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A plasmon-assisted fluoro-immunoassay using gold nanoparticle-decorated carbon nanotubes for monitoring the influenza virus

Jaewook Lee [a,e], Syed Rahin Ahmed [b,c], Sangjin Oh [c], Jeonghyo Kim [c], Tetsuro Suzuki [d], Kaushik Parmar [e], Simon S. Park [e], Jaebeom Lee [c,*], Enoch Y. Park [a,b,**]

[a] Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya Suruga-ku, Shizuoka 422-8529, Japan
[b] Department of Bioscience, Graduate School of Science and Technology, Shizuoka University, 836 Ohya Suruga-ku, Shizuoka 422-8529, Japan
[c] Department of Nano Fusion and Cogno-Mechatronics Engineering, Pusan National University, Busan 609-735, Republic of Korea
[d] Department of Infectious Diseases, Hamamatsu University School of Medicine, 1-20-1 Higashi-ku, Handa-yama, Hamamatsu 431-3192, Japan
[e] Department of Mechanical and Manufacturing Engineering, University of Calgary, Calgary, Canada, T2N 1N4

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ABSTRACT

A plasmon-assisted fluoro-immunoassay (PAFI) was developed for the detection of the influenza virus by using Au nanoparticle (Au NP)-decorated carbon nanotubes (AuCNTs) that were synthesized using phytochemical composites at room temperature in deionized water. Specific antibodies (Abs) against the influenza virus were conjugated onto the surface of AuCNTs and cadmium telluride quantum dots (QDs), which had a photoluminescence intensity that varied as a function of virus concentration and a detection limit of 0.1 pg/mL for all three types of influenza viruses examined. The clinically isolated influenza viruses (A/Yokohama/110/2009 (H3N2)) were detected in the range of 50–10,000 PFU/mL, with a detection limit of 50 PFU/mL. From a series of proof-of-concept and clinical experiments, the developed PAFI biosensing system provided robust signal production and enhancement, as well as an excellent selectivity and sensitivity for influenza viruses. This nanoparticle-based technique could be potentially developed as an efficient detection platform for the influenza virus.

1. Introduction

Many kinds of nanomaterials have been recently used in the area of nanobiotechnology research. The unique physicochemical properties of nanomaterials have found a significant number of applications in biosensing, imaging, and drug delivery system (Ahmed et al., 2013; Lee et al., 2014; Leung et al., 2012; Li and Mezzenga, 2013; Wang et al., 2013b). In particular, nanobiosensing systems have gained popularity owing to its high sensitivity, selectivity, and rapid response time (Liu et al., 2012; Yin et al., 2013). The detection techniques used in various nanobiosensing applications include magnetophoresis, electrochemical analysis, plasmonic coupling immunoassays, and fluoro-immunoassays (Draz et al., 2012; Kim et al., 2013; Li et al., 2013; Viet et al., 2013; Zhou et al., 2012, 2013). The plasmon-assisted fluoro-immunoassay (PAFI) has been used to analyze specific biomaterials (Ahmed et al., 2014; Li et al., 2012; Nooney et al., 2010; Sharma et al., 2013b). The PAFI is based on the plasmonic resonance energy transfer (PRET) phenomenon, which causes a photoluminescence (PL) enhancement from the interactions between the plasmonic nanomaterials and the semiconductor nanoparticles (Lee et al., 2004, 2005, 2007). Such hybrid structures can be used to detect the interaction between an antibody (Ab) and its antigen, because of their tuned optical properties. Although numerous plasmonic nanomaterials have been introduced (*e.g.*, gold, silver, platinum, and metal NPs), Au NP-decorated carbon nanotubes (AuCNTs) have received considerable attention, owing to their unique properties. Au NPs are able to exhibit surface plasmon resonance (Jana et al., 2001; Lee et al., 2011b). Carbon nanotubes exhibit electroconductivity and harbor many π electrons on their surfaces (Jariwala et al., 2013; Sun et al., 2011). Thus, AuCNTs are expected to show a synergistic effect owing to their roles as biosensing platforms, signal enhancers, and signal transducers (McAndrew and

* Corresponding author.
** Corresponding author at: Shizuoka University, Research Institute of Green Science and Technology, 836 Ohya Suruga-ku, Shizuoka 422-8529, Japan.
E-mail addresses: jaebeom@pusan.ac.kr (J. Lee), acypark@ipc.shizuoka.ac.jp (E.Y. Park).

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Baxendale, 2013; Sharma et al., 2013a; Wang et al., 2013a; Yick et al., 2013).

Combining the above mentioned heterogeneous materials requires sophisticated strategies in order to conserve carbon nanotube (CNT) structures and to bind two materials without the help of organic bridges. One of the well-known processes for preparing AuCNT nanostructures involves reduction with chemical reducing agents such as sodium borohydride or hydrazine (Yu et al., 2014; Zhang et al., 2013). An alternative process involves thiol- or amine-assisted interactions between Au NPs and CNT surfaces (Georgakilas et al., 2007; Li et al., 2011). Attachment of Au NPs onto CNT surfaces has been attempted using the processes of electrodeposition, DNA hybridization, and chemical reaction (Georgakilas et al., 2007; Gobbo et al., 2013; Li et al., 2011; Li and Cooper-White, 2013; Peng et al., 2009). However, these approaches can cause CNT damage and organic/biological electric resistance, which may adversely affect their electrical and mechanical properties (Hirsch, 2002; Holzinger et al., 2001).

In this study, we suggest a novel and easy method for preparing AuCNTs by using phytochemicals. The synthetic reaction was carried out in deionized (DI) water at room temperature *via* sonication and stirring processes, without resorting to external heating or application of high pressure. No electrochemistry equipment was used in the above procedure. AuCNTs were produced as follows. Au ions were first attached onto the surface of CNTs and they were then reduced to Au NPs on the CNT surface. This process was catalyzed using a mixture of gallic acid and isoflavone phytochemicals, two well-known natural antioxidants (Aruoma et al., 1993; Park et al., 2009) that served as mild reducing agents (Lee et al., 2011a). Furthermore, the generated AuCNTs were applied onto the influenza virus detection platform by using a quantum dot (QDs)-assisted PAFI.

In this study, we developed a PAFI-based detection platform for the influenza virus, using antibody-conjugated AuCNTs and CdTe QDs. In all, we tested three types of influenza viruses, *viz.* the Influenza virus A/Beijing/262/95 (H1N1), the Influenza virus/New Caledonia/20/99IvR116 (H1N1), and the clinically isolated Influenza virus A/Yokohama/110/2009 (H3N2). The minimum detection limit for the influenza virus was 0.1 pg/mL. The clinically isolated influenza virus was also monitored in the range 50–10,000 PFU/mL, with a detection limit of 50 PFU/mL. Our virus detection platform would be immensely useful not only for detecting the influenza virus, but also for detecting various other viruses and viral diseases.

2. Material and methods

2.1. Materials and instruments

$HAuCl_4 \cdot 3H_2O$, multi-walled carbon nanotubes (MWCNTs), gallic acid, EDC, NHS, cadmium perchlorate hydrate, and cysteamine were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Aluminum telluride (Al_2Te_3) was obtained from the Cerac Company (Milwaukee, WI, USA). The isoflavone was isolated from commercial soybeans. 3,3′,5,5′-tetramethylbenzidine was purchased from Dojindo (Osaka, Japan). The ECL™ anti-mouse IgG, horseradish peroxidase (HRP)-conjugated whole antibody (Ab) was obtained from GE Healthcare UK, Ltd. (Buckinghamshire, UK). Goat anti-rabbit IgG-HRP was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Anti-Influenza A virus hemagglutinin (HA) Ab Ab66189, which is a mouse monoclonal antibody [B219M] for the influenza A virus HA H1 and positive against influenza virus A/Beijing/262/95 (H1N1), A/New Caledonia/20/99 (H1N1), and A/Taiwan/1/86 (H1N1), was purchased from Abcam Inc. (Cambridge, UK). Anti-neuraminidase (NA) (New Caledonia/20/1999/(H1N1)), a rabbit polyclonal Ab, was obtained from Immune Technology Corp. (New York, NY, USA). Anti-H3 (H3N2) (Ab82454), a mouse monoclonal Ab [InA227] to H3 (H3N2) that recognizes influenza virus A HA H3, was purchased from Abcam Inc. (Cambridge, UK). Influenza virus New Caledonia/20/99IvR116 (H1N1) and A/Beijing/262/95 (H1N1) were purchased from Sino Biological Inc. (Beijing, China) and HyTest Lyd (Turku, Finland), respectively. Influenza virus A/Yokohama/110/2009 (H3N2) was isolated from a clinically isolated sample, which was kindly provided by Dr. C. Kawakami of Yokohama City Institute of Health, Japan and was used to assess the versatility of this assay system.

The absorbance of AuCNTs and the corresponding PL enhancement were measured using a filter-based multimode microplate reader (Infinite® F500, TECAN, Ltd., Männedorf, Switzerland), and the chemical reactions and surface functional groups were monitored by FT-IR spectroscopy (FT-IR 6300, JASCO, Corp. Tokyo, Japan). The morphologies and sizes of the nanostructures were characterized by TEM (JEM-2100F, JEOL, Ltd., Tokyo, Japan). An X-ray powder diffractometer (RINT ULTIMA, Rigaku, Corp., Tokyo, Japan) was used to characterize AuCNT by using CuKa radiation and a Ni filter. The data were collected from 2 theta=0–100° at a scan rate of 0.01° per step and 10 s per point. The AuCNTs and MWCNTs were analyzed by Raman spectroscopy (HR-800, Lab-RAM, HORIBA Ltd., Kyoto, Japan). In order to measure the electroconductivity, AuCNT solution was dropped on the planar interdigitated electrode (planar IDE-Pt/0.25″, Synkera, USA) and dried at room temperature. Then, the current change of the deposited area was monitored by linear sweep voltammetry from −1 V to 1 V (SP-150, BioLogic, France). A plate reader (Model 680, Bio-Rad, Hercules, USA) was used to confirm the presence of Ab-conjugated nanomaterials. The PL image of the hybrid nanostructure was observed using a confocal laser-scanning microscope (LSM 700, Carl Zeiss Microimaging, GmbH, Göttingen, Germany).

2.2. Synthesis of AuCNTs and CdTe QDs

AuCNTs were synthesized at room temperature by using commercially available reagents. Forty milligrams of MWCNT was dispersed in 100 mL of nitric acid and boiled for 5 h to prepare the hydrophilic MWCNTs. Subsequently, 0.01 mmol of $HAuCl_4 \cdot 3H_2O$ and 2 mg of acid-treated MWCNTs were dispersed in 30 mL of DI water by sonication for 30 min. Subsequently, 600 μL of GI solution was added into the MWCNT/Au ion solution, and then stirred vigorously for 1 h. The GI solution was used as a reducing agent and stabilizer, and prepared as follows: 10 mg of isoflavone was dissolved in 10 mL of the 0.01 M gallic acid solution. The cysteamine-coated CdTe QDs were synthesized as reported in detail elsewhere (Gaponik et al., 2002; Lee et al., 2010).

2.3. Preparation of antibody-conjugated AuCNTs and CdTe QDs

In order to conjugate the Abs to the AuCNTs, amine-functionalized AuCNTs and Ab-conjugated EDC/NHS were prepared. To modify the surface of the Au NPs, 1 mg of AuCNTs was dispersed in 10 mL of DI water. Then, 1 mL of 0.01 M cysteamine was added into the AuCNT solution. After 30 min of stirring, this mixture was centrifuged to separate the amine-functionalized AuCNTs. Additionally, 100 μL of the 4 mM EDC and 10 mM NHS were added in the 96-well plate and incubated and gently shaken for 30 min at 200 rpm with 1 μL of anti-HA Ab (Ab66189) (final concentration of 5 ng/mL) for the EDC/NHS coupling reaction. Finally, 30 μL of the amine-functionalized AuCNT (1 μg/μL) and activated anti-HA Ab (Ab66189) were mixed in all wells and shaken for 3 h for effective bioconjugation. Anti-NA (New Caledonia/20/1999/(H1N1)) and anti-HA (Ab82454) Abs were also conjugated to the surfaces of the Au CNTs by using the same procedure. Anti-HA (Ab66189) and