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Novel hybrid probe based on double recognition of aptamer-molecularly imprinted polymer grafted on upconversion nanoparticles for enrofloxacin sensing

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ABSTRACT

A novel luminescent "double recognition" method for the detection of enrofloxacin (ENR) is developed to overcome some of the challenges faced by conventional molecularly imprinting. Biotinylated ENR aptamers immobilised on upconversion nanoparticles (UCNPs) surface are implemented to capture and concentrate ENR as the first imprinting recognition safeguard. After correct folding of the aptamer upon the existing targets, polymerization of methacrylic acid monomers around the ENR-aptamer complexes to interact with the residual functional groups of ENR by using molecularly imprinting techniques is the second imprinting recognition safeguard. The "double recognition" imprinting cavities are formed after removal of ENR, displaying recognition properties superior to that of aptamer or traditional molecularly imprinting alone. Another interest of this method is simultaneous molecular recognition and signal conversion by fabricating the "double recognition" receptor on to the surface of UCNPs to form a hybrid sensing system of apta-MIP/UCNPs. The proposed sensing method is applied to measure ENR in different fish samples. Good recoveries between 87.05% and 96.24%, and relative standard deviation (RSD) values in the range of 1.19–4.83% are obtained, with the limits of detection and quantification of 0.04 and 0.12 ng/mL, respectively. It indicates that the sensing method is feasible for the quantification of target ENRs in real samples, and show great potential for wide-ranging application in bioassays.

1. Introduction

Biological molecular recognition, such as binding of ligand-receptor, antigen-antibody, and substrate-enzyme, are always required as highly selective tools for the detection, purification or removal of specific molecules in analysis and bio-sensing techniques. However, considering that these biomaterials have disadvantages including elevated cost, hard availability, and poor shelf-life and stability, much research is directed toward the development of suitable alternatives to overcome these limitations (Poma et al., 2015).

As one of the most suitable alternatives for biological molecular recognition, fully synthetic molecularly imprinted polymers (MIPs) have attracted great interest. Molecular imprinting is a strategy to prepare a molecular recognition unit by polymerizing functional monomers in the presence of template molecules. After removal of templates, MIPs can exhibit affinity and selectivity for the template molecules with the advantages of being chemically stable, conveniently available via chemical synthesis, and less vulnerable to denaturation.

Another promising affinity material is aptamer. Aptamer, a synthetic DNA or RNA single-chain oligonucleotide, can bind to the targets such as metal ions (Leung et al., 2015), small molecule (Entzian et al., 2016; Jiménez et al., 2015; Liu et al., 2016), biological macromolecule (Qin et al., 2016; Lin et al., 2015; Lu et al., 2016), microorganism (Chung et al., 2015), and even the cell (Li et al., 2016) with high affinity and specificity. Upon the existing targets, aptamers will undergo adaptive conformational changes and three-dimensional folding from unstructured (Chen et al., 2015). They can be selected from a combinatorial DNA library in vitro by a process known as systematic evolution of ligands by exponential enrichment (SELEX), and are referred to as chemical antibodies due to their synthetic production (Yuan et al., 2012).

Due to the above appealing characteristics, a lot of novel analysis techniques by using the MIPs or aptamers as molecular recognition elements such as electronic tongues, and sensors had been developed (Huynh et al., 2015; Figueiredo et al., 2016; Fuchs et al., 2012; Zhang et al., 2014; Chen et al., 2016; Citartan et al., 2016). Nonetheless, these techniques are only guaranteed by single recognition from MIPs or aptamers alone, and remain to be further

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breakthrough on the recognition efficiency and accuracy. A potential promising idea is to build a hybrid synergistic identification system by integrating two or more of the recognition elements. Bai and Spivak (2014) has preliminary prove the feasibility of this idea and already looked into introducing an aptamer-based imprinting strategy to synthesis a virus-responsive "double imprinted" hydrogel. However, quantification of the targets that was realized by measuring volume-shrinking of hydrogel after laser diffraction by a ruler with naked eye was still not accurate enough. Another hybrid identification system had been reported by Jolly et al. (2016). They have developed a hybrid-MIP receptor for use in an electrochemical sensor targeting the quantitative analysis of prostate specific antigen.

Currently, the robust luminescence biosensor shows promise in the development of bioassay techniques. Therefore many materials such as organic fluorescent dyes and inorganic quantum dots have been introduced as luminescence probes in sensing detection. Compared with conventional down-conversion luminescence materials, near-infrared (NIR)-to-visible up-conversion nanomaterials (UCNPs) have conceivable advantages (Liu et al., 2009). Through sequential electronic excitation and energy-transfer processes, UCNPs are capable of converting two or more NIR pump photons into a higher energy output photon (Wang et al., 2014). Because autofluorescence originating from coexisting substances in sample matrixes can not be excited by NIR light, the background light interference and detection sensitivity can be greatly improved (Tsang et al., 2015; Yang et al., 2013). In addition, UCNPs also offer more advantages, such as low toxicity, high chemical stability, high quantum yields, long lifetimes, and deep penetration depth in living tissues (Han et al., 2016; Li et al., 2013), which endow these nanoparticles with more competitive than other luminescence probes.

Enrofloxacin (ENR), a high-potency antibacterial agent, has been widely employed for disease prevention and therapy in poultry and livestock breeding, and in aquaculture practices. Residual ENR left after its use may lead to several side effects to human health (Yu et al., 2014; Dorival-García et al., 2016). This study aims to develop a luminescent "double recognition" aptamer-MIP probe on the surface of UCNPs for enrofloxacin sensing. The recognition component is at the heart of an effective sensor, because it defines the specificity, and overall quality and robustness of the results obtained. Different from a great deal of existing reports on molecular imprinting techniques, this work does not only rely on single recognition from MIPs that most of their composition is still based on the same "classic" monomers. The novelty of this study is introducing of chemically modified aptamers into the molecularly imprinted polymers composition to build a robust component with dual identification protection as the recognition center of the sensor. Unlike the studies of Bai et al. and Jolly et al. that referred above, another interest of this method is that we combine the above double recognition receptor with signal transduction of UCNPs to develop a multifunctional hybrid probe (apta-MIP/UCNPs), and thus to realize simultaneous molecular recognition and signal transduction in the process of sensing detection for accurate and rapid ENR quantification.

2. Materials and methods

2.1. Instruments and reagents

TEM imaging is performed on a Tecnai G2 F20 S-TWIN transmission electron microscope (FEI, USA) with an operating voltage of 200 KV. The crystal structure is performed using an Ultima IV X-ray diffractometer ($10° \leq 2\theta \leq 70°$; Rigaku, Japan). Fourier transform infrared (FTIR) spectra is conducted with a Scimitar 2000 Near FTIR Spectrometer (Agilent, USA) using KBr pellets. The luminescent emission spectra of UCNPs are recorded by the Hitachi F-7000 fluorescence spectrophotometer (Hitachi, Japan) attached to an external 980 nm laser instead of an internal excitation source. All measurements are performed at room temperature.

Enrofloxacin (ENR), methacrylic acid (MAA), trihydroxymethylpropyl trimethylacrylate (TRIM), azobisisobutyronitrile (AIBN), hexadecyl trimethyl ammonium bromide (CTAB), 3-Aminopropyltrimethoxysilane (APTES), tetraethyl orthosilicate (TEOS), norfloxacin, ofloxacin, ciprofloxacin and rare earthoxides, including yttrium oxide (Y_2O_3), ytterbium oxide (Yb_2O_3), and thulium oxide (Tm_2O_3) are purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The streptavidin (99%) are purchased from Beijing Solarbio Science & Technology Co., Ltd. (China). ENR aptamer is purchased from Sangon Biotechnology Inc., China, and the sequences are 5′-CCCATCAGGGGGCTAGGCTAACACGGTTCGGCTCTCTGAGCCCGGGTTATTTCAGGGGGA-biotin-3′. All other reagents are of analytical grade and used without further purification.

2.2. Preparation and surface modification of the $NaY_{0.78}F_4$:$Yb_{0.2}$, $Tm_{0.02}$ UCNPs

In brief, Y_2O_3 (0.78 mmol), Yb_2O_3 (0.2 mmol) and Tm_2O_3 (0.02 mmol) are mixed in nitric acid with heating. After dissolving, the solvent is evaporated to form the rare-earth nitrate powder and then dissolved in deionized water. EDTA (2.1273 g), and 25 mL of 1.6 g/mL CTAB-glycol solution are added in sequence. 1.5 mL hydrofluoric acid is added into the flask dropwise with vigorous stirring, followed by the addition of 3.5 mL of nitric acid. At last, the mixture is transferred to a 100 mL autoclave, sealed, and heated to 195 °C for 24 h. After the autoclave is allowed to cool to room temperature, the precipitates are separated by centrifugation, washed with ethanol three times, and then dried in an oven. The obtained powder is the rare-earth doped $NaY_{0.78}F_4$:$Yb_{0.2}$, $Tm_{0.02}$ UCNPs, and is kept in a dry condition for further modification.

The above UCNPs are modified to increase bioaffinity. Typically, 40 mL water containing 5 mL ammonia, 40 mL 3-propanol containing 120 μL TEOS is added to a 3-propanol solution of the UCNPs (100 mg in 120 mL) with vigorous stirring for 3 h. And 60 mL of 3-propanol containing 400 μL of APTES is then added dropwise into the above mixture, and the reaction is continued for 1 h. After reaction, the modified UCNPs are obtained via centrifugation, and washed three times with water and ethanol.

2.3. Hybridization of the aptamer-MIP on UCNPs

The hybridization procedure involves two major steps: the first step is grafting aptamer onto the surface of UCNPs, and the second one is the coating MIPs onto aptamer linked UCNPs. Before connection of UCNPs and aptamer, the above modified UCNPs are functionalized with streptavidin at first. Thereafter, 5 mL phosphate buffered saline (PBS, pH 7.4) containing 100 mg streptavidin linked UCNPs are incubated with 250 μL of 1μmol/L biotinylated ENR aptamer at 37 °C overnight. The resulted UCNPs-aptamer conjugates are washed with PBS for three times to remove the unbound aptamer, and collected for further use.

Prior to the molecular imprinting step, an amount of UCNPs-aptamer conjugates are incubated with ENR at 37 °C for 1 h to form complexes as the templates. The derivatized UCNPs are dispersed, and then are used straight for the synthesis of the MIPs without further storage. MAA is previously dissolved in acetonitrile, and then added to the above mixture. After moderate vibration, the polymerization is started by adding the crosslinker of TRIM and initiator of AIBN, and then is carried out at 60 °C for 24 h with a sustained oscillation. Hybrid probe of apta-MIP/UCNPs is