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Functionalized poly(pyrrole-3-carboxylic acid) nanoneedles for dual-imaging guided PDT/PTT combination therapy

Xin Liu, Huiling Su, Wei Shi*, Yang Liu, Yanan Sun, Dongtao Ge**

Key Laboratory of Biomedical Engineering of Fujian Province University/Research Center of Biomedical Engineering of Xiamen, Department of Biomaterials, College of Materials, Xiamen University, Xiamen 361005, China

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ABSTRACT

Herein, poly(pyrrole-3-carboxylic acid) (PPyCOOH) nanoneedles with abundant carboxyl groups were synthesized by aqueous dispersion polymerization method using pyrrole-3-carboxylic acid as conductive polymer monomer. The PPyCOOH nanoneedles not only owned good photothermal performance, but also more importantly showed enhanced tumor cell uptake efficiency (1.64 fold) compared with size and zeta-potential matched nanospheres. After loading photosensitizer aluminum phthalocyanine tetrasulfonate ($AlPCS_4$) and modifying with poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA) onto the PPyCOOH nanoneedles, novel nanoneedle complexes ($AlPCS_4$@PPyCONH-PAH-PAA) integrating photodynamic therapy (PDT) and photothermal therapy (PTT) were successfully fabricated. The as-prepared nanoneedle complexes improved uptake efficiency of $AlPCS_4$ both *in vitro* and *in vivo*. Moreover, the nanoneedle complexes have infrared thermal and fluorescent properties. By combined PDT/PTT under the guiding of dual modal imaging, the tumors in mice were completely eliminated and no recurrence was observed in 30 days after treatment, indicating that PPyCOOH nanoneedles have great potential as a novel drug carrier for constructing multifunctional nanoplatform used for cancer treatment.

1. Introduction

Phototherapy is a promising cancer therapeutic approach that selectively kills cancer cells *via* light triggered cytotoxicity [1,2]. Compared with traditional cancer therapies like surgery excision, chemotherapies and ionizing radiation therapies, phototherapy is preferable for its minimal invasiveness, spatio-temporal controllability and quick recovery after operation [3,4]. Photodynamic therapy (PDT) and photothermal therapy (PTT) as two major classes of phototherapy have attracted great interest in various cancer treatments, where PDT induces the cell death by photosensitizers which generate cytotoxic reactive oxygen species (ROS) under light excitation [5–9], and PTT kills the cancer cells *via* hyperthermia induced by light excited photothermal agents [10–14]. Thus, integration of PDT and PTT in one system by using photothermal nanoparticles (NPs) as carriers for photosensitizers would delivery ROS and heat simultaneously to target sites under light irradiation. It has been proved that combined PDT/PTT treatment not only improves photosensitizers and oxygen uptake efficiency in the tumor tissues but also exhibits high superiority to PDT and PTT alone [15–19].

Nevertheless, majority of commonly used photothermal agents in combined PDT/PTT systems are inorganic nanomaterials, like copper sulfide NPs, gold NPs, Pd nanosheets, graphene oxide, carbon nanotubes and so on [20–24], which show poor photostability and potential long-term toxicity [25,26]. As an ideal alternative, conductive polymer polypyrrole (PPy), owing to its excellent biocompatibility, photostability and high photothermal conversion efficiency, shows great potential in fabricating PDT/PTT combination systems [27–29]. However, PPy NPs often show poor stability in physiological environments, which restricts their applications in the biomedical fields [30]. In order to overcome this deficiency, hydrophilic polymers like polyvinyl alcohol need to be covalently grafted onto the surface of PPy [31]. But majority of the existed PPy NPs are hard to be further modification, due to the lack of surface functional groups. Therefore, a complicated process is often required for modifying PPy-based nano-agents. In addition, as a conjugated polymer, PPy is also suitable for loading

* Corresponding author.
** Corresponding author.
E-mail addresses: shiwei@xmu.edu.cn (W. Shi), gedt@xmu.edu.cn (D. Ge).

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photosensitizers with aromatic structure. But the drug loading efficiency (DLE) of PPy to photosensitizers is not satisfactory by the weak hydrophobic interactions, which reduces the amounts of photosensitizers delivered to tumor sites and makes the therapeutic efficacy of PDT insufficient. Hence, exploring novel PPy NPs with abundant surface functional groups and high loading efficiency for photosensitizers is of great value.

Besides excellent biocompatibility, good photostability and abundant functional groups, high cell uptake efficiency is also required for the carriers of combined PDT/PTT treatment. Singlet oxygen is known to have a short half-life less than 40 ns and a confined diffusion range about 10–20 nm in physiological environment, which makes cellular internalization of photosensitizers necessary for effective PDT [32–34]. Recently, many researchers have found that the morphology of NPs has a great influence on cellular uptake. Comparing to size and surface properties matched spherical NPs, rod-shape ones were more apt to enter and accumulate in tumor cells [35–39]. On account of the shortest dimension of rod shape NPs, they can penetrate tumors *via* the vessel pores and reach the tumor cells more rapidly [40]. In addition, rod-shape NPs induce minimal vascular collision, owing to their excellent alignment with the blood flow, leading to longer circulation time *in vivo* and enhanced accumulation in tumors *via* EPR effects [41,42]. However, to the best of our knowledge, rod-shape phototherapy carriers with functional groups based on conductive polymer have not been reported so far.

In this work, poly(pyrrole-3-carboxylic acid) (PPyCOOH) nanoneedles with abundant carboxyl groups and ideal tumor cell uptake efficiency were prepared for the first time, and further modified with photosensitizer aluminum phthalocyanine tetrasulfonate ($AlPCS_4$), poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), as schematically shown in Scheme 1. The obtained $AlPCS_4$@PPyCONH-PAH-PAA nanoneedle complexes showed great stability, excellent biocompatibility, good photostability and high DLE. Moreover, infrared thermal and fluorescent imaging could monitor the *in vivo* bio-distribution and PTT efficacy of nanoneedle complexes, which contributed to guiding PDT and PTT treatments. These features ensure that the as-fabricated nanoneedle complexes achieved high efficient tumor treatment of synergistic PTT and PDT both *in vitro* and *in vivo*.

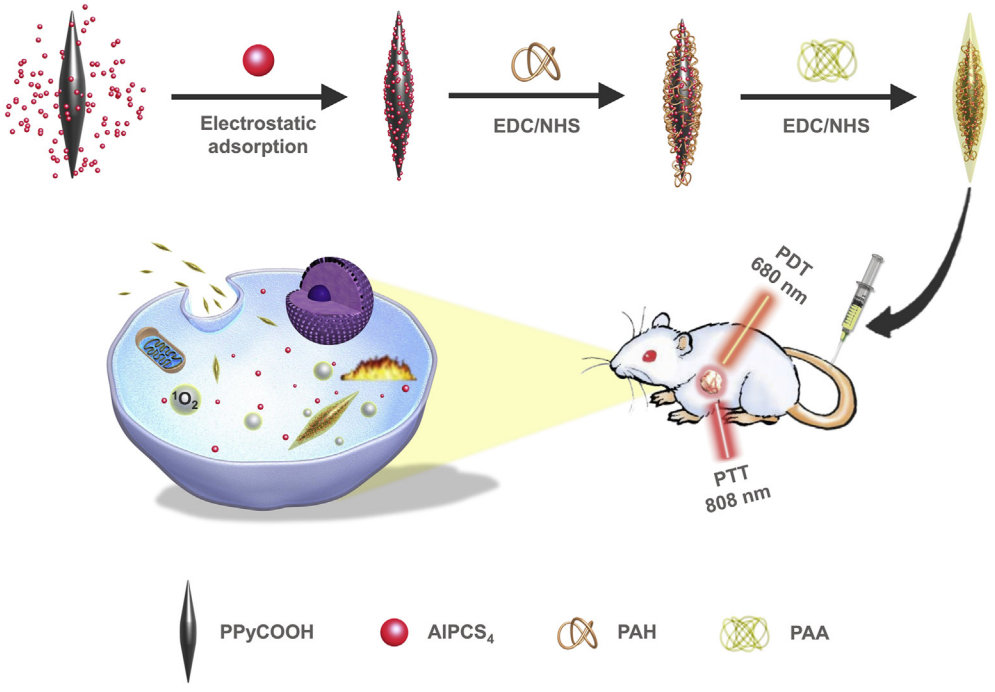
2. Experimental section

2.1. Materials

Pyrrole-3-carboxylic acid (Py-3-COOH, 98%) was purchased from Soochow Boke Chemical Co. Ltd.. Rhodamine B (RhB, 99%), Sodium hydroxide (99%) and $FeCl_3 \cdot 6H_2O$ (99%) were obtained from Sinopharm Chemical Reagent Co. Ltd.. Al(III) phthalocyanine chloride tetrasulfonic acid ($AlPCS_4$) was purchased from Frontier Scientific Inc.. Poly(acrylic acid) (PAA, 35 wt% in H_2O, M_w~100,000) was obtained from Sigma-Aldrich Co.. Poly(allylamine hydrochloride) (PAH, M_w~15,000), 1,3-diphenylisobenzofuran (DPBF, 97%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from J&K Scientific Ltd.. All chemicals were directly used as received without further purification. Hela and RAW 264.7 cells were bought from Shanghai Gefan Biotechnology Co. Ltd. and had passaged for 10 times during the experiments.

2.2. Synthesis of PPyCOOH nanoneedles

PPyCOOH nanoneedles were synthesized through aqueous dispersion polymerization, without any template. Firstly, 66.6 mg Py-3-COOH was dissolved in 50 ml water, followed by going through a 0.22 μm syringe filter to remove any insoluble compound. 24 mg NaOH was added to the above solution under vigorous mechanical stirring for 1 h. Then, 324 mg $FeCl_3 \cdot 6H_2O$ was added and stirred for 30 min. Finally, the temperature was risen to 60 °C. After polymerization for another 15 h, the PPyCOOH nanoneedles were separated from the resulting dark brown dispersion by centrifugation (13,000 rpm, 20 min) and washed (four times by



Scheme 1. Schematic diagram of the synthesis of $AlPCS_4$@PPyCONH-PAH-PAA nanoneedle and its application in combined PDT/PTT therapy.