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Turn-on theranostic fluorescent nanoprobe by electrostatic self-assembly of carbon dots with doxorubicin for targeted cancer cell imaging, in vivo hyaluronidase analysis, and targeted drug delivery

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ABSTRACT

This paper reports a turn-on theranostic fluorescent nanoprobe P-CDs/HA-Dox obtained by electrostatic assembly of polyethylenimine (PEI)-modified carbon dots (P-CDs) and Hyaluronic acid (HA)-conjugated doxorubicin (Dox) for hyaluronidase (HAase) detection, self-targeted imaging and drug delivery. P-CDs/HA-Dox show weak emission in a physiological environment. By utilizing the high affinity of HA to CD44 receptors overexpressed on many cancer cells, P-CDs/HA-Dox are capable of targeting and penetrating into cancer cells, where they are activated by HAase. As a result, HA-Dox can be digested into small fragments, causing the release of Dox and thereby restoring the fluorescence of P-CDs. The theranostic fluorescent nanoprobe can effectively distinguish cancer cells from normal cells. The as-prepared nanoprobe achieves a sensitive assay of HAase with a detection limit of 0.65 U mL^{-1}. Furthermore, upon Dox release, the Dox could efficiently induce apoptosis in HeLa cells, as confirmed by MTT assay. The design of such a turn-on theranostic fluorescent probe provides a new strategy for self-targeted and image-guided chemotherapy.

1. Introduction

Hyaluronic acid (HA) is a negatively charged polysaccharide made of repeating disaccharide units, N-acetyl-D-glucosamine and D-glucuronic acid (Lapcik et al., 1998; Lokeshwar et al., 2001). It is abundant in the extracellular matrix and synovial fluids of all vertebrates and participates in several biological processes such as tissue hydration, and cell motility, proliferation and differentiation (Toole and Slomiany, 2008). Importantly, HA exhibits a high specific affinity for CD44 receptors overexpressed on the surfaces of many cancer cells, and can enter cells through receptor-mediated endocytosis (Kim et al., 2010). Hyaluronidase (HAase), a family of endoglycosidases, can degrade HA by cleaving its internal β-N-acetyl-D-glucosamine bonds (Lokeshwara and Selzer, 2008). It has been reported that HAase has a close relationship with the presence of many malignant tumors (Wang et al., 2015). The expressed levels of HAase greatly increase in cancer cells, whereas HAase is less distributed in normal cells (Lokeshwar et al., 1996). Therefore, it is of considerable importance to construct a theranostic fluorescent probe for the evaluation of HAase level, and subsequently, in vivo drug delivery and cancer diagnosis and therapy (Bhang et al., 2009; Chen et al., 2015; Han et al., 2016; Ma et al., 2012; Swierczewska et al., 2012).

Carbon dots (CDs) represent a new class of biocompatible fluorescent carbon materials for various biological applications because of their fascinating properties, such as small sizes, good aqueous solubility and tunable optical properties by optimized precursors and synthetic procedure (Ge et al., 2015; Gong et al., 2016; Shen et al., 2012). A number of studies have explored CDs as fluorescent nanoprobes for intracellular imaging (Goh et al., 2012; Hsu and Chang, 2012; Kong et al., 2012; Liu et al., 2009; Zhao et al., 2015). However, most of these methods are either "fluorescence always on" or "fluorescence turn-off" due to non-specific endocytosis or nonradiative electron transfer between carbon dots and target molecules. It is well known that "fluorescence always on" nanoprobes and "turn-off" nanoprobes may either lead to false positive signals or suffer potential interference from the environment, which may be problematic for utilization in quantitative measurements (B.Y. Feng et al., 2013; Verma and Stellacci, 2010; Zhang et al., 2009). In contrast, "turn-on fluorescence"

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nanoprobes, whose signals is activated only by the targeted molecule or cancer cell, not only address the above-mentioned problems, but also enhance optical signal in microscopy (D. Feng et al., 2013; Pan et al., 2013; Silvers et al., 2012; Wang et al., 2013). Thus, it is desirable to construct turn-on fluorescent CD-based nanoprobe for targeted cells detection. Moreover, it is great interesting to design and construct a multifunctional CDs-based composite that combines diagnostic, self-targeting, and drug-delivery functions to achieve accurate treatment and avoid drug side reaction, especially in anticancer treatment (Gao et al., 2016; Wójcik et al., 2015).

The electrostatic self-assembly method provides a facile and versatile strategy to build multi-functional CDs composites by cyclic adsorption of charged polyelectrolyte and oppositely charged CDs (Caruso et al., 1998; Caruso and Mohwald, 1999; Medley et al., 2008; Zhao et al., 2013). This strategy makes electrostatic self-assembly of CDs-based composites containing doxorubicin (Dox) modified HA suitable for constructing turn-on theranostic fluorescent nanoprobe for the evaluation of HAase levels, and for cancer cell treatment. We have developed a turn-on theranostic fluorescent nanoprobe, In which, CDs, Dox, and HA are designed as fluorescent agent, energy acceptor, and cell-targeting moiety, respectively (Fig. S1). First, polyethylenimine modified CDs (P-CDs) emit strong fluorescence, and its fluorescence intensity is insusceptible of biological environments. Second, Doxorubicin (Dox), a selected chemotherapy medication model, interacts with DNA by binding to a DNA-associated enzyme and intercalating in the DNA pair, and it also inhibits macromolecular biosynthesis (Tacar et al., 2012; Yang et al., 2016). Moreover, Tang et al. reported an efficient Förster resonance energy transfer (FRET) process between CDs and Dox (Tang et al., 2013). Third, HA is a typical cancer-cell targeting agent because it can bind to several different cancer cell receptors, including CD44. In addition, Lokeshwar et al. showed that HAase significantly elevation (3–10 fold) in tumor tissues compared to the normal adult prostate (Lokeshwar et al., 1996). The level of HAase in different cells allows us to self-target and distinguish cancer cells from normal cell. To the best our knowledge, this type of multi-functional CDs-based composites has not been reported previously.

Scheme 1 depicts the design of the electrostatic self-assembly of the multi-functional CDs-based composite and its fluorescence turn-on response to HAase in cancer cells. First, CDs are modified with a positively charged polyelectrolyte, PEI via electrostatic interaction, and then functionalized P-CDs with HA-Dox (reaction product of HA with Dox conventional EDC chemistry). The theranostic fluorescent nanoprobe produces a negligible fluorescence signal due to the Förster resonance energy transfer from P-CDs to Dox. As shown in Scheme 1, the theranostic fluorescent nanoprobe could be activated by HAase in cancer cells, The HA can be extensively digested into tetrasaccharides or small fragments in the presence of HAase, resulting in release of Dox for drug delivery, and subsequent recovery of fluorescence of P-CDs. Furthermore, the release of Dox can efficiently induce apoptosis in HeLa cells, as confirmed by the MTT assay.

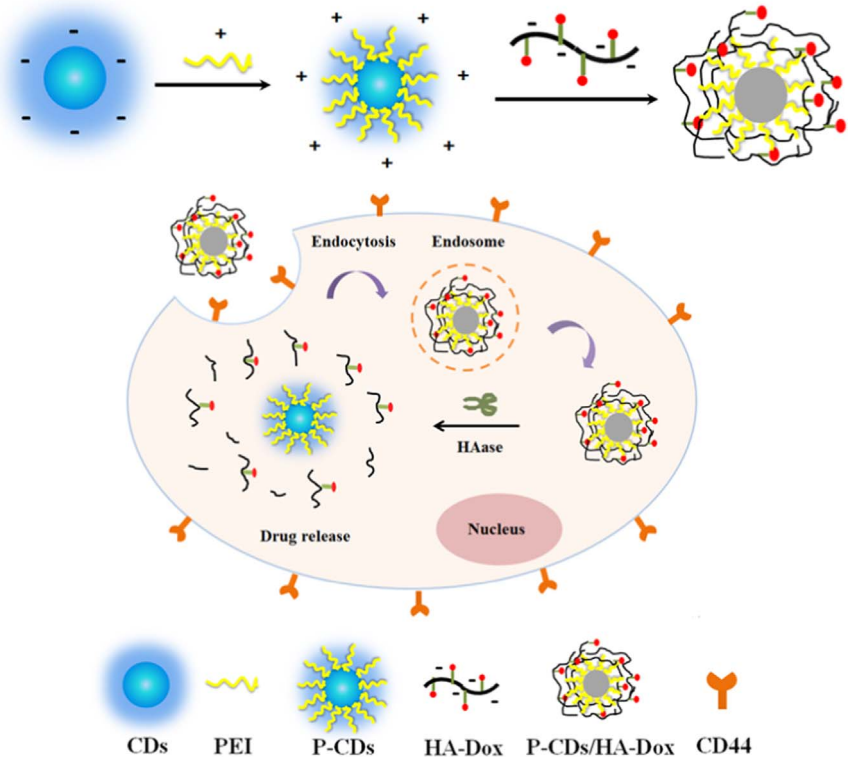
2. Experimental section

2.1. Synthesis of PEI-CDs

All materials are purchased and used without further purification (see Supporting information). In a typical synthesis, 0.5 g ATP was mixed with 10 mL deionized water to form a clear solution. Then the mixture was heated hydrothermally in a Nabertherm Muffle furnace (Germany) at 300 °C for 4 h to form the CDs. After the light yellow solution cooled to room temperature, CDs were obtained by filtering through a 0.22 μm membrane. The CDs were then dialysed several times against deionized water with stirring. Finally, 1 mL of CDs solution, 30 μL of PEI and 4 mL of phosphate buffered saline (PBS, pH 7.4) were mixed and reacted for 3 h at 60 °C to form the PEI-CDs.

2.2. Preparation of HA-Dox conjugate

First, HA (50 mg, 0.012 mmol) was dispersed in 10 mL of a mixture of DMSO and H_2O. Then, 0.07 mmol of EDC (20 mg/mL) and 0.16 mmol NHS (aq) (10 mg/mL) were added to the HA solution, followed by stirring for about 30 min. Subsequently, 0.0086 mmol of Dox·HCl (aq) (5 mg/mL) was added, and the mixture was further



Scheme 1. Schematic illustration of the formation of PEI-CDs/HA-Dox, and the nanoprobe used for targeted cancer cell imaging and drug delivery.