Conjugated Polymer Nanoparticles toward In Vivo Theranostics – Focus on Targeting, Imaging, Therapy, and the Importance of Clearance

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Conjugated polymer nanoparticles are highly fluorescent colloids with tunable emission colors ranging from the visible deep into the near infrared spectrum. Conjugated polymer nanoparticles are easy to prepare, tunable in their size, and virtually nonbleachable. Conjugated polymer particles can also be designed to give off heat upon irradiation. All these properties make conjugated polymer particles ideal materials for biomedical fluorescence and photoacoustic imaging as well as for theranostic applications. Here, different examples of surface functionalization to attach pathological homing devices, imaging modalities, as well as the emerging possibilities for therapeutic measures are discussed. Furthermore, clearance of the particles is considered, which is important to ultimately apply the materials for in vivo theranostics. Due to the conjugated backbone of the conjugated polymers, established degradation strategies, as known from hydrophilic nonconjugated polymer carriers, cannot be applied. Bioinspired strategies and potential pathways for degradation and clearance via structural changes upon triggers such as pH, oxidation, and temperature are also discussed in this progress report.

1. Introduction

Medical diagnostics has always benefitted from new imaging modalities, leading to improved biomedical understanding as well as improved patient treatment. The impelling aim is to enhance imaging resolution, reduce invasiveness, and enable specific mapping of desired pathological markers and morphologies. To achieve these advancements, new imaging techniques are developed often in conjunction with the possibility to also apply the respective technique for treatment. This will allow for precise imaging and diagnosis combined with therapeutic effects. There exists a plethora of different approaches ranging from biological carriers, such as proteins and peptides or synthetic macromolecules, to capsules, nanoparticles, and water soluble microgels. These entities can be loaded with drug molecules and functionalized with contrast agents and biomolecular recognition motifs for specific homing to the site of disease. Conjugated organic molecules represent powerful molecules for imaging, because they can be used for fluorescence or photoacoustic imaging. Furthermore, some of these organic conjugated materials allow photothermal and photodynamic therapy. Due to the hydrophobic molecular framework of these molecules, they need to be functionalized to improve their solubility in aqueous media or the molecules aggregate into particles. Conjugated polymer nanoparticles (CPNs) smaller representatives below 20 nm in diameter are sometimes also termed semiconducting polymer dots or Pdots utilizing this hydrophobicity of the conjugated polymer backbone for particle formation. Several strategies to impart colloidal stability in water have been developed. CPNs exhibit stable fluorescence or photoacoustic response and are virtually nonbleaching due to the large number of chromophores in a single nanoparticle. Due to their hydrophobic character, CPNs present interesting materials as carriers for hydrophobic drugs with high loading capacities. In contrast to many existing carrier systems, CPNs can bind hydrophobic drugs physically via van der Waals, π-stacking, and hydrophobic interactions. Many anticancer drugs are hydrophobic and difficult to administer due to their low solubility in water. Such anticancer drugs exhibit cytotoxicity, making targeted delivery important to avoid damage to healthy tissue and to increase the local dose. Consequently, CPNs present ideal delivery vehicles for such active compounds. Several comprehensive reviews on CPNs are available, covering synthetic approaches as well as their applicability for biomedical imaging and theranostics. In this short overview, I will focus on the different synthetic strategies toward CPNs and the possibilities for their surface functionalization to introduce specific recognition motifs for homing to the pathological site. I will then concentrate on the potential of CPNs for therapeutic applications in conjunction with their imaging qualities. A critical challenge for the ultimate application of CPNs for in vivo theranostics is to develop strategies that prevent accumulation of the particles in the body. So far, this problem has hardly been addressed by the community. Therefore, I will devise approaches, which could in the future be used to allow clearance of CPNs from the body.
2. Synthesis of Conjugated Polymer Nanoparticles

Synthetic pathways to produce CPNs can be discriminated into two distinct approaches, namely postpolymerization particle formation and direct polymerization into particles. Postpolymerization approaches produce particles from conjugated polymers, which have previously been synthesized via standard solution mediated processes. These conjugated polymers can subsequently be converted into particles using miniemulsification, nanoprecipitation, or self-assembly processes (see Figure 1).[19]

For the miniemulsification approach, the conjugated polymer is dissolved in a good solvent. The polymer solution is miniemulsified in a nonsolvent for the conjugated polymer, which is also immiscible with the conjugated polymer solvent.[21,22] This nonsolvent is usually water, whereas the good solvent is often toluene or chloroform. The miniemulsion is stabilized using surfactants and the particles are obtained after the dispersed solvent has been evaporated and the surfactant molecules then stabilize the CPNs in the continuous (aqueous) nonsolvent. Particle sizes can vary between 20 nm and several hundreds of nanometers.

In the nanoprecipitation approach, we also start with a solution of conjugated polymer, which is injected into a nonsolvent for the conjugated polymer.[23–25] By contrast, this nonsolvent is miscible with the solvent of the conjugated polymer, leading to fine precipitation of CPNs of diameters down to <10 nm.[26] Here also, surfactants can be added to control the size of the CPNs and impart colloidal stability after nanoprecipitation.

Conjugated polymer self-assembly can be applied for charged conjugated polymers, so called \( \pi \)-conjugated polyelectrolytes.[27,28] By addition of molecules, polymers, or nanoparticles carrying the opposite charge, the conjugated polyelectrolyte coacervates with the other charged entity to form charge neutral nanoparticles. Alternatively, conjugated block copolymers with a hydrophobic and a hydrophilic block self-assemble into block copolymer micelles with a solid hydrophobic core.[29,30] In cases where the conjugated polymer has sufficient molecular weight, single chains can form charge stabilized “quantum dot” sized nanoparticles (~5–10 nm).[31,32]

![Figure 1. Synthetic strategies for the preparation of conjugated polymer nanoparticles. In postpolymerization approaches, previously synthesized conjugated polymers are formed into particles. This can be done via micro- and miniemulsification of a conjugated polymer solution in a nonsolvent. Alternatively, the conjugated polymer solution can be injected into a nonsolvent for the conjugated polymer (which is miscible with the solvent of the conjugated polymer). Here conjugated polymer particles nanoprecipitate. Finally, specific conjugated polymer geometries such as amphiphilic block copolymers or conjugated polyelectrolytes can be self-assembled into conjugated polymer nanoparticles. The schematic here shows a positively charged conjugated polymer, which is precipitated by addition of a negatively charged (flocculation agent) polymer. By contrast, in direct polymerization approaches, we start from the conjugated monomers and form the conjugated polymer nanoparticles in situ. The monomers can be emulsified in a micro- or miniemulsion, where the polymerization is performed in the stabilized droplets and delivers polydisperse particles. Alternatively, the polymerization is performed in a solvent which is good for the monomers but poor for the conjugated polymer, leading to precipitation during polymerization. Added stabilizers prevent aggregation and maintain the particles in dispersed form. Finally, when the monomers are liquid, emulsion polymerization can be performed, where particle growth is started in micelles.](image-url)
All of these approaches yield CPNs with broad size distributions. The above mentioned approaches have also been performed in microfluidics to better control the particle formation environments and indeed the size dispersity can be improved; however, the throughput is relatively low, leading to long preparation times.

In the direct polymerization approaches, polymerization of conjugated monomers and particle formation take place at the same time (see Figure 1). Miniemulsions and microemulsions are not just useful for making particles from previously prepared conjugated polymers as discussed above; but, they can also be used as templated reaction environments for the synthesis of CPNs from \( \pi \)-conjugated monomers. Here, the polymerization takes place inside the mini- or microemulsion droplets suspended in the continuous immiscible phase. The difference between mini- and microemulsion polymerization is in the stability of the emulsion droplets. While the microemulsion is thermodynamically stable and forms spontaneously, the miniemulsion is only metastable and accessible through shearing of the immiscible phases. This is most commonly done using ultrasonication or homogenization. In microemulsions, particle diameters of down to 5 nm can be obtained, whereas in miniemulsions, particle diameters are typically larger than 50 nm. Due to the polydispersity of the micro- and miniemulsion droplets, also the resulting particles become polydisperse as discussed above for the postpolymerization approach. The only approaches, which mechanistically allow for the generation of monodisperse particles, are emulsion polymerization and dispersion polymerization (see Figure 1). In the literature, the nomenclature for the production of CPNs is often inconsistent and “emulsion polymerization” is often used incorrectly for what should read “polymerization in miniemulsion.” The particle generation mechanism is fundamentally different: In emulsion polymerization, a liquid monomer possesses limited solubility in a continuous phase with added surfactant above the critical micelle concentration. The polymerization is initiated and begins in the continuous phase. The solubility of the produced oligomers decreases even further as compared to the monomers. This decrease in solubility drives the oligomers inside of the micelles, where polymerization can continue, supported by steady diffusion of new monomer into the micelles. However, this strategy can only rarely be applied because conjugated monomers are often solid at the reaction temperature. This problem can be circumvented when using liquid non-conjugated monomers, where the resulting polymer can subsequently be converted into a \( \pi \)-conjugated polymer. Similar approaches are also possible in dispersion polymerization. The monomers can be solids in the dispersion polymerization as it starts as a homogeneous solution of monomers, catalyst, and stabilizers. The solvent is chosen so that the monomers are soluble but the conjugated polymer is not. This leads to eventual nucleation of insoluble polymer chains during polymerization. This nucleation is followed by condensation of more and more polymer chains, reaching the critical molecular weight of dissolution. Condensation onto the nuclei constitutes growth to form the nanoparticles. While monodisperse particles are easily accessible using this technique, the particles are often larger than 150 nm in diameter and the reaction conditions can be tuned to produce particles with diameters as large as micrometers.

CPNs are often stabilized using soluble nonconjugated polymers and charged or nonionic surfactants or phospholipids. The stabilizers can be added during the particle production step; however, they can also be added subsequently to improve or adjust stability, if the reaction medium is exchanged for a different continuous phase. There are also examples of surfactant free approaches where the colloidal stability, which prevents particle aggregation, is attributed to oxidative defects, for example, fluorenone formation on fluorene containing CPNs and consequential electrostatic charging of the particle surface. There have also been approaches to attach water soluble oligomers and polymers to the surface to convey steric stability to the particles. The same strategies can also be pursued to attach biological recognition motifs to the surface of the particles. The above mentioned phospholipid dispersant already conveys some biomimicry to enable colloidal stability and biocompatibility for relevant in vivo applications. The different approaches for CPN surface functionalization will be discussed in the following paragraph.

3. Approaches for Surface Functionalization and Targeting

The functionalization approaches can be categorized into two pathways, namely before and after particle formation. While the preparticularization approaches facilitate a wider variety of chemical coupling and ligation strategies, postparticle functionalization is more economic as no hidden motifs are produced, which turn out inaccessible in the core of the particle.

A variety of conjugated polymers with amine functionalities have been prepared. Upon particle formation, some of these amine functionalities will reside on the surface of the particle and acquire positive charge around neutral pH. This helps to charge stabilize the particles and also offers (postparticularization) coupling sites. Positively charged particles can be used for uptake into cells and transfection, as negatively charged genes can be bound ionically to the particles. Furthermore, such conjugated polymers have been functionalized with fatty acid residues for uptake into the cytoplasm. Alternatively, conjugated multiblock copolymers have been terminally functionalized with folic acid groups. Such multiblock copolymers self-assemble into phase-separated nanoparticles with a large fraction of folate receptors on the surface. These particles can be applied to target tumors where they bind to overexpressed folate receptors. In mice experiments, large amounts of such CNPs have been found in the tumor, which allow imaging using fluorescence tomography. However, a substantial fraction of CNPs also ends up in the liver, kidneys, and lungs of the mice. With particle sizes above 20 nm in diameter, these particles are not cleared (e.g., renally) and will reside in the organism virtually forever. This exemplifies the need for new clearance strategies in the field of CNPs.

The above described amine functionalized polymer particles can be transformed into specific probes by coupling surface accessible amine moieties to biorecognition motifs. Another strategy is the coprecipitation of functional
nonconjugated polymers together with π-conjugated polymers. For example, hyaluronic acid,[55] poly(lactic acid),[56] poly(styrene-co-maleic acid anhydride),[57,58] or carboxylic acid functionalized polystyrene[59] (~20%) have been coprecipitated with conjugated polymers (~80%). Some of the acid groups are accessible for active ester EDC–NHS coupling (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-N-hydroxysuccinimide). Following this strategy, folic acid,[56,60] antibodies,[57,59,61] or chlorotoxin peptides[58] could be attached to the surface of the CPNs for tumor targeting. Instead of acid carrying nonconjugated polymer, also azide functionalized polymers have been coprecipitated with conjugated polymers. The azide moiety can take part in azide–alkyne click chemistry (Huisgen 1,3-dipolar cycloaddition). In the literature, this has been used to attach alkyne functionalized antibodies[62] for tumor targeting or drugs (such as plerixafor) to address transmembrane proteins.[63] Furthermore, thiolyne click chemistry has been utilized for attachment of RGD (arginyl-glycyl-aspartic acid) peptides to the surface of fluorescent acrylate-acylencopolymer for targeting of overexpressed integrins on HUVECs (human umbilical vein endothelial cells).[31,41] Upon binding of RGD to the cell membrane, the CPNs are taken up quickly via endocytosis. This uptake mechanism is comparable to what has been observed for other colloidal particles such as quantum dots, gold nanoparticles, and silica-coated magnetic nanoparticles. Uptake kinetics depend on the surface charge,[65] functionality, size,[65] and the cell type.[66] However, the bright fluorescence of CPNs and the lack of cytoxicity render CPNs superior to other colloidal and molecular fluorescence probes. A relatively new approach for CPN surface functionalization is to avoid covalent attachment and opt for anchor peptides such as hydrophobins instead. Hydrophobins adhere to the hydrophobic CPNs and also acts as a steric stabilizer. Hydrophobins can be introduced during particle generation, e.g., as stabilizers during miniemulsion. In the future, hydrophobins could be ligated with biological recognition motifs to allow binding of the CPNs to the pathological site.[67]

CPNs are highly advantageous as imaging probes as they exhibit no apparent impact on intracellular processes.[64] The functionalization after particle formation might on first sight lead to particles with low surface coverage of the functional moieties; however, it has been shown that lower amounts of surface attached recognition motifs are beneficial for colloidal stability and binding.[62]

4. Modalities of Imaging with Conjugated Polymer Nanoparticles

Conjugated polymers in their solid state – this means in powder form, as thin films, or as particles – exhibit high fluorescence yield. Further beneficial properties such as low cytotoxicity, inertness to intracellular processes, and resistance toward bleaching render conjugated polymer particles interesting for cell culture experiments. Fluorescent particles are of interest as they bring the additional quality of providing their own resonator geometry, allowing amplified emission from single fluorescent particles.[45,68] This could be of interest for tracking of individual cells in larger united cell structures.[69,70] However, for this whispering gallery resonance to take place, the particle diameter needs to be in the range of several micrometers to support the lasing modes. With these sizes, the particles are currently less important for in vivo applications; however, there might be solutions to make single particle laser resonator smaller by confining the fluorescence and gain to subwavelength surface plasmon polaritons.[71,72] In fact, the CPNs discussed so far exhibit an ideal size range for in vivo application as they are small enough to allow access also to smaller capillary blood vessels and large enough to not be cleared immediately, which impact therapeutic strategies and the associated pharmacokinetics, which we will discuss later. Most of the above presented conjugated polymer particles exhibit fluorescence in the visible spectrum. For future in vivo applications, the excitation source as well as the response from CPNs would ideally be situated in a spectral interval, where there is little absorption from tissue and biological fluids. This transparent tissue interval is usually termed the ‘near-infrared (NIR) window in biological tissue’ or the ‘therapeutic window’, which spans from 650 to about 1200 nm. With CPNs that have the absorption as well as their emission profiles within these boundaries, deep tissue penetration is possible.[73] Beneficially, in this NIR window, the radiation poses almost no photodamage to biological material and there is minimum interference from background autofluorescence by biomolecules in the living systems.[74] Due to the small electronic bandgap of the NIR spectral range, organic molecules often exhibit thermal relaxation pathways, which are in competition with radiative relaxation. As a result, CPNs with a large NIR absorption cross-section either fluoresce in the NIR spectrum or they produce heat in their direct environment.[9,75,76] Serendipitously, both effects can be utilized for medical imaging (see Figure 2). NIR fluorescent CPNs can be utilized for whole body fluorescence imaging or fluorescence molecular tomography.[77,78] By contrast, photoacoustic imaging relies on a pulsed NIR excitation beam, which is absorbed by the thermally relaxing contrast agent leading to thermal expansion of the surrounding tissue at the pulse frequency of the excitation laser. This expansion–contraction oscillation can be picked up by an ultrasound transducer. There are many endogenous probes in the body; however, for strong photoacoustic contrast, artificial agents and probes have to be administered.[79,80] There exist several approaches to fluorencently monitor oxygen and reactive oxygen species (ROS) in vivo using CPNs (see Figure 2).[81,82] ROS are often the result of oxidative stress and a viable indicator for inflammation or cancerous tumor tissue. I will discuss some examples in the context of the respective imaging mode.

5. Imaging and Therapy – Conjugated Polymer Nanoparticle Theranostics

It needs to be pointed out that there is a plethora of carrier and probe materials with potential for theranostic application.[83] CPNs represent only one class of potential candidates for these theranostic agents. Other materials are already much further advanced with some of them that have made it into clinical applications. Today, drug delivery and theranostics is maturing as a field and consolidation has set in, with only the most
promising and lucrative strategies progressing into the clinic. Therefore, the community needs to identify unmet needs in the field of theranostics, which can potentially be answered using CNPs to develop their full potential and to advance the field. Combined with the inherent imaging properties of CPNs, we have to contemplate which type of theranostic nanomedicine we wish to develop and what effect we wish to image: is it drug delivery, or drug release, or drug efficacy – or do we wish to image the dimensions of a tumor with every administration?\cite{84}

As described above, the hydrophobicity of conjugated polymers can be considered an asset as it allows for incorporation of hydrophobic drugs via noncovalent, mere physical interactions. In contrast to liposomes or micelles, where the entrapped drug is released in a burst, CPNs could potentially be useful for drug delivery with slow release profiles, where the liberation of drug is the result of surface erosion of the CPNs. Surface erosion is typical for drug delivery using solid polymer nanoparticles and there is a variety of diseases, which require such release kinetics.\cite{85–87} To achieve nanoparticle surface erosion, the community relies mostly on enzymatic degradation of specific peptide linkers, pH, or oxidation induced bond scission. Depending on the targeted environment, the particles are tailored to release their cargo. However, in CPNs, these strategies are currently almost nonexistent, mainly due to the somewhat inert and rigid backbone of the conjugated polymers. We need to develop respective pathways, which enable the release of active agents or drugs and at the same time allow for clearance of the resulting degradation products from the body. Due to the broad absence of such strategies in the current literature, we will discuss the few existing examples as well as potential future pathways for CPN degradation in the final section. First, we will discuss some of the therapeutic approaches from the literature, which utilize CPNs.

5.1. Drug Delivery

Above, we have already mentioned the potential of charged CPNs with regards to transfection. This pathway can also be used for therapeutic applications, where CPNs are used to monitor transfection.\cite{12} Deep red emitting CPNs made from dimethylamine functionalized conjugated oligomers have been equipped with cucurbituril unity on the surface to incorporate camptothecin, a cytostatic drug.\cite{88} The drug is released at pH <5, which also induces charging of the dimethyl amine groups (potentially) making the oligomer water soluble (see Figure 3a). This example represents a promising route for drug delivery as well as a pathway for clearing the degradation products. Alternatively, highly potent cisplatin can be complexed with negatively charged hyaluronic acid. During complexation, not all acid groups are consumed so that positively charged conjugated arylene–ethynylene polymers can be precipitated as CPNs. During particle formation, the conjugated polymers crystallize and the fluorescence is quenched.\cite{55} The particles have diameters in the range of 50 nm and are readily taken up by cells via CD44 receptor mediated endocytosis. CD44 binds to hyaluronic acid and is expressed
on many types of cancer cells. Cisplatin can be released by enzymatic degradation of the hyaluronic acid. The degradation of the particles also releases the conjugated polymer, which in its solvated form recovers its fluorescence. In this way, drug release can be monitored following the fluorescence of the conjugated polymer. In a similar approach, poly(fluorenedivinylene-benzothiadiazole) (PFVBT) with tetraalkyl ammonium side chains and terminally linked doxorubicin with an oxidation labile linker has been prepared.\[89\] The polymers self-assemble into ≈120 nm nanoparticles in water. The particles can be decorated with cyclic RGD peptides via EDC–NHS coupling of amine functionalized RGD to carboxylic acid terminated side chains (see Figure 3e). Upon irradiation, ROS are generated via photoinduced electron transfer (PET) from the excited state on the conjugated polymer to ground state oxygen.\[90,91\] The resulting ROS cleave the oxidation sensitive linker and releases the drug. Alternatively, the oxidative stress in the tumor environment can be used to cleave off the drug from the CPNs. This mechanism has been shown for PEGylated conjugated polymers, where chloromethin is liberated after oxidative cleavage of an arylboronate linker.\[92\] This strategy holds also promise for changing the solubility of conjugated polymers upon oxidation and might become relevant for the clearance problem. Drug delivery can also be triggered by heat. A photothermally active isoindigo-alt-bithiophene polymer has been combined with a polyethylene glycol-block-poly(hexyl ethylene phosphate) (PEG-block-PHEP) and doxorubicin to form CPNs using the miniemulsification procedure. PEG-block-PHEP is thermoresponsive and allows release of doxorubicin upon photothermal heating (see Figure 3b).\[93\] These CPNs can be applied for anticancer drug delivery as well as photothermal therapy as explained in the following section.

5.2. Photothermal Therapy

As mentioned above, thermal relaxation of the excited state can be used for photoacoustic imaging; however, at longer exposure...
times, the photothermal effect (which is also the foundation for photoacoustic imaging) can be used for therapy. Oxidative polymerization of a (ProDOT) thiophene sulfonic acid leads to ≈200 nm spheres, which are charge stabilized and do not require any surfactants or stabilizers. Antibodies against CD44 can be attached to the surface to allow for tumor homing of the particles. The resulting particles fluoresce in the NIR spectrum and can be used for photothermal ablation of the CD44 expressing cells in the tumor. When the particles are irradiated with NIR laser light, the local temperature in the vicinity of the particles rises by several degrees, which damages the respective cells such as cancerous mammospheres in a tumor mouse model. However, the fact that the polythiophene nanoparticles exhibit NIR fluorescence as well as photothermal efficiency, shows that none of the two relaxation pathways is predominant. It might be advantageous when the CPNs only relax via thermal pathways for better efficiency of photothermal therapy and photoacoustic imaging rather than having competing fluorescence. To improve the photoacoustic efficiency, electron acceptors such as fullerenes can be added to the CPNs. Upon excitation of the polythiophene based CPN, the electron is transferred (via PET) to the admixed fullerene and the fluorescence is greatly reduced. At the same time, the photothermal (and photoacoustic) efficiency increases (see Figure 3c).

In a similar approach, a combination of conjugated polymers has been used to form CPNs, following the postpolymerization miniemulsion technique. The two combined conjugated polymers are an amine functionalized PFVBT, which is good for fluorescence imaging and produces ROS in the presence of oxygen. The second polymer has a more complex thiophene-based backbone (PIDTTTQ), which is active for photothermal therapy (see Figure 3d). Additionally, a maleimide carrying block copolymer stabilizer has been added during particle formation. The free maleimide groups can be used to attach anti-HER2 affibodies or RGD peptides to the surface of the particles for tumor cell targeting. The particles fluoresce red ($\lambda_{em} \approx 612$ nm) when excited in the green-blue spectrum. Furthermore, PFVBT in the excited state produces ROS upon reaction with ground-state oxygen, which can be used for photodynamic therapy (see Figure 3d). The second polymer PIDTTTQ has an absorption spectrum ranging from 620 to 1100 nm and shows no fluorescence when excited in this spectral range. This absence affirms the potential for photothermal therapy. Anti-HER2 CPNs have been injected into an SKBR-3 tumor-bearing mouse model where they home in on the tumor. When excited by NIR radiation, the tumor is locally heated (e.g., up to 60 °C for 6 min), which leads to thermal necrosis and apoptosis in the direct vicinity of the CPNs. When excited in the green-blue spectrum, the CPNs produce ROS to oxidize surrounding tumor cells. There are also examples where both effects can be induced at the same irradiation wavelength. The particles consist of conjugated polymer with bithiophene as donor and bis(oxoindolyl)benzo difuranidione (BT-BIBDF) as acceptor and a PEG-polycaprolactone block copolymer for stabilization. The CPNs have diameters of ≈100 nm and can be excited at 785 nm, which is beneficial as this is right in the therapeutic window. Upon irradiation, the particles perform both; first, photoinduced heating, which can be used for photoacoustic imaging and for photothermal therapy and second, the generation of singlet oxygen, which can be used for photodynamic therapy. I will discuss further approaches for photothermal therapy in the following section.

5.3. Photodynamic Therapy

Besides the previously named examples of ROS producing PFVBT and BT-BIBDF based CPNs, there are also examples, where the particles are more selective in producing singlet oxygen versus heating. Singlet oxygen is reactive and can be used to kill cancer cells. Triphenylene diiophenene benzo-thiadiazole polymers that coassemble with pluronic block copolymer surfactants into CPNs can generate singlet oxygen upon irradiation with UV light (254 nm). However, this wavelength is unsuitable for cell experiments and CPNs with an efficient photodynamic effect at visible or ideally NIR wavelengths are more desirable. There are examples where metal coordinating centers are introduced into the conjugated polymer architecture to stabilize the required triplet state and facilitate electron transfer to ground state oxygen to enhance the ROS generation efficiency. Platinum cored porphyrin units have been introduced into fluorene based CPNs. The porphyrins are either admixed or covalently introduced as a comonomer in the conjugated polymer backbone. In the literature, these examples have also been applied for oxygen sensing; however, sensing is conducted through the decrease in photoluminescence intensity. This decrease is the result of PET to ground state (triplet) oxygen and therefore ROS generation. These particles, both with admixed and covalently introduced platinum porphyrin, represent powerful singlet oxygen generators for photodynamic therapy. By contrast, superoxide anions (also a ROS) can be sensed using positively charged CPNs with imidazopyrazinone units in the periphery. Naturally, also iridium complexing conjugated polymer particles have been applied for both ratiometric oxygen sensing as well as photodynamic cancer therapy.

Summing up, CPNs can be functionalized via simple ligand exchange techniques, which are well established in the biomedical sciences, e.g., click chemistries and active ester coupling protocols. Depending of the molecular architecture, CPNs relax their excited states via fluorescence, phosphorescence with the possibility for PET to produce ROS, or thermally with the potential for photoacoustic imaging and photothermal therapy. Several approaches for drug delivery have been pursued ranging from (oxidation sensitive) covalent linkage to CPNs to supramolecular host–guest incorporation of the drugs. While some potential pathways for degradation or clearance of the conjugated polymers have been scratched, there is no real debate about clearance of CPNs and the cytotoxicity of the degradation products.

6. Clearance of Conjugated Polymer Particles

For the ultimate goal of applying CPNs for in vivo administration and to transfer the theranostic nanoparticles into clinical applications, the pathways for clearance need to be discussed. For oral administration, attention needs to be paid that no
unwanted byproducts or carrier entities are taken up via the intestine; however, for clearance, the size of the microscopic drug delivery vehicle is relatively unimportant. By contrast, for intravenous administration, size matters and entities smaller than 5 nm are quickly cleared through the renal system. Consequently, to improve the pharmacokinetics of small drug delivery vehicles, they need to be functionalized to home in on the pathological site and bind there to avoid immediate clearance without effect. By contrast, larger drug delivery vehicles such as CPNs have long circulation times as they are too large to pass the kidney membranes. This means that CPNs would have to be degraded or they will reside in the body, where they could cause adverse effects. A few examples for CPN decomposition have been touched in the previous sections. When we look at existing nonconjugated polymer drug delivery vehicles, we realize that a wide variety of degradation and clearance pathways has been described. The degradation of these nonconjugated entities is usually triggered by:

1. a change in pH, which is sensible as the pH varies in healthy and pathological tissue,
2. enzymatically, where usually specific peptide sequences are introduced into the particle and disease specific enzymes can then cleave the peptide sequence,
3. oxidation, either enzymatic or because of oxidative stress (ROS), for example, caused by tumor associated macrophages,[85,104]
4. or reduction, either enzymatic or due to high local concentrations of reductive signal molecules such as glutathione.[105]

To develop new degradation strategies for conjugated polymer clearance, it is instructive to look at nature and its examples to deal with π-conjugated entities. From bioluminescent organisms, a variety of conjugated dye molecules are known. It is intriguing that in luciferin based molecules such as coelenterazine,[106] vargulin,[107] as well as in green

Figure 4. Strategies for CPN clearance. Different triggers such as pH, reactive oxygen species, and enzymes can be utilized to disintegrate conjugated polymer particles and solubilize the building blocks. Deprotection and solubilization, de-crosslinking, and backbone degradation into soluble units are general strategies, which can be induced by the above mentioned triggers. Here, three examples as discussed in the text are shown.
fluorescent protein,\textsuperscript{[108]} we find imidazole units as part of the conjugated dye scaffold. The imidazole unit is the result of coupling of phenylalanine, or tyrosine with serine and glycine. The imidazole unit is biodegradable through oxidation (enzymatic or ROS), leading to scission of the imidazole ring. This ring scission produces amino acids and amides, which can be metabolized by the respective organism.\textsuperscript{[109,110]} It is difficult to find representatives of conjugated polymers in the realms of natural materials; however, melanin can be considered a conjugated polymer despite its ill-defined and crosslinked nature. \textit{Aspergillus fumigatus}, a fungus found in compost and soil, can degrade melanin.\textsuperscript{[111]} The degradation is most probably performed enzymatically by cytochrome P-450, which is known to degrade polycyclic aromatic hydrocarbons.\textsuperscript{[112]}

Effectively, these strategies could in the future be utilized to produce biodegradable conjugated polymers and CPNs (see Figure 4). Different imidazole and other aromatic N-heterocycle units have been examined for their susceptibility toward oxidative degradation.\textsuperscript{[113,114]} CPNs with incorporated labile aromatic N-heterocycles could be incorporated into the polymer backbone of CPNs. These could then be degraded by direct enzymatic oxidation or by ROS from activated macrophages or other chemical and enzymatic processes (see Figure 4, backbone degradation). Alternatively, short and water soluble conjugated units can be linked together by ester, containing segments, which are cleavable by lysosomal enzymes.\textsuperscript{[115]} Such nanoparticles can also be used to release drugs while they are enzymatically decomposed. There are also examples, where oxidative stress is utilized to cleave bonds in the side chain of conjugated polymers.\textsuperscript{[90]} In this way, the conjugated polymers in CPNs could be rendered water soluble to clear them from the body. We have previously seen that conjugated polymers or oligomers, which carry charges, are soluble in aqueous media. Protection groups, which prevent charging of amine or carboxylic acid moieties, can be used to render conjugated materials hydrophobic or crosslink shorter conjugated segments (see Figure 4, de-crosslinking into soluble units). At reduced pH, the protection group can be removed producing the respective moiety, which will collect charge and produce a water soluble conjugated molecule.\textsuperscript{[32]} Charged amine functionalized conjugated oligomers have been discussed in conjunction with cucurbituril based host–guest delivery systems.\textsuperscript{[88]} Conjugated polymers with Boc protection groups on amine periphery have also been reported previously.\textsuperscript{[12]} The Boc group can be removed at low pH, yielding water soluble ammonium functionalized conjugated polymers.\textsuperscript{[116]} Alternatively, esters can be saponified to produce negatively charged and soluble conjugated polymers (see Figure 4, deprotection and solubilization).\textsuperscript{[36,117]}

The degradation products, namely either charged polymers or water soluble small conjugated molecules must not be cytotoxic to guarantee fast and unimpeded clearance. Despite the cytotoxicity of charged transfection agents such as polyethyleneimine,\textsuperscript{[118]} charged conjugated polymers show much reduced cytotoxicity (up to $40 \times 10^{-6}$ μl).\textsuperscript{[119]} If the small degradation products are decorated with water soluble periphery such as oligo- or polyethylene glycol or sulfobetaine, the cytotoxicity of small $\pi$-conjugated molecules can be controlled to be low.\textsuperscript{[120,121]}

Apart from a few seminal examples toward CPN clearance, strategies for CPN degradation are widely absent. Adding this extra dimension of CPN decomposition to future research efforts will enable new manners of slow rate drug release and strengthen the position of CPNs in the world of theranostic agents. The community has developed some amazing examples of extremely complex and finely tunable geometries. In the future, the focus needs to lie with simplification of current synthetic approaches, while maintaining specific targeting, good signal to noise ratios in the respective imaging type, and high therapeutic efficiency or drug loading capacity. As theranostics is intimately linked to personalized medicine, robust syntheses and formulation procedures need to be advanced. In comparison with other theranostic agents, CPNs have the potential to outshine in most of the postulated and here defined requirements. The future for conjugated polymer nanoparticles theranostics is – like the nanoparticles themselves – bright and almost impossible to bleach.

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Conflict of Interest

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