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Sensitive semi-quantitative detection of respiratory syncytial virus by dark-field light scattering imaging of the infected host cells

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Abstract A novel sensitive semi-quantitative virus detection technique was developed using the respiratory syncytial virus (RSV) as an example, through dark-field light scattering imaging of the surface state of the virus-invaded host cells. In this method, anti-RSV-antibody modified gold nanoparticles (AuNPs) could bind with the invading virus on the cell membrane of the infected host cells through the specific antibody-antigen binding. Then, the host cells could be imaged by the localized surface plasmon resonance light scattering properties of AuNPs under a dark-field light scattering microscopy, which could be further used to semi-quantify the invading virus.

Keywords Virus · Living cells · Gold nanoparticles · Dark-field light scattering imaging · Semi-quantitation

1 Introduction

There is an increasing requirement for rapid virus detection techniques in the field of clinical diagnosis, due to the many existing and emerging viral diseases. Most of the quantitative methods for virus detection are based on enzyme-linked immunosorbent assays (ELISA) [1–5]. The ELISA technique is considered the "gold standard" for quantification of antigens based on the specific antibody-antigen binding. Conventionally, the measurable signal of ELISA is generated by conversion of the enzyme substrate into a coloured molecule [6–8]. Unfortunately, natural enzymes have several intrinsic shortcomings, including limited lifetime, high cost and complex experimental conditions [9–12]. Although various inorganic nanoparticles with enzyme mimetic activities, such as carbon materials [13–15], metal oxides [16, 17], and noble metal nanoparticles or nanoclusters [18–20] could be used to replace the natural enzymes, some heavy metal ions, such as Ag^{+}, Hg^{2+}, Pb^{2+} or Pt^{4+}, need to be added to enhance the enzymatic activity of the inorganic nanoparticles [2, 21, 22]. These heavy metal ions usually as exhibit toxicity towards the virus being analysed, as well the environment. Therefore, it is of utmost importance to develop simple and effective method to detect virus.

Herein, we developed a new dark-field microscopic imaging (iDFM) method for the semi-quantitative detection of virus by using living cells as the substrates, gold nanoparticles (AuNPs) modified with anti-virus-antibody as the dark-field light scattering probes, and respiratory syncytial virus (RSV) as a proof-of-concept target. RSV is a single-stranded RNA virus of the paramyxovirus family, which mainly infects the infants and the elderly and causes serious complications [23–25]. Thus, RSV is generally considered as a major global public health problem, which would benefit from a new simple and rapid detection method. Our semi-quantitative strategy for RSV detection is shown in Scheme 1. Human epidermis larynx carcinoma cell lines (HEp-2) cells were cultured in 24-well plate (Scheme 1a) before RSV infection (Scheme 1b), and then anti-RSV-antibody modified AuNPs were added to the

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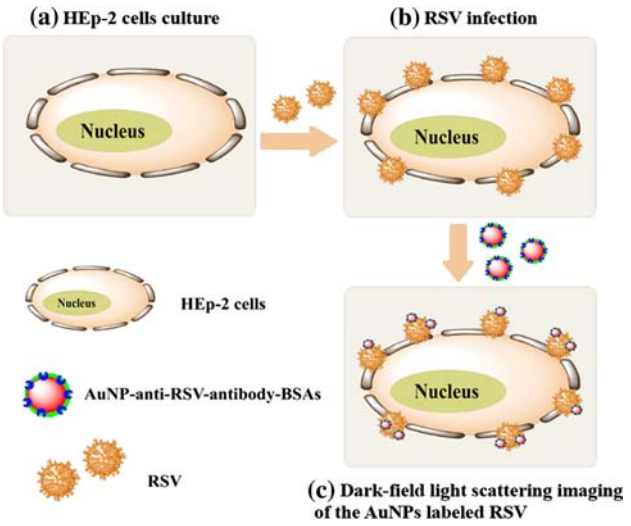

Scheme 1 Schematic representation of the HEp-2 cell-based semi-quantitative analysis of RSV. **a** The HEp-2 cells were cultured in 24-well plates. Then, RSV was added and attached onto the cell membrane surfaces (**b**), which were then imaged and detected using the scattered light signals of anti-RSV-antibody modified AuNPs (**c**)

cells. The AuNPs would target and link onto the surface of cell membrane where the virus attached (Scheme 1c) through the specific binding between antibody and RSV. As the localized surface plasmon resonance (LSPR) light scattering features of AuNPs are strong, the RSV could be semi-quantitated based on the percentage of the scattering signal of AuNPs on the cell membrane. Compared to ELISA, our method does not require the addition of other metal ions to assist viral detection, and it can detect viruses without any pretreatment steps. Additionally, one of the key advantages of AuNPs is that its scattering images can be obtained without photobleaching effects [26–29], thus allowing this method to be potentially applied for long-time imaging analysis.

2 Experimental

2.1 Materials

Human epidermis larynx carcinoma cell lines (HEp-2 cells) and RSV strain were cultivated in our laboratory. Anti-RSV-antibody was obtained from Abcam (Cambridge, MA, USA). Bovine serum albumin (BSA) was obtained from Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). Trisodium citrate and $HAuCl_4$ were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) and used without further purification. All of the solutions were prepared with doubly deionized water (18.2 MΩ, Millipore, USA).

2.2 Preparation of AuNPs

The AuNPs with diameters of 50 nm were prepared by the following method: 0.5 mL $HAuCl_4$ (1 %, w:w) and 49.5 mL water were mixed and heated to boiling under the magnetic stirring. Then 500 μL of 1 % trisodium citrate was added to the solution quickly, which was refluxed for 15 min and cooled to room temperature under stirring. Finally, the resulting solution of AuNPs was filtered by a 0.22 μm membrane and stored in a 4 °C refrigerator.

2.3 Conjugation and characterization of AuNP-anti-RSV-antibody-BSA

AuNPs were conjugated with the anti-RSV-antibody by incubating 1 mL of the as-prepared AuNPs solution with 10 μg/L anti-RSV-antibody at room temperature for about 60 min. The concentration of the as-prepared AuNPs was calculated to be 2.69×10^{-11} mol/L. Subsequently, 20 μg/L BSA was added and reacted for 30 min to block the excess binding sites on the surface of AuNPs. UV-Vis absorption spectra of AuNPs, AuNP-anti-RSV-antibody and AuNP-anti-RSV-antibody-BSA were measured with a U-3010 UV-Vis spectrophotometer (Hitachi, Tokyo, Japan), and their hydrodynamic diameters were measured by a Zetasizer Nano ZS90 (Malvern Instruments). An S-4800 scanning electron microscope (Hitachi) was employed to capture the scanning electron microscopy (SEM) images of AuNPs.

2.4 Cell culture and virus propagation

RPMI 1640 culture medium containing 10 % (w:v) fetal bovine serum (FBS) was used for the culture of HEp-2 cells. The propagation of RSV was conducted in the monolayer culture of HEp-2 cells in RPMI 1640 culture medium containing 2 % FBS with 5 % CO_2 at 37 °C. When the cytopathic effect (CPE) were formed after 1–2 d post infection, 2–3 rounds of freeze-thaw cycles of the cells were performed to release RSV. Then, the cell debris was removed after centrifugation at 3,000 *g* for 10 min. Finally, the harvested RSV was stored at −80 °C.

2.5 Dark-field light scattering imaging

Before RSV infection, HEp-2 cells were cultured for about 24 h to reach 50 % confluence. Next, 200 μL of different amounts of RSV (0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 pg/mL) was successively added to HEp-2 cells and incubated at 4 °C for 30 min. After removing the spare virus solution, the cells were rinsed with PBS. Then,