5–32 μM     | 24 h            | Dark                     | Human cervical (HeLa) | [24]     |
| PFPPhim            | 1–16 μM       | Not described   | Dark                     | Hela      | [97]      |
| P3HT-lm            | 1–20 μM       | 24 h            | Dark and blue light      | MCF7      | [98]      |
| BT-substituted fluorenone-phenylene | 3 and 5 μM | 6 h | Dark and light irradiation (25 mW cm⁻², 15 min) | Human breast cancer (MCF7) | [18] |
| PFVBET and CTAB CPNs | 0.5–10 μg mL⁻¹ | 48 h | Dark | MCF7 | [82] |
| PFE-CN-2           | 0.5–10 μM     | 24 h            | Dark                     | MCF7      | [20]      |
| Cationic PPV-M     | 1.5–200 μM    | 8 h             | Dark                     | MCF7      | [83]      |
| PPFPBA             | 4–20 μM       | 16 h            | Dark and light irradiation (45 mW cm⁻², 5–40 min) | Human renal carcinoma (A498) | [25] |
| Cationic PBF       | 5–20 μM       | 24 h            | Dark and light irradiation (45 mW cm⁻², 5–40 min) | Jurkat cells (human T lymphocytes) | [99] |
| Quaternary ammonium containing PPV | 10–60 μM | 30 min | Dark and under white light illumination (45 mW cm⁻², 10 min) | Jurkat cells (human T lymphocytes) and human pulmonary fibroblasts | [99] |

**Table 3.** In vitro safety assessment settings of conjugated polymers investigated as antimicrobial phototherapy agents.
chemical composition, degree of polymerization, and length of side chains in PPE derivatives were directly correlated with disturbances on vesicles mimicking mammalian and bacterial cellular membranes.[115] The authors reported that anionic polymers and low-molecular-weight cationic oligomers showed no vesicle perturbation, whereas polymers with high positive charge density and oligomers bearing cationic functionalization at the ends of the carbonic chain presented no selectivity.[115]

5. Conclusion

Conjugated polymers are versatile materials that have gained increased research interest in the biomedical field. Their use as antimicrobial agents activated by light is related to their inherent capability of generating ROS and/or heat, causing damage to bacteria through multiple targeted mechanisms. With the increasing interest in exploring conjugated polymers as phototherapy agents, materials with variable chemical composition and structure have been studied to optimize their performance. Conjugated polymers with donor–acceptor structure as well as homopolymers (e.g., PPV and polythiophene derivatives) generated sufficient ROS/heat to enable bacteria inactivation. The association of conjugated polymers with distinct capabilities of ROS and heat generation caused cytoxicity against bacteria through both mechanisms. Hydrophobic conjugated polymers have been assembled into nanoparticles using amphiphilic stabilizing agents and their entrapment into CPNs has not compromised their performance as aPDT and aPTT agents. Functionalization with cationic groups has been broadly used to increase their water solubility and promote interaction with negatively charged bacteria. Some cationic conjugated polymers displayed toxicity in the dark against bacteria, but most of them required light illumination to eradicate bacteria. It is noteworthy that the outer structure of bacteria played an important role in their susceptibility against cationic conjugated polymers, with gram-positive bacteria being more vulnerable toward such compounds than gram-negative strains. Apart from their activity upon light illumination, conjugated polymers could also tackle biofilms under light irradiation. A few literature reports showed the efficacy of conjugated polymers as phototherapy agents in vivo, but most studies have been conducted in vitro with variable settings. Conjugated polymer preparations showed selective interaction with bacteria when simultaneously exposed to mammalian cell lines in vitro. As to their preclinical safety assessment, most conjugated polymers designed for bacteria inactivation upon light illumination showed good biocompatibility in vitro with human cell lines and in vivo with mice models. For the evaluation of the biocompatibility, the cell metabolic activity in vitro based on tetrazolium assays has been widely used and, despite the promising results, a more detailed investigation of their early safety screening in vitro would greatly benefit their future clinical application. We anticipate that the content of this Review will give insightful contribution toward the use of conjugated polymers in the biomedical field and may be of interest for a wider audience interested in new materials for antimicrobial phototherapy.

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Conflict of Interest

The authors declare no conflict of interest.

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