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Fabrication of aggregation-induced emission based fluorescent nanoparticles and their biological imaging application: recent progress and perspectives

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Aggregation-induced emission (AIE) dyes have received wide-spread concern since their inception. Several types of AIE-based fluorescent nanoparticle (FNP) have been developed, and the potential applications of these FNPs have also been explored. Recent studies of AIE-based FNPs in biological areas have suggested that they show promise as bio-materials for cell imaging and other biomedical applications. This article reviews recent progress in the synthesis of AIE-based FNPs via non-covalent, covalent and novel one-pot strategies, and the subsequent cell-imaging of those AIE-based FNPs. Many successes have been achieved, and there is still plenty of space for the development of AIE-based FNPs as new bio-materials.

Introduction

Biological imaging is a useful tool to provide visual information on organs and biological processes in living systems [1–6]. Common biological imaging technologies including magnetic resonance imaging (MRI), X-ray phase tomography, radionuclide imaging, positron emission tomography, fluorescence imaging, etc. have been used and play important roles in several biological applications such as cancer detection, stem cell transplantation and tissue engineering. Among them, fluorescence imaging has attracted much research interest because the use of fluorescence as an output signal can provide strong signal intensity and high resolution images at subcellular levels, and the fluorescent probes are usually biocompatible and designable [7,8]. Compared with small molecular dyes, fluorescent nanoparticles (FNPs) are the better choice for fluorescence imaging, due to their tunable size, superior photostability, desirable pharmacokinetic behavior, and multifunctional potentials. Fluorescent inorganic nanoparticles (FINs) have been developed over the past few decades, such as fluorescent carbon dots, photoluminescent silicon nanoparticles, semiconductor quantum dots, and metallic nanoclusters [9–12]. Many successes have been achieved in the research of FINs, however, it cannot be ignored that many FINs are not biodegradable and might be potentially toxic to living organisms. Thus, a novel type of FNPs, fluorescent organic nanoparticles (FONs), has recently been developed. Compared with FINs, the FONs have better biocompatibility and biodegradability; they may also be modified with greater ease. So far, several FONs based on organic dyes conjugated with polymers, metal coordination luminescent polymers, fluorescent proteins, etc. have been studied, and the improvement of fluorescent intensity is still an important focus of research in the development of FONs [13–16]. Unfortunately, most fluorescent organic dyes are hydrophobic, therefore, the dye moieties in organic dye-decorated polymers tend to aggregate together to form a hydrophobic core, which induces strong intermolecular π–π interactions, resulting in fluorescence quenching and photobleaching because of the notorious aggregation-caused quenching (ACQ) [17–19], and severely limiting the bioimaging and tracing applications of FONs under physiological conditions. Different from traditional organic dyes, a type of unique organic dye which can emit much stronger luminescence in the aggregation state was discovered by Tang et al. in 2001, and named aggregation-induced

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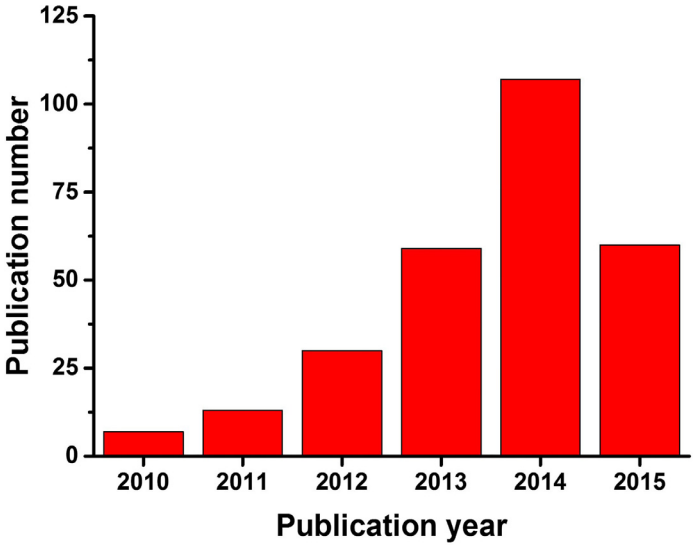

FIGURE 1

Histograms showing the annual numbers of publications on the topic including both 'aggregation-induced emission' and 'cell imaging' from 2010 to present (data obtained from Web of Science on 30 June 2015).

emission (AIE) dyes [18,20]. Since then, a lot of AIE molecules such as siloles, tetraphenylethene, triphenylethene, cyano-substituted diarylethene, distyrylanthracene derivatives, etc. have been successfully developed [21–27]. Meanwhile, several possible molecular mechanisms have been put forward to explain the interesting AIE phenomena. Among them, restriction of intramolecular motion (RIM) processes has been recognized as the major cause for the distinct AIE phenomena [26,20]. Because of their unique AIE properties, AIE-based FNPs have drawn considerable attention in the research area of biological imaging, especially cell imaging [28–36]. Several types of AIE-based FNPs have been developed as cell imaging agents, and many papers have been published in the last five years (Fig. 1). In this review, we will focus on the development of the fabrication of AIE-based FNPs and their application as biological imaging agents over the past few years. Common fabrication strategies including non-covalent or covalent approaches, such as direct covalent/non-covalent linkage of AIE dyes in the polymer chains, copolymerization of AIE-based monomers via controlled radical polymerizations (CRPs), etc. will be presented. Moreover, the novel one-pot polymerization strategy for the fabrication of AIE-based FNPs will also be discussed.

Non-covalent strategy

Almost all AIE dyes are hydrophobic. To increase their dispersibility in an aqueous environment, amphiphilic polymers have been used to self-assemble with AIE dyes to form AIE-based FNPs. In 2012, Wei et al. reported a new fluorescent bioprobe system based on nanoaggregates combining AIE-based organic fluorogens **An18** (derivatized from 9,10-distyrylanthracene with an alkoxyl endgroup) and surfactant Pluronic F127 (Fig. 2) [37]. The surfactant Pluronic F127 can surround the An18 to achieve **An18-F127** FNPs. F127 can change the hydrophobic surface of FNPs to hydrophilic in character, thus the **An18-F127** composite exhibits good water dispersibility and the AIE property. Nanoparticles with diameters ranging from 400 to 600 nm could be obviously observed from the transmission electron microscopy (TEM) image, further confirming the successful fabrication of the **An18-F127** FNPs. Due to the AIE property of the **An18** molecules in the core of the FNPs, the obtained **An18-F127** FNPs show no obvious fluorescence in pure THF solution but strong fluorescence in water after the self-assembly. The biocompatibility of **An18-F127** was subsequently evaluated, and cell viability results from cell counting kit-8 (CCK-8) assay further suggested the excellent biocompatibility of the **An18-F127** FNPs. The **An18-F127** FNPs were then applied in cell imaging. Bright yellow fluorescence could be observed when cells were incubated with 40 $\mu g\ mL^{-1}$ of **An18-F127** for 3 hours. The confocal result shows that the **An18-F127** FNPs could easily translocate into A549 cells and locate at cytoplasm. It is noticeable that the concentration of FNPs (40 $\mu g\ mL^{-1}$) used for cell imaging is much lower than that for cell viability testing (100 $\mu g\ mL^{-1}$), suggesting their potential for biological imaging applications.

However, it is still difficult to control the surface properties of such AIE-based FNPs due to the lack of designability of commercially available surfactants. Furthermore, most surfactants suffer from toxicity when used in living organisms. Thus, synthetic polymers might be suitable alternatives and more promising candidates for bio-applications because of their better controllable and designable properties. In 2013, Wei et al. reported novel synthetic copolymers as surfactants used for encapsulating the AIE molecule **An18** to form PEGylated AIE-based FNPs. The copolymers were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization using stearyl methacrylate (SMA) and poly(ethylene glycol) methacrylate (PEGMA) as comonomers. The molecule weight and composition of the synthetic copolymers could be easily tuned by changing the feeding ratio of the two monomers and adjusting the conversion of RAFT polymerization. The obtained copolymers and **An18** could self-assemble to form AIE-based FNPs with diameters less than 100 nm. Besides, the obtained copolymers have also been used to fabricate AIE-based FNPs along with other AIE molecules such as a cyano-substituted diarylethene derivate dye [38].

Surfactants used in the non-covalent fabrication of AIE-based FNPs could not only encapsulate the AIE molecules, but they also have some special functions. In 2011, Liu et al. applied a two-photon absorbing (TPA) molecule (named as **T1**), which also has the AIE property, for cell imaging. They reported a one-step strategy to prepare hydrophobic TPA chromophore-doped FNPs with surface-modified folic acid by encapsulating the TPA AIE molecule in a mixture of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine N-[methoxy(polyethylene glycol)-2000] (**DSPE-PEG$_{2000}$**) and **DSPE-PEG$_{5000}$-folate** [39,40]. The formation of the **DSPE-PEG** encapsulated TPA NPs (FTNPs) is easy and efficient via a modified nanoprecipitation method [41]. During the formation process, the hydrophobic DSPE segments tend to be entangled into the hydrophobic core while the hydrophilic PEG-folate chains extend into the aqueous phase. Using **DSPE-PEG$_{2000}$** and **DSPE-PEG$_{5000}$-folate** mixture as the encapsulation matrix makes the obtained FNPs biocompatible with well-controlled surface functional groups. Meanwhile, the folate density on FTNPs surface can be facilely adjusted by varying the feed ratio of **DSPE-PEG$_{5000}$-folate** to **DSPE-PEG$_{2000}$**. Due to the specific interaction between folate acid on the FTNPs and the