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Minireview

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Highly Luminescent Conjugated Polymer Nanoparticles for Imaging and Therapy

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Abstract: In recent years, water soluble conjugated polymer nanoparticles (CPNs) have gained widespread attention as potential therapeutic devices and fluorescent imaging probes. Characteristic of CPNs are their high fluorescent quantum yields, resistance to photobleaching, and low cytotoxicity, enabling their use as effective bioprobes. Among many applications of CPNs are their uses in cellular labeling and biomedical imaging due to their tunable multi-color emissions. In addition, CPNs with functional side chains can form complexes with drugs and gene, creating nanostructures of 10 to 100 nm. They allow for controlled, continuous release of their cargo, a highly desirable characteristic for use in gene therapy and targeted anti-tumor therapy. In this review, we briefly summarize recent progress in CPNs, highlighting their applications in imaging and drug delivery

Keywords: Nanomaterials, Conjugated Polymers, Imaging, Drug Delivery, Photodynamic Antimicrobial Chemotherapy

1. INTRODUCTION

Much interest has been generated in synthesizing novel highly fluorescent nanoparticles for use in biomedical imaging due to their biocompatibility, high sensitivity, and photostability [1-6]. In particular, semiconducting quantum dots (Qdots) have gained widespread attention [1, 3-4]. Qdots have been used as ultrasensitive imaging probes and show greater fluorescent quantum yields and greater tolerance to photobleaching compared to conventional organic dyes. However, Qdots show leakage of metal ions, inducing cytotoxicity. Water soluble conjugated polymer nanoparticles (CPNs) address the pitfalls of Qdots while retaining their advantageous properties. CPNs are characterized by their delocalized electronic structures, allowing for fluorescence [7-13]. They exhibit high signal intensities and a strong

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resistance to photobleaching, giving them the same advantages Qdots have over traditional organic dyes. However, unlike Qdots, CPNs show low cytotoxicity. Their biocompatible and biodegradable characteristics enable the use of CPNs in vitro, without risk to cellular viability. Combined with fluorescent dyes, CPNs are ideal candidates as biosensors. In addition, CPNs often have a hydrophobic backbone, allowing them to effectively mix with hydrophobic drugs. This system facilitates a controlled continuous release of the drug, which can be monitored by fluorescent imaging. Due to the limited page of this review, we can only focus on parts of CPNs and their biological applications. The readers who want more examples and references are directed to also read other recent reviews [14-18].

2. IMAGING WITH CPNs

The backbone of conjugated polymers behaves as numerous light-harvesting units that exhibit a larger optical cross section compared to small organic molecule dyes. CPNs have been widely used in cell imaging due to their large extinction coefficients, high brightness, superior photostability, low cytotoxicity, facile chemical synthesis, tunable spectral properties, and versatile surface modification [19-20]. The high fluorescence of CPNs enabled their use at a low concentration in a cell culture to further improve their biocompatibility. For example, Poly[(9,9-dioctylfluorenyl-2,7-diyl)-*co*-(1,4-benzo-{2,1′,3}-thiadazole)] (PFBT) nanoparticles have shown efficient uptake in vitro [21]. Using flow cytometry, the fluorescence per cell was determined to have a linear relationship with nanoparticle concentrations as low as 155 pM. Cellular uptake slowed at low temperatures, which was consistent with endocytosis, rather than diffusion across the plasma membrane. PFBT nanoparticles that were mixed with Texas Red dextran were taken up by the cells via endocytosis, and accumulate in lysosomes. This was consistent with results of immunostaining the PFBT nanoparticles with an anti-LAMP-1 antibody, which showed fluorescence in lysosomes and late endosomes. A comparison of PFBT treated cells to control cells showed little decrease in cell viability. Nanoparticles have also been used encapsulate dyes for bioimaging. Fluorescent core-shell silica nanoparticles were observed to be brighter and more photostable than the dye alone, while showing low toxicity in vitro. [22-23]. In order to meet the needs of multiplexed biological imaging, CPNs have also been made for multicolor cell imaging. 60-120 nm particles of highly fluorescent poly(arylene ethynylene)s that were copolymerized with dibromo-substituted fluorenone showed emissions ranging from blue to orange color [24]. Fluorescent quantum yields increased with dye incorporation from 44% to over 60% and photostability studies showed a high tolerance of the CPNs to bleaching; after 500s of irradiation, there was no decrease in fluorescence. Cell viability was not affected by these processes. Further progress in multicolor emitting CPNs has been made in 2012 for cell labeling. Cationic 50 to 100 nm CPNs were designed to show multicolor emissions by tuning FRET efficiencies to a single excitation wavelength [25]. Three polymers were bound closely together on a cellular outer membrane. A blue-emitting polymer acted as a donor for the green-emitting and red-emitting polymers. The green-emitting polymer acted as an acceptor for the blue-emitting polymer and a donor for the red-emitting polymer. The red-emitting polymer acted as an acceptor to the blue-emitting and green-emitting polymers. Efficient intermolecular FRET between neighboring polymers occurred upon excitation of a blue-emitting polymer (Figure 1). When irradiated for 20 seconds, the CPNs maintained their original fluorescence for 60 seconds, demonstrating the photostability of the CPNs. Fine-tuning FRET among the three CPNs resulted in 12 color-encoded microparticles, allowing for efficient labeling of cells. The identification of multi-color microparticle-encoded cells was performed by flow cytometry.

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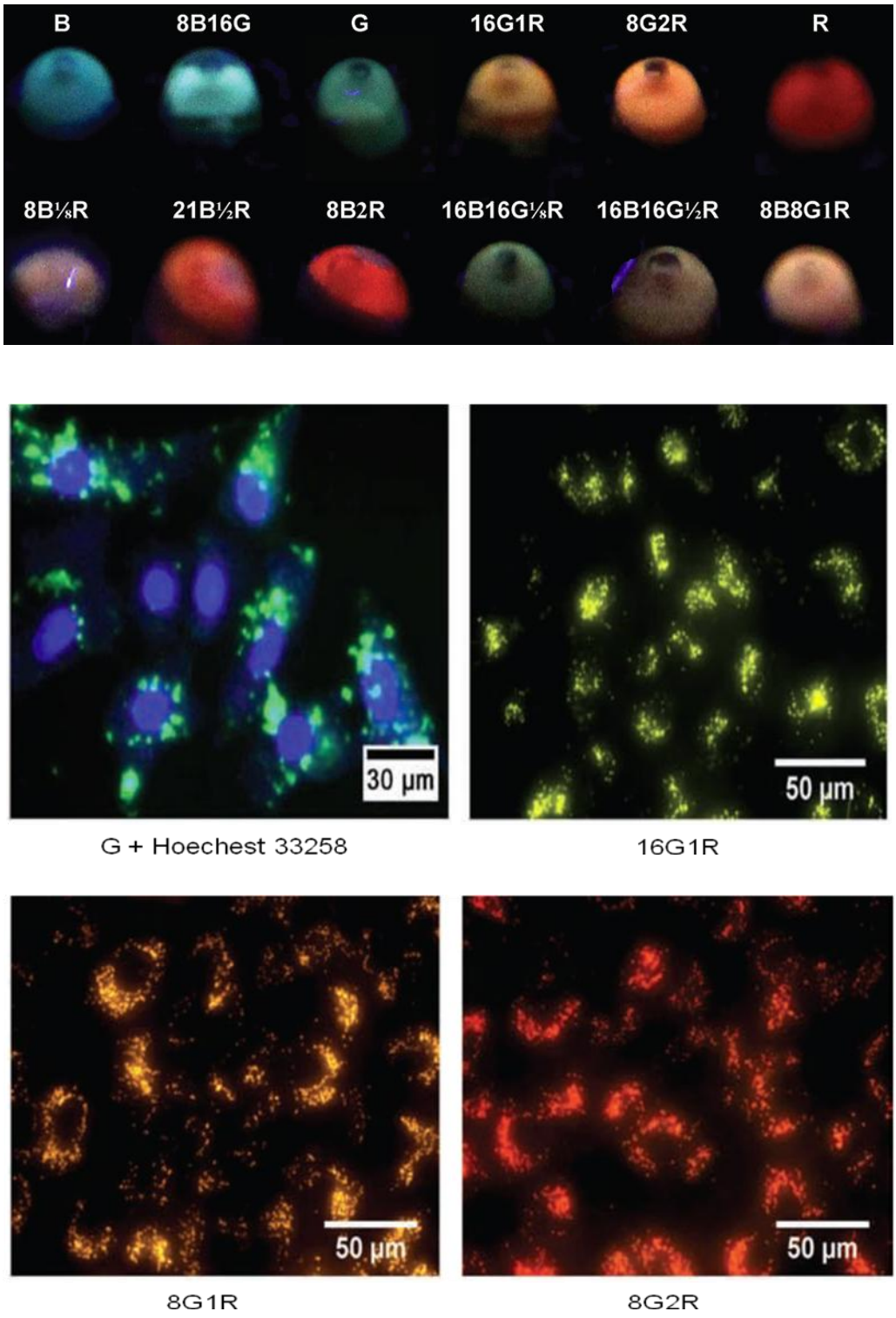

Figure 1. Various color-barcoded microparticles by mixing E. coli and CPNs (Top). The overlay images of fluorescence of A549 cells treated with green-emitting CPNs and Hoechest 33258 dye, and the overlay fluorescent images of A549 cells treated with barcoded microparticles (16G1R, 8G1R, and 8G2R) (middle and bottom). Reproduced with permission from Ref. [25].

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3. DRUG AND GENE DELIVERY

Multifunctional CPNs have diverse applications not only for cell imaging but also for drug and gene delivery [26, 27]. A 50 nm CPN was designed from a positively charged conjugated polymer (PFO) and negatively charged poly(L-glutamic acid) (PG). The "turn off" state of the complex occurred when the fluorescence of PFO was quenched by Dox. When the CPN was taken up by cancer cells or exposed to carboxypeptidase, Dox was released from the complex, and the PFO showed a fluorescent quantum yield of 24% in water, activating the "turn on" state (Figure 2A). Photostability was observed even after 30s of continuous radiation; the PFO fluorescence remained consistent at 60%. Little cytotoxicity was observed and the CPNs allowed for real-time monitoring of drug release while the PFO remained in the "turn on" state. Simultaneous in vivo imaging and drug tracking has also been shown in CPNs that were densely packed with poly(ethylene glycol) (PEG). A conjugated polyelectrolyte (CPE) that was densely grafted with PEG was complexed with an anti-cancer drug cisplatin (Pt) [28]. The efficiency of the drug release was tracked by the fluorescence of the CPE-PEG-Pt nanoparticles. The drug loading payload of 8.6 ± 0.9% was attributed to the dense PEG chains. The cytotoxicity of the CPE-PEG-Pt nanoparticles was comparable to that of the free drug. Amphiphilic and hydrophilic semiconducting polymer nanoparticles have also been explored for gene delivery. Lipid-modified cationic poly(fluorenylene phenylene) polymer (PFPL) with ammonium pendant groups was synthesized and bound to DNA through electrostatic interactions [29]. The pCX-EGFP plasmid encoding the green fluorescent protein (GFP) was associated with the PFPL nanoparticles to form a nanoparticle/plasmid complex that was taken up by A549 cells through endocytosis. Green fluorescence from the GFP was observed (Figure 2B), thus indicating the nanoparticles successfully delivered plasmids into cells for further transcription and translation.

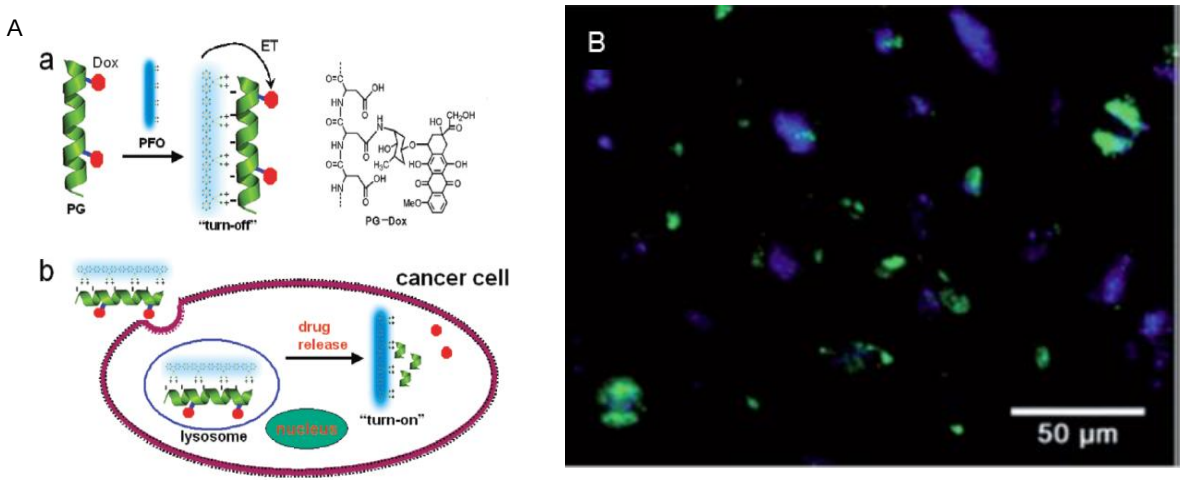

Figure 2. Conjugated polymer nanoparticles for drug (A) and gene delivery (B). A-a).The fluorescence of PFO was quenched by Dox in the PFO/PG/Dox "turn off" state. A-b).When taken up by a cancer cell, Dox was released and the PFO was in its "turn-on" state. B) Lipid-modified cationic PFPL nanoparticles for delivery of pCX-EGFP plasmids encoding the GFP. Fluorescence imaging of A549 cells after incubation with PFPL/pCX-EGFP nanoparticles showed both blue fluorescence from PFPL and green fluorescence from GFP, thus indicating the PFPL nanoparticles successfully delivered plasmids into cells. Reproduced with permission from Ref. [27, 29].

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Fluorescent CPNs have also been designed to deliver small interfering RNA (siRNA) [30]. Amine-containing hydrophilic PPEs were prepared into loosely aggregated particles. These particles formed stable complexes with siRNA. The complexes were delivered to HeLa cells and a significant downregulation of a target gene was observed.

4. CPNs ASSISTED THERAPY

Photodynamic antimicrobial chemotherapy (PACT) has been shown to effectively kill a wide range of Gram-positive and Gram-negative bacteria [31]. A divalent vancomycin-porphyrin conjugate was developed to deactivate and image vancomycin resistant bacteria [32]. The structure showed multiple advantages, including ease of preparation, selective adhesion of the divalent vancomycin to bacterial surfaces, and the use of vancomycin as a fluorescent probe to monitor bacterial behavior. Whitten's group pioneered killing bacteria with cationic conjugated polymers, where cationic conjugated polymers could bind to the surface of bacteria and function as singlet oxygen photosensitizers to kill the bacteria [33]. In addition to direct sensitization of oxygen molecules by conjugated polymers, energy transfer from conjugated polymers to photosensitizers (e.g., porphyrin) was another efficient method to further improve singlet oxygen generation efficiency. Additional work in PACT was made in 2009. Anionic polythiophene (PTP) and cationic porphyrric (TPPN) formed PTP/TPPN particles via electrostatic interactions. When irradiated by white light, energy transfer from PTP to TPPN was followed by sensitization of oxygen molecules, greatly improving the ability of PTP/TPPN to adsorb on bacterial membranes. The process was extremely efficient (Figure 3). Upon irradiation by 5 minutes of white light, bacterial viability decreased 70%.

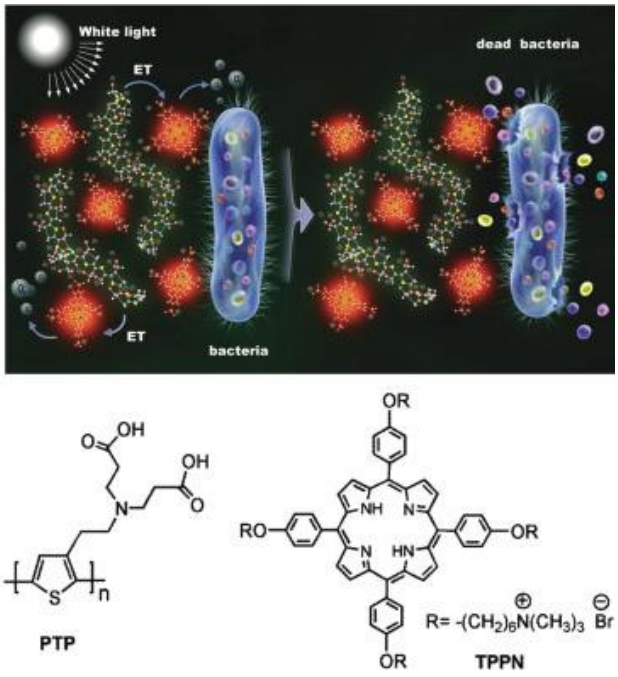

Figure 3. Chemical structures of PTP and TPPN and antibacterial mechanism of PTP/TPPN complex particles. Reproduced with permission from Ref. [34].

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5. CONCLUSIONS

CPNs show a wide range of applications in both drug delivery and imaging. The low cytotoxicity and high luminescence of CPNs make them ideal candidates for these two roles. Specifically, novel CPNs have shown efficient gene delivery and antitumor therapy and continuous monitoring of drug release as well as fluorescent imaging and multicolor imaging in vivo. The progress that has been made in the vast number of CPN applications has shown the utility and versatility in the biomedical areas.

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