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Metal enhanced fluorescence on nanoporous gold leaf-based assay platform for virus detection

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ABSTRACT

In the present study, a rapid, sensitive and quantitative detection of influenza A virus targeting hemagglutinin (HA) was developed using hybrid structure of quantum dots (QDs) and nanoporous gold leaf (NPGL). NPGL film was prepared by dealloying bimetallic film where its surface morphology and roughness were fairly controlled. Anti-influenza A virus HA antibody (ab66189) was bound with NPGL and amine ($-NH_2$) terminated QDs. These biofunctionalized NPGL and QDs formed a complex with the influenza virus A/Beijing/262/95 (H1N1) and the photoluminescence (PL) intensities of QDs were linearly correlated with the concentrations of the virus up to 1 ng/mL while no PL was observed in the absence of the virus, or in bovine serum albumin (BSA, 1 μg/mL) alone. In addition, it was demonstrated that this assay detected successfully influenza virus A/Yokohama/110/2009 (H3N2) that is isolated from a clinical sample, at a concentration of ca. 50 plaque forming units (PFU)/mL. This detection limit is 2-order more sensitive than a commercially available rapid influenza diagnostic test. From these results, the proposed assay may offer a new strategy to monitor influenza virus for public health.

1. Introduction

Epidemic diseases via transmission of the virus are becoming a threatening fear for public health system; e.g., the pandemic influenza A (H1N1) 2009 virus was firstly identified in Mexico in 2009 and caused rapid outbreaks, resulting in ca. 18,000 casualties around the world (Kawai et al., 2012; Panning et al., 2009). It continues to expand globally and causes significant rates of morbidity and mortality, particularly in the elderly and children. A rapid diagnosis of influenza viruses is vital for prevention and timely control of influenza epidemics. Currently forefront tests, i.e., immunosensors and genosensors for monitoring influenza viruses at initial stage usually require professional skill, equipment, multiple processes, and low sensitivity, resulting in retardation to clinical decision (Bonanni et al., 2010; Choi et al., 2010; Deng et al., 2011; Drexler et al., 2009; Druce et al., 2005; Egashira et al., 2008; Kok et al., 2010; Kukol et al., 2008; Owen et al., 2007; Pavlovic et al., 2008; Rahman et al., 2008; van Elden et al., 2001). Numerous technologies for higher sensitivity are emerging for virus detection.

In particular, it has been attractive to utilize photoluminescence (PL) enhancement based on the near-field plasmonic effect at metallic nanostructures (Driskell et al., 2011; Gramotnev and Bozhevolnyi, 2010; Schuller et al., 2010). The interaction between metal and semiconductor nanostructure offers attractive opportunities for tuning the optical properties of such composites based on exciton–plasmon coupling. Such composite structures feature complementary optical properties; e.g., semiconductor nanostructures give rise to high emission yields and light-harvesting capabilities, whereas the metallic surface is particularly effective for local probing, confined excitation, non-linear optics and intense PL enhancement (Achermann, 2010; Lee et al., 2006, 2007). Surface roughness has long been considered as one of the

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critical parameters for optimizing metal enhanced fluorescence and has enabled precise control of localized surface plasmon resonance (LSPR) as well as surface plasmon polariton (SPP). In rough metallic surface, the scattering of SPP mode can produce photons that can decrease diffraction limit and resolve the sub-wavelength structure, thereby unlocking the prospect of utilizing metal–semiconductor nanocomposite films for enhancing PL emission (Ahmed et al., 2012; Leong et al., 2010; Okamoto et al., 2004).

Nanoporous gold film has unique physical properties such as excellent stability, biocompatibility, as well as high specific surface area to form self-assembled monolayers from thiols, sulfides and disulfides (Biener et al., 2008; Huang and Sun, 2005). Usually a dealloying technique is utilized to prepare nanoporous structures with controlled pore size and ligaments. By exploiting the dealloying method, PL enhancement in the vicinity of metal nanostructures can be achieved with delicate control of the morphology of the surface on the scale of a few hundreds nanometers in conjunction with interconnected-porous structures (Ciesielski et al., 2008; Detsi et al., 2011).

In the present study, the fabrication of metallic surfaces with tunable roughness and controlled structures is reported using the dealloying method. The procedure for fabrication of metal–semiconductor hybrid nanostructures was achieved by means of self-assembly techniques, and the importance of the metallic surface morphology for PL enhancement is illustrated. Furthermore, this physical study expanded to develop a highly sensitive metal–semiconductor hybrid nanostructure for the detection of influenza virus (Fig. 1).

2. Materials and methods

2.1. Materials

3-Mercaptopropionic acid (MPA; 99%), poly-diallyldimethylammonium chloride (PDDA; M.W. 400,000–500,000), polyacrylic acid (PAA; M.W.,~450,000), cadmium perchlorate hydrate, thioglycolic acid (TGA), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Milwaukee, USA). Aluminum telluride (Al_2Te_3) was acquired from Cerac Company (Milwaukee, USA) at the highest purity available. The chromogenic substrate, 3,3′, 5,5′-tetramethylbenzidine (TMB) was obtained from Dojindo (Osaka, Japan). Gold leaf films were purchased from Giusto Manetti Inc. (Campi Bisenzio, Italy). Anti-Influenza A virus HA H1 antibody [B219M] (ab661189, Lot: GR40088-11), anti-Swine Influenza A (H1N1) HA antibody (ab91530, Lot: 942815), and anti-H3 (H3N2) antibody [InA227] (ab82454, Lot: GR84403-3) were purchased from Abcam Inc. (Cambridge, UK). Recombinant influenza A virus HA (H1N1) (New Caledonia/20/1999; Cat: 11683-V08H) and influenza virus A/Beijing/262/95 (H1N1) (Cat: 81N73-2) were purchased from Sino Biological Inc. (Beijing, China) and HyTest Lyd. (Turku, Finland), respectively. Influenza virus A/Yokohama/110/2009 (H3N2) that was isolated from a clinical sample was kindly provided by Dr. C. Kawakami of the Yokohama City Institute of Health, Japan, and was used for confirming the versatility of the assay system. ECL™ anti-mouse IgG, horseradish peroxidase (HRP) linked whole antibody (from sheep) was purchased from GE Healthcare UK Ltd. (Buckinghamshire, UK). All other chemicals were obtained from Wako Pure Chem. Ind. Ltd. (Osaka, Japan). All experiments were carried out using high purity deionized (DI) water ($>$ 18 MΩ).

2.2. Preparation of NPGL and semiconductor nanoparticles

The dealloying process of NPGL film has previously been described (Ciesielski et al., 2008). In this study, a gold/silver leaf was gently placed on a microscope slide. This slide was then slowly immersed into a beaker of concentrated nitric acid in order to float the leaf at the air–acid interface. The glass slide was removed when the leaf floated freely on the surface of the nitric acid solution. Subsequently, it was dealloyed for the desired time intervals of 5, 10, 30, and 60 min, and labeled as NPGL05, NPGL10, NPGL30 and NPGL60, respectively. The leaf was removed from the acid using a glass slide and transferred into a beaker containing deionized water, where the leaf was rinsed by floating for 30 min. The dealloyed leaf was withdrawn on a glass substrate that had

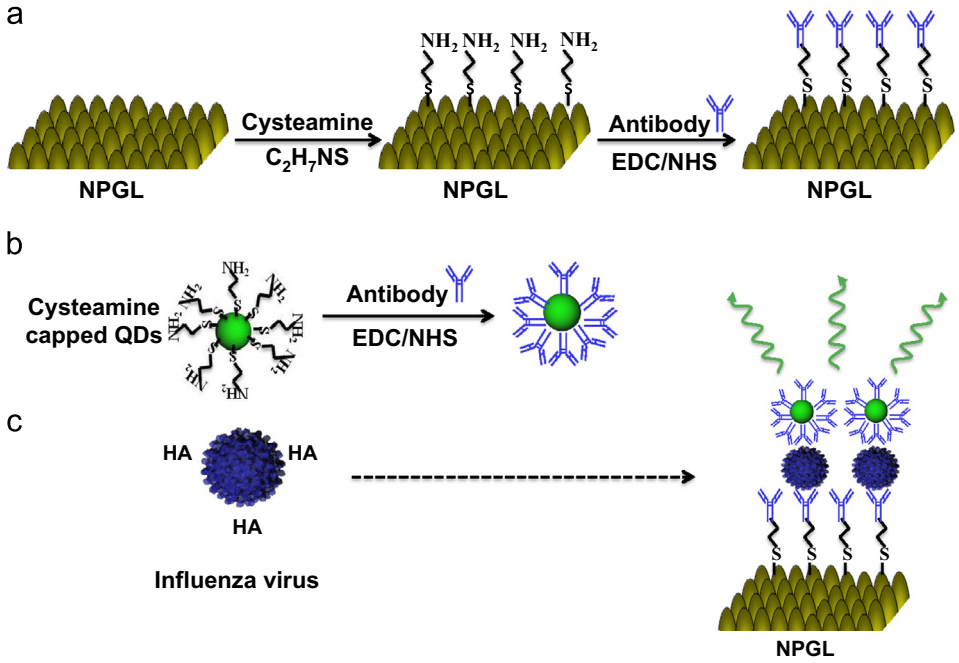

Fig. 1. Schematic of virus detection using nanoporous gold leaf (NPGL) film. The NPGL (a) and quantum dots (QDs) (b) were firstly conjugated with anti-hemagglutinin (HA) antibodies (anti-HA Ab, Y shape) by the reaction of ethylcarbodiimide (EDC)/*N*-hydroxysuccinimide (NHS). Then anti-HA Ab-conjugated with NPGL and QDs form complex (c) in presence of HA on the surface of influenza virus, finally enhancing PL intensity.