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A multifunctional molecularly imprinted polymer-based biosensor for direct detection of doxycycline in food samples

Jon Ashley, XiaoTong Feng, Yi Sun*

Department of Micro, and Nanotechnology, Technical University of Denmark, Ørsteds Plads, DK-2800 Kgs Lyngby, Denmark

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ABSTRACT

In this study, we developed a new type of multifunctional molecularly imprinted polymer (MIP) composite as an all-in-one biosensor for the low-cost, rapid and sensitive detection of doxycycline in pig plasma. The MIP composite consisted of a magnetic core for ease of manipulation, and a shell of fluorescent MIPs for selective recognition of doxycycline. By simply incorporating a small amount of fluorescent monomer (fluorescein-O-acrylate), the fluorescent MIP layer was successfully grafted onto the magnetic core via a surface imprinting technique. The resultant MIP composites showed significant doxycycline-dependent fluorescence quenching in an aqueous environment. Good linearity ranging from 0.2 to 6 μM was achieved, and the limit of detection was determined to be 117 nM. The biosensor also showed good selectivity towards doxycycline when compared to other common antibiotic residues. The multifunctional MIP composites were used to directly extract doxycycline from spiked pig plasma samples and quantify the antibiotics based on the quenched fluorescence signals. Recoveries of doxycycline were found in the range of 88–107%.

1. Introduction

The overuse of antibiotics is of considerable concern to public health, as these drugs can possibly enter the food chain. These residues can then accumulate in the body to cause adverse health effects as well as increasing the likelihood of antibiotic resistance. Doxycycline, which is one of a broad class of tetracycline antibiotics, has been found to be overprescribed by veterinary professionals to effectively control bacterial pneumonia in pigs and other livestock which has contributed to the bacteria resistance [1]. Screening of doxycycline is compulsory for a number of food matrices. According to the EU commission regulation No 37/2010, the maximum residue limits (MRLs) of doxycycline is 100 μg/kg in muscle, 300 μg/kg in skin, fat and liver, and 600 μg/kg in Kidney [2].

As such, there is a need for the development of rapid, sensitive and low cost analytical methods to accurately detect doxycycline and other tetracycline based antibiotics within different food matrices. Current analytical methods can be split into two general groups, namely confirmatory and screening [3]. Confirmatory methods such as high performance liquid chromatography (HPLC) with UV detection [4], liquid chromatography-mass spectrometry (LC-MS) [5], and capillary electrophoresis (CE) [6,7], have demonstrated high sensitivity towards doxycycline with a high degree of accuracy and precision. However these techniques are often time-consuming, require expensive instrumentation as well as trained personnel to operate them.

Alternatively, screening based methods such as bioassays and biosensors have allowed for the semi-quantitative analysis of drug residues [8–10]. In particular, a number of biosensors for the detection of tetracycline based antibiotic residues in food products have been reported in the literature [11]. They have the potential to be used before confirmatory methods and have shown great potential for antibiotic analysis at the point-of-need due to their high throughput analysis, rapid analysis times and miniaturization [12–14]. However, most of the assays rely on bioreceptors such as antibodies and enzymes for specific recognition. In spite of the high specificity and affinity, these bioreceptors are expensive and inherently unstable due to their biological origin.

Recently, molecularly imprinted polymers (MIPs), the synthetic materials with recognition sites that can specifically bind to target molecules, have attracted immense attention. MIPs targeting tetracycline antibiotics have been synthesized by radical polymerization or precipitation polymerization for use in biosensing platforms or as sorbents for solid phase extraction [15,16]. Compared to conventional bioreceptors, MIPs offer apparent advantages including higher robustness and stability under a wide range of conditions, ease of synthesis and low production costs. More interestingly, different active molecules can be incorporated during synthesis, so that multifunctional MIP composites can be formed [17,18]. MIPs have been grafted onto the

* Corresponding author.
E-mail address: sun.yi@nanotech.dtu.dk (Y. Sun).

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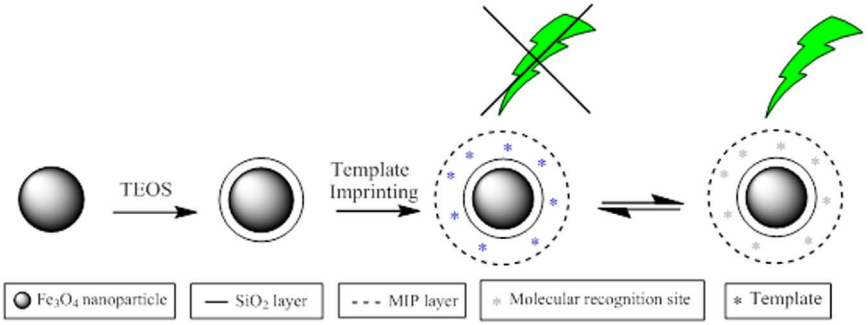

Fig. 1. Overview of core-shell molecular imprinting of the MIP composites.

surface of magnetic nanoparticles, resulting in magnetic MIPs which possess both magnetically susceptible characteristics and good selectivity for the target molecules [19–21]. Fluorescent molecules such as fluorescent dyes, quantum dots or carbon dots have also been integrated into MIPs, which effectively turns the MIP composites into fluorescence-based sensors [22,23]. Several reports have even demonstrated the combined use of magnetic nanoparticles and fluorophores with MIPs [24,25]. However, there are still technical challenges for synthesizing such multifunctional MIPs. For instance, many examples thus far have used cadmium-based quantum dots which are highly toxic [26]. In addition, the incorporation of fluorescence dyes or quantum dots into the MIP layer might lead to interference in MIP binding. Some work tried to avoid the problem by incorporating quantum dots into a separate silica layer, but this could in turn result in decrease in quenching ability due to the distance between the binding site and fluorescent molecules [22].

In this study, we developed a new type of multifunctional MIP composite for the recognition and detection of doxycycline. The MIP composite was synthesized by a core-shell imprinting technique, and consisted of a magnetic core and a shell of fluorescent MIP (FMIP) **(**Fig. 1**)**. Instead of incorporating quantum dots or fluorescent dyes in MIPs, we developed a facile polymerization method to produce FMIPs by introducing fluorescein-O-acrylate monomer into the molecular imprinting formulation. With a set of combined properties, the new MIP composites were used as a biosensor to directly detect doxycycline in pork plasma samples. Attributed to the magnetic core, the composite could be easily separated and collected using an external magnetic field. The high selectivity of MIPs ensured specific recognition of doxycycline. Upon binding, a fluorescence quenching effect was induced via the interaction between the fluorescein-O-acrylate and the analyte, thereby providing a convenient methodology for optical detection. The use of a fluorescent monomer greatly simplified the synthesis procedures, and the sensitivity was similar or higher when compared to previous methods. The MIP composite-based biosensor was demonstrated to be rapid, simple, selective, and applicable to raw samples. To the best of our knowledge, this is the first reported example of a multifunctional MIP composite for the detection of doxycycline in plasma samples.

2. Experimental

2.1. Materials

Ferric chloride $FeCl_3{\cdot}6H_2O$, ethylene glycol, sodium acetate, polyethylene glycol (PEG, MW 2000), tetraethoxysilane (TEOS), 35% ammonium hydroxide, methacryloxypropyltrimethoxysilane (MPA), doxycycline (DO), cloxacillin (CA), spiramycin (SP), gentamycin (GE), tetracycline (TC), amoxicillin (AC), ethylene glycol dimethacrylate (EDGMA), methacrylic acid (MAA) and azo(bis)isobutyronitrile (AIBN), fluorescein-o-acrylate and porcine plasma were purchased from Sigma Aldrich. Inhibitors were removed from all monomers prior to polymerization using pre-packed columns from Sigma Aldrich. All solvents were analytical grade and used without further purification.

2.2. Synthesis of FeO_x@SiO_2-FMIPs

The synthesis procedures are illustrated in Fig. 1. The iron oxide (FeO_x) nanoparticles were synthesized using the thermal solvent method. $FeCl_3{\cdot}6H_2O$ (1.36 g) was dissolved in ethylene glycol (40 ml). Anhydrous sodium acetate (3.6 g) and PEG (MW 2000, 0.2 g) were added sequentially. The mixture was stirred for 30 mins to form a homogenous orange suspension. The solution was then degassed under vacuum for 30 mins, transferred to a 100 ml autoclave reactor and heated in a furnace oven at 200 °C for 10 h. The resultant black precipitate was washed several times with ethanol and dried under vacuum for 6 h.

0.5 g of the resultant FeO_x microspheres were re-suspended in ethanol (60 ml) and water (12 ml). The black solution was then sonicated using a probe sonicator for at least 30 mins followed by the addition of 35% ammonia hydroxide (2 ml) and TEOS (2 ml). The solution was allowed to react under pulse sonication for 4 h before transferring to an orbital shaker for 12 h. The resultant FeOx@SiO_2 microspheres were washed several times with water until the pH had returned to neutral. This was followed by washing with ethanol several times and dried under vacuum for 12 h.

The resultant FeO_x@SiO_2 (0.4 g) were mixed with 4% (v: v) MPA: anhydrous toluene. The solution was sonicated for 15 mins and then heated to 60 °C with overhead stirring under nitrogen overnight. The resultant FeO_x@SiO_2-MPA microspheres were washed several times using ethanol and dried for 10 h under vacuum.

The synthesis of the MIP layer is based on a previous report with some modifications [27]. A solution containing MAA (4 mmol), EDGMA (8 mmol), doxycycline (1 mmol), and fluorescein-o-acrylate (0.1 mM) in methanol (60 ml) was prepared and degassed by bubbling nitrogen for 30 mins. The solution was added to a three-way round bottom flask containing 0.2 g of FeO_x@SiO_2-MPA, sonicated for 15 mins and stirred for one hr at room temperature. The polymerization reaction was initiated by adding AIBN (40 mg) and the mixture was heated to 60 °C for 16 h. The resultant FeO_x@SiO_2-FMIPs were transferred to a soxhlet extractor and continually washed using 9:1 (v: v) methanol: acetic acid over a 24 h period to remove the template. The removal of the template was then confirmed by checking the fluorescence of the microspheres. FeO_x@SiO_2-FMIPs were finally washed with methanol several times to remove residual acetic acid, and dried under vacuum. The non-imprinted polymers (NIPs) were prepared in the same manner as for the MIPs but in the absence of the template.

2.3. Instrumentation

All scanning electron microscopy (SEM) images were taken using a Quanta FEG SEM (FEI, Oregen USA). All transmission electron microscopy (TEM) images were taken using a Tecnai T20 G2 (FEI, Oregen USA) transmission electron microscope. IR spectra were taken using a Spectrum 100 (Perkin Elmer, MA, USA). XPS spectra were measured using a K-alpha XPS (Thermo Fisher Scientific, MA, USA**).** All fluorescence-based quenching experiments were performed on a Tecan