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Photodynamic Therapy with Conjugated Polymer Nanoparticles: Recent Advances and Therapeutic Considerations

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ABSTRACT

Many types of cancers require elevated levels of Reactive Oxygen Species (ROS) for uncontrolled proliferation. However, this also makes tumor cells more susceptible to ROS induced cell death by additional oxidative stress caused by external stimuli. By selectively targeting cancer cells and tumors that exhibit high levels of ROS with nanotechnology-based materials to trigger further elevation of ROS therapeutic possibilities become available. Specifically, light-activated treatment through Photodynamic Therapy (PDT) has been demonstrated as a feasible approach with Photophryn as the leading FDA approved sensitizer. However, such small molecule sensitizers do still have significant hurdles to overcome, including poor solubility, non-targeted delivery, and low absorption of light. In this mini review, recent advances in the development of Conjugated Polymer Nanoparticles (CP-NPs) solving these issues are discussed, including amplified ROS generation, tumor targeting, theranostic capabilities, and multimodal CP-NPs. We also give an outlook towards further needs in CP-NP development for clinical application.

Introduction

Reactive Oxygen Species (ROS) are essential participants for normal functioning of cells such as cell signaling and apoptosis, but have also been identified as playing a major role in promoting carcinogenesis[1]. The Akt pathway ensures resistance to senescence and ROS mediated apoptosis in normal cells, but in human cancers that protection is removed in order to promote uncontrolled proliferation. Thus, ROS induced apoptosis in cancer cells is facilitated compared to normal cells[2,3]. This double-edged sword for human cancer growth and proliferation opens avenues to selectively target and eliminate cancer cells and tumors that exhibit high levels of ROS through Photodynamic Therapy (PDT).

Attempts to utilize this mechanism in therapeutic approaches have been reported based on inorganic (quantum dots, metal oxide nanoparticles) and metal nanoparticles (gold, silver, and iron)[4,5]. In these instances intracellular damage and cell death typically occurred due to lipid peroxidation of mitochondria. However, the lack of biocompatibility of these nanomaterials is of concern, and in some cases such as quantum dots there is known cytotoxicity. It is thus unlikely that these approaches will lead to commercial products and therapies.

Distinct advantages of CP-NPs include tunable surface functionalization, minimal need for purification, biocompatibility and buffer dispersibility. There are some disadvantages, however, such as that the particles could disintegrate before reaching the target since they are not cross-linked, and that the PDT photochemistry bleaches the particles i.e. no longer trackable by fluorescence imaging.

Biophotonics based on novel nanoparticle designs has brought significant advances to sensing, imaging, and therapeutic modalities for biological applications. Recently, there has been a surge in the development of organic polymer-based nanoparticles as PDT sensitizers. McNeil's group was at the forefront in reporting conjugated polymer nanoparticles (CP-NPs) for bioimaging and PDT applications,[6] which was extended by our and other groups into CP-NPs with multimodal capabilities. These advances will be discussed in this mini review.

CP-NPs Preparation Requires Minimal Chemistry

The reprecipitation method has recently become the most commonly used method for CP-NPs fabrication,[7,8] although laser ablation of microcrystals has also made a significant impact on the field[9]. Both methods are illustrated in Figure 1. The straightforward fabrication of CP-NPs by the reprecipitation method is attractive since it is a simple solution-processing technique in which a solution of organic material in good solvent is injected into a very poor solvent for the organic material. By managing solvent types and starting concentration of the conjugated polymer stock solutions it is also possible to gain control over particle size (Figure 1). The reprecipitation method finds its origin in the preparation of dye molecule nanoparticles[10]. Upon injection, a stable suspension of nanoparticles is formed due to hydrophobic aggregation of the organic compounds when entering the water phase. Zeta potential measurements for CP-NPs typically reveal a slightly negative charge that stabilizes the hydrophobic CP-NPs in water and buffer suspension when the CP-NPs have no additional surface coating[11]. Surface modification of the CP-NPs provides further control on their surface charge[12]. This can be accomplished through careful design of the polymers[12-15] or by application of mild chemistry to surface functional groups, which is further discussed below (vide infra). Particle size, hydrophobicity, and surface charge directly affect quantity and internalization mechanism of CP-NPs by cancer cells[12,16-18].

Mechanism of ROS formation and PDT effects

ROS are formed during the native function of cells. During the oxidative metabolism in mitochondria, single electron reduction of molecular oxygen to superoxide anion occurs in the cell body[19]. Other cell organelles such as the endoplasmic reticulum and the nuclear membrane also have electron transport systems that lead to formation of ROS[20].

Similar mechanisms for ROS formation are at play when exogenous ROS sensitizers are introduced for PDT. By providing a light source to the PDT sensitizer that matches its absorption spectrum the photosensitizer will enter the singlet exited state[14,21]. From there either relaxation to the ground state or intersystem crossing to the triplet state occurs. The latter is desired for PDT, especially for conjugated polymer sensitizers, because of the similarity of their triplet energy with the excited state of oxygen (singlet oxygen). This feature enables both efficient energy and charge transfer processes towards formation of ROS

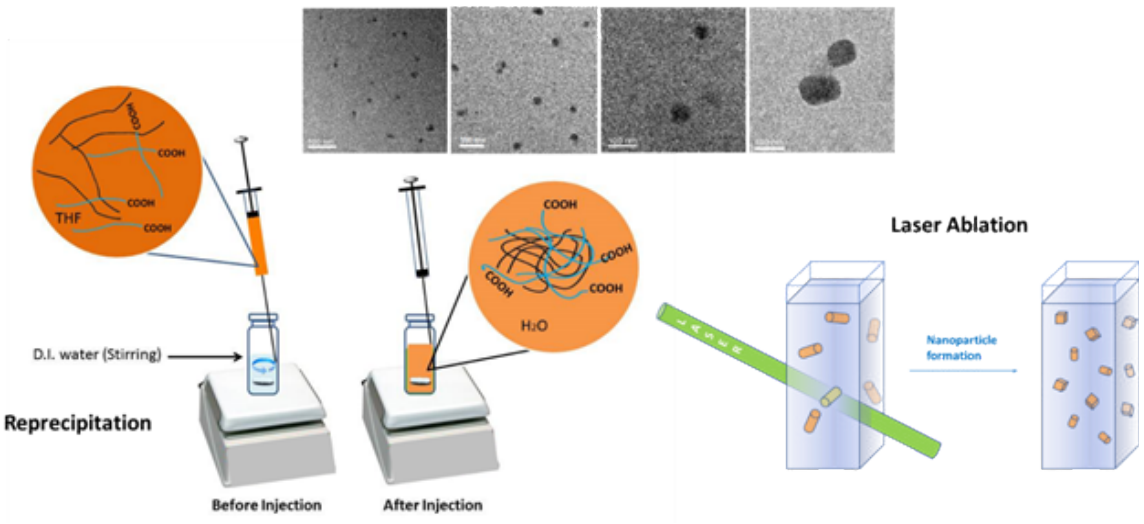

Figure 1: Schematic representation of the reprecipitation and laser ablation methods for CP-NPs preparation. In the reprecipitation method, surface modalities can be introduced by co-precipitation. The TEM images in the upper section show the possibility for size control (unpublished results). Scale bar is 100 nm.

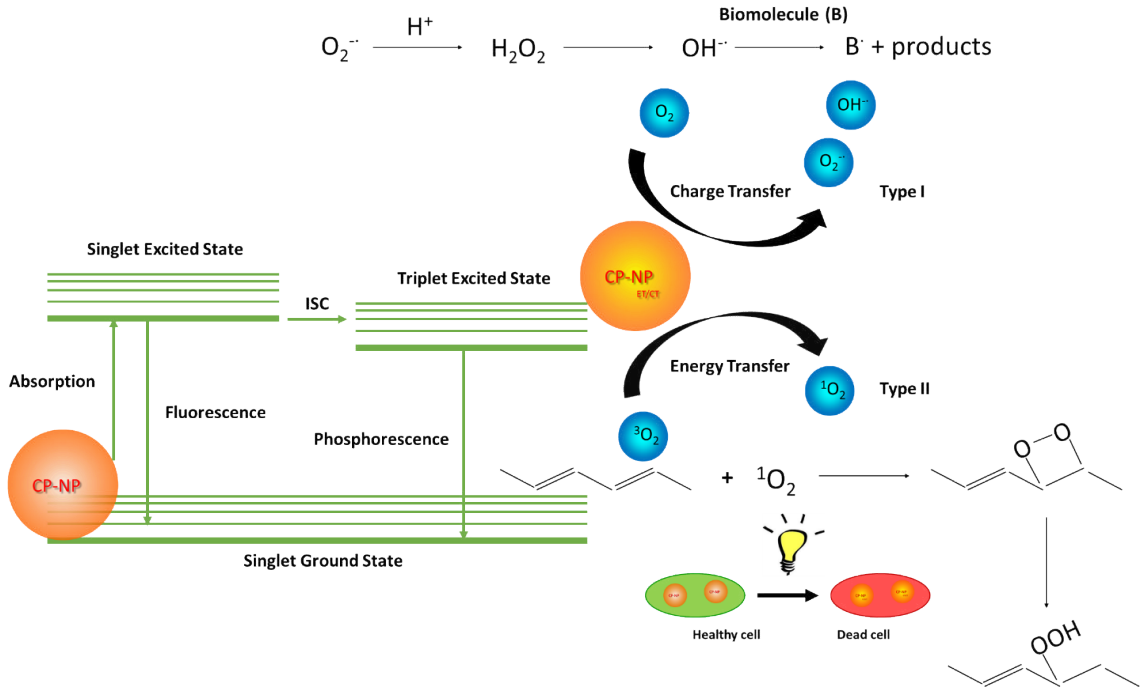

Figure 2: Diagram of Photodynamic Therapy with CP-NPs as photosensitizer. Once the CP-NPs reach the triplet excited state charge transfer to oxygen or biomolecules occurs (Type I mechanism) or energy transfer takes place to form singlet oxygen (Type II mechanism). Either mechanism leads to elevated ROS under illumination and potential for therapeutics.

that can be used therapeutically in cancer treatment[22-25]. There are two well-known pathways for ROS formation[21,26]. In the type I mechanism, charge transfer occurs from the triplet state of the photosensitizer to oxygen to produce a superoxide radical anion. This mechanism also works with biomolecules and solvent molecules such as those found in cells. In the type II mechanism, energy transfer occurs from the triplet state of the photosensitizer to oxygen to yield singlet oxygen[14,25,27,28]. The resulting elevated levels of ROS induce oxidative stress that lead to cell damage and cell death (Figure 2)[8,14,23,25-27].

CP-NPs Photophysics Enables Enhanced PDT Properties

Conjugated polymers are multichromophoric systems that typically contain a few tens to over a hundred chromophores depending on molecular weight and have well-known singlet and triplet properties. Their corresponding nanoparticles contain from a few to several tens of polymer chains. Considering these properties, it becomes clear why CP-NPs are significant as a next generation of PDT sensitizers. CP-NPs have major advantages over current small molecule sensitizers (including FDA approved ones) given the CP-NPs' (i) large extinction coefficients (ε > 10^7 L mol^{-1} cm^{-1}), (ii) efficient triplet formation as necessary for PDT, with triplet energies that are close to that of oxygen,[29] (iii) intrinsic ROS formation (no need for sensitizing dopants or encapsulation of conventional sensitizers), (iv) low to absent cytotoxicity found in vitro (in dark), (v) buffer dispersibility and stability, (vi) intrinsic fluorescence for tracking of delivery (no need for dye dopant or attachment), and (vii) adaptable design for targeted delivery. The large extinction coefficient ε indicates that the photon absorption rate is 2 to 3 orders of magnitude higher than current small molecule sensitizers such as Phthalocyanine, Porphyrine, and Photophryn (where the best sensitizers have an extinction coefficient of several 10^5 at best). These properties result in triplet generation rates (through intersystem crossing, ISC) that are 2 to 3 orders of magnitude greater than those reported for current small molecule sensitizers. The intrinsic fluorescence has been an additional attractive feature since it allows for tracking of CP-NPs in-vitro and in-vivo by optical imaging[22,24]. Reports have also started appearing that investigate the tunability of CP-NPs' optical properties[27].

The development of blended CP-NPs has in some cases lead to additional improvement in singlet oxygen or radical anion formation. In such cases, the CP-NPs contain additional small molecule photosensitizers. Amplification of singlet oxygen generation from CP-NPs has recently been reported by the Palacios group[25,28]. The authors reported that poly(9,9-dioctylfluorene-alt-benzothiadiazole) (F8BT) doped with platinum octaethylporphyrin (PtOEP) produces singlet oxygen from F8BT rather than from

PtOEP. The dopant serves to increase the quantum yield of singlet oxygen formation through the intricacies of conjugated polymer photophysics. Downconversion has also been employed by several groups to enhance singlet oxygen generation. In this CP-NP design, photosensitizer dopants are loaded inside the CP-NPs. The CP-NPs then act as antenna systems for the PDT sensitizer. After light absorption by the CP-NPs, energy is transferred by FRET to the triplet sensitizer, either in a single step or in a multi-step energy transfer cascade. This approach has been completed for different conjugated polymer-sensitizer combination, including for Rose Bengal molecules attached on the surface of coumarin 153-dye-doped poly[N-vinyl carbazole], Rose Bengal and Rose Bengal Methyl Ester blended with poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt-co-(1,4-benzo-{2,10,3}-thiadiazole)], poly[(9,9-dioctylfluorenyl-2,7-diyl)-alt-co-(1,4-benzo-{2,1′,3}-thiadiazole)] blended with porphyrin, and for the conjugated polymers conjugated polymers poly(9,9-dihexylfluorene), poly(9,9-dioctylfluorenyl-2,7-diyl) and poly[{9,9-dioctyl-2,7-divinylene-fluoreneylene}-alt-co-{2-methoxy-5-(2-ethylhexyloxy)-1,4-phenylene}] (PFBT) blended with Rose Bengal or porphyrin for instance[6,30-34]. With this approach, sensitizers that are not buffer compatible can be solubilized in aqueous media for increased bioavailability while enhancing their performance. A charge transfer cascade was reported by our group through the use of fullerene dopants loaded into (poly[2-methoxy-5-(2-ethylhexyl-oxy)-p-phenylenevinylene] (MEH-PPV) CP-NPs[35]. Besides enhanced PDT effect, an interesting observation was the absence of fullerene-induced cytotoxicity, suggesting that encapsulation in CP-NPs can increase biocompatibility of PDT sensitizers[36].

Multimodal CP-NPs: Tumor Targeting and Multifunctional Designs

Targeted delivery is an important consideration for therapeutic impact and clinical relevance. For CP-NPs the constituent polymer usually does not have functional groups that allow for easy ligand binding chemistry. A co-polymer with for example carboxyl or amine moieties is often used to allow for targeting ligand attachment. Once the CP-NPs reach the target site and accumulate, typically by EPR effect, the CP-NPs undergo receptor-mediated endocytosis. This approach has been demonstrated for small molecule ligands and peptides[23,37], which promises advanced therapeutic applications of targeted CP-NPs. The Xu group recently reported significant advances using peptide functionalized MEH-PPV CP-NPs for in-vivo theranostics of triple-negative breast cancer[38].

Multimodality has also received a lot of attention in the last few years. There has been a major increase in work reported on novel nanoparticles that have multiple imaging, sensing or therapeutic modalities, including combinations thereof. This also holds true for CP-NPs. Multifunctional CP-NPs that are tumor targeted, fluorescent, and are able to deliver combined PDT and photothermal therapy (PTT) were reported recently, developed from the conjugated polymers poly[9,9-bis(2-(2-(2-methoxyethoxy) ethoxy)-ethyl)fluorenyldivinylene]-alt-4,7-(2,1,3-benzothiadiazole) (PFVBT) and poly[(4,4,9,9-tetrakis(4-(octyloxy)phenyl)-4,9-dihydro-s-indacenol-dithiophene-2,7-diyl)-alt-co-4,9-bis(thiophen-2-yl)-6,7-bis(4-(hexyloxy)phenyl)-thiadiazolo-quinoxaline] (PIDTTTQ), and from D–A-type alkyl-chain-grafted bithiophene-(3E,7E)-3,7-bis(2-oxoindolin-3-ylidene)benzo-[1,2-b:4,5-b′]-difuran-2,6(3H,7H)-dione[39,40]. At the basis of the design are CP-NPs with strong near-infrared absorption, which will be discussed further below (vide infra). Multimodality through combined imaging and biosensing, including pH, gas, ion, and temperature sensing, has also been investigated extensively for CP-NPs[41,42]. Novel therapeutics have been incorporated into CP-NPs by the Moon group specially for gene delivery[43,44]. The investigators applied their multimodal CP-NPs as imaging and siRNA delivery vehicles.

In-vivo application of CP-NPs: Looking Ahead

Substantial progress has been made to bring CP-NPs towards clinical relevance, but further advances are required. These include the development of near-infrared (NIR) absorbing/emitting nanoparticles and in vivo studies on CP-NPs. So far, donor-acceptor conjugated polymers were included in CP-NPs for synergistic PDT and PTT under NIR excitation and detection[40]. An NIR theranostic approach was also reported in which NIR tracking of poly(1,2-bis(4-((6-bromohexyl)oxy)phenyl)-1,2-diphenylethene-co-alt-9,10-anthraquinone) CP-NPs was followed by PDT[45]. In-vivo targeted delivery, tracking and therapeutics have been shown as well. Brain tumor targeted PFBT CP-NPs exhibited accumulation at the tumor site in genetically engineered mouse models[46]. Similar observations were made for subcutaneous RCC (human renal cell carcinoma) xenografts[24]. PFBT CP-NPs were applied in vivo with human gastric adenocarcinoma xenografts, and a comparison between intravenous and intratumoral injection was made. Significant tumor inhibition and eradication were observed, respectively[34]. SKBR-3 breast cancer tumor-bearing mice were treated with anti-HER2 functionalized NIR absorbing PFVBT/PIDTTTQ CP-NPs.[39] Excellent tumor targeting and accumulation of the anti-HER2 CP-NPs at the tumor site were observed. Synergistic PDT/PTT showed near complete inhibition of tumor growth.

Current advances in multiphoton excitation and bioluminescence energy transfer (BRET) capabilities for CP-NPs can lead to even further progress. Multiphoton excitation allows the use of NIR light sources to activate visible absorbing PDT sensitizers. This approach has

been successful for a variety of CP-NPs designs, including tumor targeted and activatable CP-NPs with high two-photon cross sections such as from poly{9,9-bis[6''-(bromohexyl)-fluorene-2,7-ylenevinylene]-co-alt-1,4-(2,5-dicyanophenylene)} as conjugated polymers[6,47-49]. Antitumor activity of BRET-based CP-NPs where luminol was incorporated as a bioluminescence precursor was studied. An over 50% tumor inhibition ratio was found for HeLa (cervical cancer) tumor bearing nude mice[50]. Similarly, strong tumor growth inhibition was found for luciferase modified BRET CP-NPs applied in vivo to breast cancer (MCF-7) and cervical cancer (HeLa) tumor model[s51]. These BRET systems have demonstrated clear promise in deep tissue imaging and light activated tumor treatment. Near-infrared optical properties, multiphoton excitation and BRET will be important factors in the success of CP-NPs as high impact anticancer theranostics.

Conclusion

In recent years, CP-NPs have developed from a novelty in bioimaging to a full-fledged multimodal platform for nanomedicine. At present, CP-NPs are tumor targeted, brightly fluorescent, and capable of intracellular sensing and cargo delivery. The future looks even brighter with the advent of near-infrared abilities for both imaging and therapeutics, including through multiphoton excitation, and the use of bioluminescent materials in CP-NPs as built-in light sources for BRET schemes. These developments are bringing CP-NPs into the NIR I biological window, which opens up avenues for deeper tissue in vivo theranostics and a positive outlook for clinical viability.

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Conflict of Interest Statement

The authors have no conflict of interest to disclose.

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