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Size-controlled preparation of peroxidase-like graphene-gold nanoparticle hybrids for the visible detection of norovirus-like particles

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ABSTRACT

A hybrid structure of graphene-gold nanoparticles (Grp-Au NPs) was designed as a new nanoprobe for colorimetric immunoassays. This hybrid structure was prepared using chloroauric acid, sodium formate and Grp flakes at room temperature. Au NPs attached strongly onto the Grp surface, and their size was controlled by varying the sodium formate concentration. The Raman intensity of the Grp-Au NP hybrids was significantly enhanced at 1567 cm^{-1} and 2730 cm^{-1} compared with those of pristine Grp because of the electronic interaction between Au NPs and Grp. The Grp-Au NPs with a hybrid structure catalyzed the oxidation of the peroxidase substrate 3,3,5,5-tetramethylbenzidine (TMB) with H_2O_2, developing a blue color in aqueous solution. This catalytic activity was utilized to detect norovirus-like particles (NoV-LPs) in human serum. The enhanced colorimetric response was monitored using Ab-conjugated-Grp-Au NPs and found to depend on the NoV-LP concentration, exhibiting a linear response from 100 pg/mL to 10 μg/mL. The limit of detection (LOD) of this proposed method was 92.7 pg/mL, 112 times lower than that of a conventional enzyme-linked immunosorbent assay (ELISA). The sensitivity of this test was also 41 times greater than that of a commercial diagnostic kit. The selectivity of the Grp-Au NPs was tested with other viruses, and no color changes were observed. Therefore, the proposed system will facilitate the utilization of the intrinsic peroxidase-like activity of Grp-Au NPs in medical diagnostics. We believe that the engineered catalytic Grp-Au NP hybrids could find potential applications in the future development of biocatalysts and bioassays.

1. Introduction

Nanomaterial-based enzyme mimics have attracted research interest and have recently been shown to have potential applications in bioanalysis and environmental detection (Lin et al., 2014; Wei and Wang, 2103). Although natural enzymes, such as horseradish peroxidase (HRP), have been frequently used in recent decades, they have critical limitations for industrial applications, such as low stability under harsh conditions (temperature and pH) and relatively high costs of preparation, purification, and storage (Gao et al., 2007; Zhao et al., 2016). To overcome these limitations, the research interest in enzyme mimics has expanded from molecular to inorganic nanoscale materials, and as a result, highly organized artificial enzymes have been synthesized for a wide range of applications.

In particular, metal-based artificial enzymes, such as gold nanoparticles (Au NPs), magnetic NPs (Fe_3O_4 NPs) and platinum NPs (Pt NPs) have been discovered to possess intrinsic peroxidase-like activities (Ahmed et al., 2016a, 2016b; Gao et al., 2013; Liang et al., 2013; Sun et al., 2012; Zhao et al., 2015). In addition to single NPs, nanohybrids combining two or more NPs in single entity have also been intensively studied (Ahmed et al., 2016a, 2016b; Gao et al.,

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2014; Wang et al., 2014). Surprisingly, nanohybrids often exhibit synergistic effects that significantly enhance the catalytic performance and detection of analytes in the field of biosensors and immunoassays. To keep pace with developing sensitive detection of analytes, new generation of hybrid nanostructures with enhanced catalytic activity, high stability and low toxicity remains highly desirable to replace conventional peroxidase systems for practical applications.

Graphene (Grp)-based materials have been considered extremely versatile because of the extraordinary physicochemical properties that originate from Grp's unique structure, including high mechanical strength, large surface area, good biocompatibility, high chemical stability and catalytic properties. Grp-based materials have shown promise for several advanced technological applications in the past few years (Li et al., 2008; Qu et al., 2011; Singh et al., 2011; Yang et al., 2013). Similarly, Au NPs have emerged as an interesting area of scientific research because of their favorable optical properties, biocompatibility, low toxicity, high intrinsic peroxidase-like activity and significant applications in photonics, catalysis, electronics and biomedicine (Daniel et al., 2004; Yeh et al., 2012). Based on these attractive properties, hybrid structures of Grp and Au NPs should enable the preparation of nanoprobes with great potential applications in colorimetric immunoassays. To date, several methods have been used to prepare hybrid structures of Grp-Au NPs, including thermal treatment, chemical reduction, and electrochemical or microwave-assisted methods. However, these methods have some limitations: they are time consuming, require high temperatures or involve complicated procedures.

Herein, a facile approach to prepare Grp-Au NP hybrids using sodium formate as a reducing and stabilizing agent of Au NPs on a Grp surface is presented. Because individual Grp and Au NPs have peroxidase-like activities, enhanced peroxidase-like performance was expected to be achieved via the rational combination of Au NPs and Grp. The attachment of Au NPs onto the Grp surface facilitated the conjugation of biomolecules, such as antibodies, onto the hybrid. Thus, a Grp-Au NP hybrid was further conjugated with an antiviral antibody (Ab) to obtain Ab-conjugated-Grp-Au NPs (Ab-Grp-Au NPs). These Ab-Grp-Au NPs were used as a robust nanoprobe for the quantitative, selective, and colorimetric detection of virus particles through the superior peroxidase-like activity of the hybrid (Scheme 1). In this study, norovirus-like particles (NoV-LPs) was chosen as a model analyte because it is a leading cause of viral gastroenteritis outbreaks worldwide. NoVs are commonly transmitted through shellfish consumption and food and waterborne routes. However, the levels of enteric viruses in mussels are generally low (Morton et al., 2009; Schultz et al., 2007). Polymerase chain reaction (PCR)-based RNA detection has been widely used to identify causative agents. Simple and highly sensitive diagnostic systems for the detection of NoV antigens have not been established to date. To the best of our knowledge, this report is first to describe the sensitive colorimetric detection of NoV-LPs using inorganic nanohybrids as an artificial enzyme.

2. Experimental section

2.1. Materials

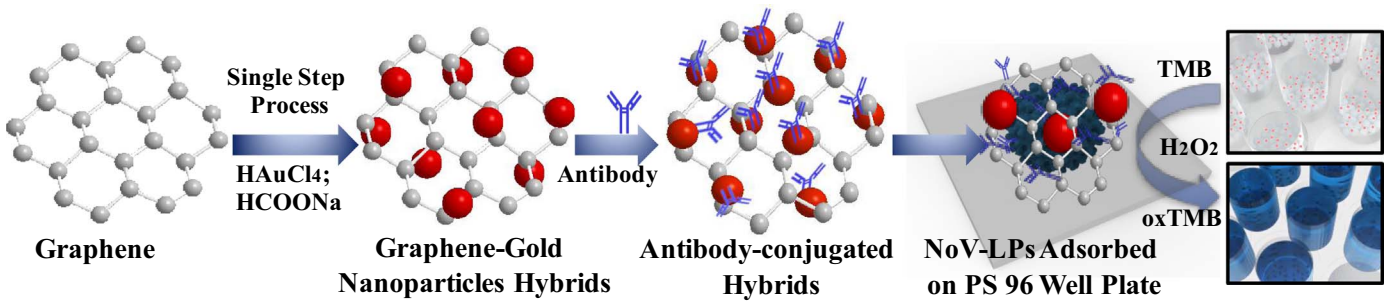
Gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$) and human serum (containing extremely complex biological matrices in dL, such as iron 35–180 μg, cholesterol 110–210 mg, triglyceride 30–175 mg, glucose 60–140 mg, endotoxin < 10 EU, and hemoglobin < 20 mg) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Grp flakes (Model AO-3) were purchased from Graphene Supermarket (Calverton, NY, USA). Sodium formate (HCOONa), sodium acetate (NaOAc) and hydrogen peroxide (H_2O_2) were purchased from Wako Pure Chemical, Inc. (Osaka, Japan). N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). The chromogenic substrate, 3,3′,5,5′-tetramethylbenzidine (TMB) was obtained from Dojindo (Osaka, Japan). The anti-influenza A virus hemagglutinin (HA) H1 Ab [B219M] (ab661189, Lot: GR40088–11) was obtained from Abcam, Inc. (Cambridge, UK). The influenza A (H3N2) HA monoclonal Ab (Anti-HA H3N2 MAb, Lot: HB04N0160) was acquired from Sino Biological, Inc. (Beijing, China). The anti-NoV Ab (NS-14), which is broadly reactive with the NoV genogroup II, was developed and characterized as previously described (Kitamoto et al., 2002; Kou et al., 2015). All experiments were conducted using high-purity deionized (DI) water (> 18 MΩ cm).

2.2. Preparation of Grp-Au NP hybrids

The one-step preparation of in situ hybrid nanostructures of Grp-Au NPs was conducted as follows: 1 mL of 20-mM $HAuCl_4 \cdot 3H_2O$ and 2 mg of Grp flakes were mixed for 5 min under gentle stirring. Subsequently, 2 mL of 200–500-mM HCOONa was added, and the mixture was maintained at room temperature for 1 h. The Au cations attach to the Grp flakes via electrostatic interactions, are reduced by HCOONa to Au NPs, and are subsequently stabilized on the Grp surface.

2.3. Physicochemical properties of Grp-Au NP hybrids

Transmission electron microscopy (TEM) images were obtained using a TEM microscope (JEM-2100F, JEOL, Ltd., Tokyo, Japan) operated at 100 kV. The ultraviolet-visible (UV–vis) spectra of the nanostructured films were recorded using a Tecan Infinite M200 spectrophotometer (Infinites F500, TECAN, Ltd., Männedorf,



Scheme 1. A schematic illustration of one-step preparation of Grp-Au NP hybrids using Graphene flakes, HCOONa and $HAuCl_4$. The antibody was conjugated with Grp-Au NP hybrids through amide bonding, and the peroxidase-like activity was established based on the colorimetric detection of the virus deposited on 96-well plates. In the absence of Grp-Au NP hybrids, the TMB-H_2O_2 mixed solution was colorless. By contrast, in the presence of the Grp-Au NP hybrids, the oxidized TMB (oxTMB)-H_2O_2 solution produced a strong blue color. (For interpretation of the references to color in this scheme legend, the reader is referred to the web version of this article.)