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Research paper

Preparation of optical functional composite films and their application in protein detection

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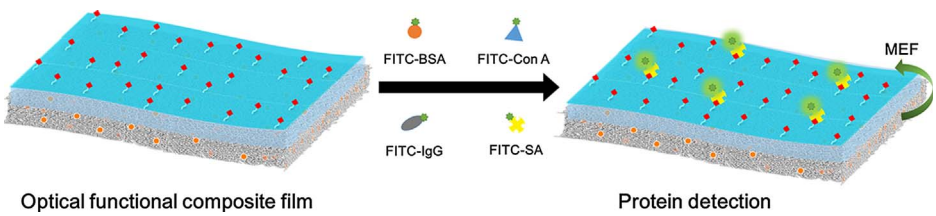
GRAPHICAL ABSTRACT



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ABSTRACT

In this work, an optical functional composite film was prepared through self-assembly technique for specific protein detection. The Au nanostructures with metal enhanced fluorescence (MEF) effect were prepared via the *in situ* reduction of Au ions in a filter paper. Then, a multilayer film was prepared on the Au nanostructures by layer-by-layer adsorption of poly-(ethylenimine), poly-(glycolic acid) and biotinylated poly(L-lysine)-graft-poly (ethylene glycol) (PLL-*g*-PEG-biotin). Through adjusting the structure of multilayer film, an optimum interaction distance between Au nanostructures and fluorophores for the MEF effect was achieved. Meanwhile, the surface PLL-*g*-PEG-biotin layer allows the film to capture specific streptavidin through biotin-streptavidin interaction. Owing to the MEF effect of Au nanostructures, a significant enhancement in the fluorescence of fluorescein isothiocyanate (FITC) that labeled streptavidin was successfully obtained. This optical functional composite film with enhanced fluorescence could be used to recognize specific protein in a facile and efficient way.

1. Introduction

Fluorescence detection technology is a dominant tool in biological and medical research, mainly due to its high sensitivity, high efficiency, and ease of use [1–5]. It has become massively popular in analytical sciences, particularly in the protein detection based on antibody-antigen specific recognition [6–9]. With excellent optical properties, organic fluorophores [10–12], conjugated polymers [13–17], quantum dots [18–20], metal nanostructures [21–23] and upconversion nanoparticles [24–26] have been used as fluorescent probes in fluorescence detection. Currently, to meet the increasing demands of high-throughput assay, solid-phase detection system begins to be developed, such as paper-based sensors, gene chips and microarrays [27–31]. However, the applications of fluorescent probes in solid-phase system are often limited by aggregation-induced fluorescence quenching, low fluorescence quantum yield, and low resistance to photobleaching. Introducing metal nanoparticles into solid matrices will provide a means to bypass the limitation [32–34].

Metal nanoparticles including gold (Au), silver, aluminum and copper nanoparticles are known as plasmonic nanoparticles [35–38]. They exhibit an extraordinary capability to enhance the fluorescence intensity of nearby fluorophores. The strong interaction of excitation

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light with metallic nanostructures leads to the collective oscillations of conduction-band electrons and then an amplified near-field. The amplified near-field results in significant improvements in the optical properties of excited fluorophores. This phenomenon is named as metal-enhanced fluorescence (MEF) effect [39]. Direct synthesis of nanoparticles in solid matrixes is an efficient way for preparing metallic nanostructures that induces MEF effect [40–42]. Cellulose fibers are composed of microfibrils which form three-dimensional network structure and exhibit porous structures [43]. Such structural features can provide a unique microreactor for synthesizing nanoparticles in an *in situ* way and make cellulose fibers as ideal matrixes.

More importantly, MEF effect has a distance-dependent nature [44], only affecting the fluorophores which reside in the region of the amplified near-field produced by metal nanoparticles. However, if the fluorophores directly contact with metallic nanostructures, energy and/or charge transfer occurs that result in significant quenching of fluorophores by metallic nanostructures [45]. To avoid fluorescence quenching, nanometer-thick film is usually desired to separate fluorophores and metal nanostructures. Layer-by-layer (LbL) multilayer films [46–50], whose thickness can be accurately controlled at the nanometer scale, can be used as interlayer between fluorophores and metal nanostructures to achieve the optimal MEF effect. Moreover, in the preparation of LbL multilayer films, it is easy to introduce biological functional groups to the film system [51–53], which facilitates their further application in biological analysis and detection.

In this work, we prepared an optical functional composite film based on LbL self-assembly for protein detection. The film structures and its detection mechanism for special protein are illustrated in Scheme 1. The Au nanoparticles performing MEF effect were prepared via the *in situ* reduction of Au ions in a filter paper. Through self-assembly technology, a multilayer film containing poly-(ethylenimine) (PEI), poly-(glycolic acid) (PGA) and biotinylated poly(L-lysine)-graft-poly(ethylene glycol) (PLL-*g*-PEG-biotin) was covered on the Au nanostructures. Adjusting the structure of multilayer film can effectively optimize the interaction distance of the MEF effect. Taking advantage of biotin-streptavidin (SA) coupling [54], assembled PLL-*g*-PEG-biotin layer specifically captured fluorescein isothiocyanate-labled SA (FITC-SA). With the MEF effect in our composite film, the excited FITC produced an enhanced fluorescent signal in response to SA binding allowing specific protein detection. The easily prepared optical functional composite film is expected to serve as novel detection system for sensitive protein detection.

2. Experimental

2.1. Materials

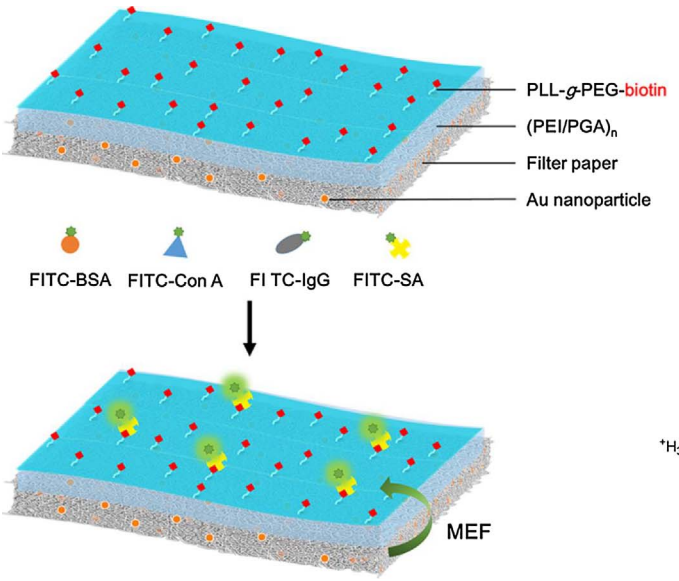
Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4{\cdot}3H_2O$), PEI (M_n = 60,000), PGA, FITC-labeled lectin protein concanavalin A (FITC-Con A), FITC-SA, FITC-labeled goat anti-mouse IgG (FITC-IgG) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Whatman brand filter paper (grade 2, ϕ70 mm) and HyClone Phosphate Buffered Saline (PBS) were purchased from GE Healthcare Life Sciences. Ascorbic acid was purchased from Beijing ABOXING Bio-Tech Co., Ltd. PLL-*g*-PEG-biotin was purchased from SoSuS AG (Dübendorf, Switzerland). FITC-labeled bovine serum albumin (FITC-BSA) was purchased from Sangon Biotech. Ultrapure Millipore water (18.6 MΩ cm) was used throughout the experiments. All reagents were used without further purification unless otherwise stated.

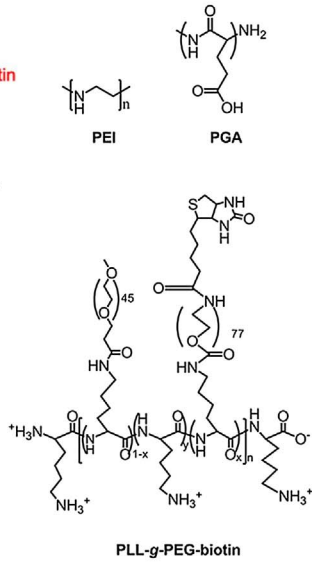
2.2. Measurements

The morphologies of samples were characterized by scanning electron micro-scope (SEM, Carl Zeiss Jena, SUPRA 55 SAPPHIRE). The prepared samples were sputter-coated with carbon to enhance the contrast of the SEM images. Ultraviolet visible (UV–vis) near-infrared absorption spectra were recorded with the Varian Cary 5000 UV-vis near-infrared spectrophotometer. UV–vis absorption spectra were characterized with a Hitachi U3900 spectrophotometer. Fluorescence spectra were recorded with a Hitachi F-7000 fluorescence spectrometer equipped with a Xenon lamp excitation source at room temperature. Time-domain lifetime measurements were performed by an Edinburgh Instruments F900 spectrometer with excitation at 490 nm. Photographs of samples were captured by a Nikon D-7000 camera with excitation at 365 nm provided by the portable UV lamp ZF-7A.

2.3. Preparation of optical functional composite films

Filter paper was cut into 1 cm × 3 cm size. Ascorbic acid and $HAuCl_4$ were respectively dissolved to form 30 mM ascorbic solution and 30 mM $HAuCl_4$ solution. The above solutions were stored at 4 °C for further use. Quartz slides were immersed in piranha solution (H_2O_2/H_2SO_4 = 1:3 v/v) for 30 min, washed three times with deionized water and ethanol, and then dried with a gentle stream of nitrogen gas. (CAUTION: "Piranha" solution reacts violently with organic materials; it must be handled with extreme care.) The cleaned slides were placed





Scheme 1. Schematic illustration of the optical functional composite film for protein detection and molecule structures of PEI, PGA and PLL-*g*-PEG-biotin.