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Labeling viral envelope lipids with quantum dots by harnessing the biotinylated lipid-self-inserted cellular membrane

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ABSTRACT

Highly efficient labeling of viruses with quantum dots (QDs) is the prerequisite for the long-term tracking of virus invasion at the single virus level to reveal mechanisms of virus infection. As one of the structural components of viruses, viral envelope lipids are hard to be labeled with QDs due to the lack of efficient methods to modify viral envelope lipids. Moreover, it is still a challenge to maintain the intactness and infectivity of labeled viruses. Herein, a mild method has been developed to label viral envelope lipids with QDs by harnessing the biotinylated lipid-self-inserted cellular membrane. Biotinylated lipids can spontaneously insert in cellular membranes of host cells during culture and then be naturally assembled on progeny Pseudorabies virus (PrV) *via* propagation. The biotinylated PrV can be labeled with streptavidin-conjugated QDs, with a labeling efficiency of ~90%. Such a strategy to label lipids with QDs can retain the intactness and infectivity of labeled viruses to the largest extent, facilitating the study of mechanisms of virus infection at the single virus level.

1. Introduction

Labeling viruses with fluorescent materials is the prerequisite for exploring virus-cell interactions at the single virus level, which can offer opportunities for dissecting mechanisms of virus infection so as to prevent virus-related diseases [1–3]. Compared with organic dyes and fluorescent proteins, quantum dots (QDs) are excellent for long-term single-particle tracking due to their unique photostability and high brightness [4–9]. Recently, QDs have been widely used to label viral envelopes [10–14], capsids [15] and genomes [16]. Viral envelopes, generally composed of lipids and proteins [17], are essential for attachment and entry of enveloped viruses to/into their host cells. Currently, most developed strategies to label viral envelopes with QDs involve chemical modification of envelope proteins, which might affect native structures of proteins, interfering virus attachment [18]. However, so far as we know, few reports on labeling of viral envelope lipids, the most abundant structural component of viruses, with QDs have appeared most probably owing to the lack of efficient methods to functionalize the lipids [19,20].

Based on the strategy to label lipids with the assistance of cells, we have developed methods to label viral lipids with organic dyes, and the lipids of cell-derived microvesicles with QDs [21,22]. Nevertheless, because of the steric hindrance caused by envelope proteins and the size of QDs, it is still a challenge to label viral envelope lipids with QDs. Thus, in this work, taking Pseudorabies virus (PrV) as a model [23,24], a mild strategy to efficiently label viral envelope lipids with QDs is proposed by harnessing the biotinylated lipids self-inserted cellular membranes to monitor virus-cell interactions in real time.

As shown in Fig. 1A, biotinylated lipids can spontaneously insert in cellular membranes of wild-type BHK-21 cells (Baby Hamster Syrian Kidney, W-BHK) by simply feeding cells with commercial DSPE-PEG2000-biotin. When wild-type PrV (W-PrV) infects the biotinylated BHK-21 cells (B-BHK) to propagate itself, these biotinylated lipids can naturally assemble on the envelopes of progeny PrV since PrV virions inherit their envelopes from cellular membranes of host cells. Subsequently, QDs labeled PrV (QDs-PrV) can be obtained by simply incubating biotinylated PrV (B-PrV) with

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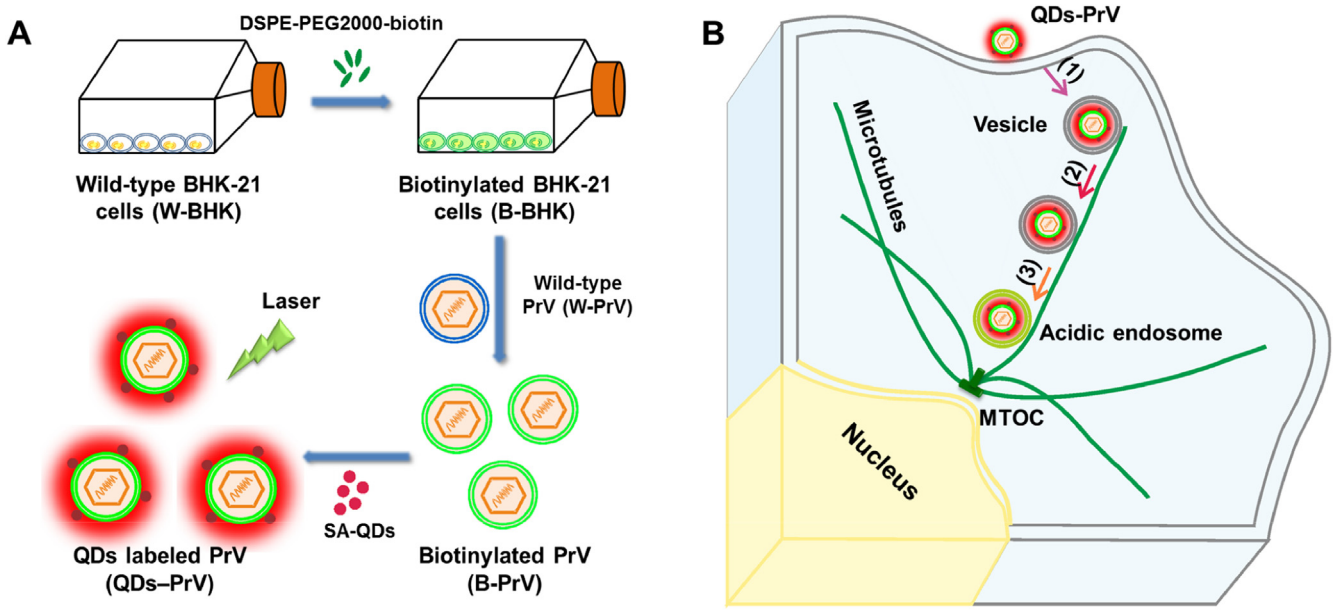

Fig. 1. (A) Schematic illustration of PrV biotinylation in host cells and labeling with SA-QDs. (B) Scheme of QDs-PrV infection routes in a living HeLa cell. Wild-type BHK-21 cells: W-BHK, biotinylated BHK-21 cells: B-BHK, wild-type PrV: W-PrV, biotinylated PrV: B-PrV, QDs labeled streptavidin: SA-QDs, SA-QDs labeled PrV: QDs-PrV.

streptavidin-conjugated QDs (SA-QDs). According to references [25,26], PrV infecting HeLa cells through endocytosis would experience processes including PrV entering HeLa cells in a vesicle (1), moving towards perinuclear region along microtubules (2), and entering acidic endosomes (3). However, these processes have never been dynamically monitored in previous PrV studies. In our work, these critical endocytic processes can be illustrated in real time (Fig. 1B).

2. Materials and methods

2.1. Biotinylation of host cells and viruses

Wild-type BHK-21 cells (W-BHK) were cultured in Minimum Essential Medium (MEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco) at 37 °C, 5% CO_2. Biotinylated BHK-21 cells (B-BHK) were produced by culturing W-BHK cells in MEM containing DSPE-PEG2000-biotin (AVANTI) and 10% (v/v) FBS at 37 °C, 5% CO_2. Wild-type PrV (W-PrV) was propagated in W-BHK cells in MEM supplemented with 2% (v/v) FBS for 48 h at 37 °C, 5% CO_2, while biotinylated PrV (B-PrV) was propagated in B-BHK cells under the same conditions.

B-BHK cells were fixed with 4% (w/v) paraformaldehyde for 20 min at room temperature and washed with 1 × PBS. Then the cells were incubated with 5 nM SA-QDs (Wuhan Jiayuan Quantum Dots Co. Ltd.) for 10 min at room temperature and washed with 1 × PBS. The orthogonal fluorescence image was acquired by a spinning-disk confocal microscope (Andor Revolution XD) and analyzed by Andor IQ software (Andor Technology).

2.2. High-performance liquid chromatography (HPLC) assay

Lipids of W-BHK and B-BHK cells were extracted according to a chloroform/methanol extraction protocol [27] and detected by HPLC. HPLC analysis was performed on a Shimadzu LC-6AD semi-preparative system with a conventional XAqua C18 column (150 mm × 4.6 mm, i. d. 5.0 μm) and an ultraviolet–visible (UV–vis) detector (SPD-20A, Shimadzu). Absorbance was measured at 206 nm. Methanol was chosen as the mobile phase at a flow rate of 0.5 mL/min to perform the preparative operation.

2.3. Labeling efficiency of biotinylated host cells with QDs

W-BHK and B-BHK cells were incubated with 5 nM SA-QDs at room temperature for 10 min and then excessively washed with 1 × PBS. Confocal images were acquired by a spinning-disk confocal microscope (Andor Revolution XD). Labeling efficiency was detected by Flow cytometer (FACSAria III BD). The results were analyzed by Flowjo software.

2.4. Purification and labeling of viruses

After two freeze and thaw cycles, the cell debris was removed by centrifuging at 5000 rpm for 30 min. Then, PrV was concentrated by ultracentrifugation at 30000 rpm for 2.5 h in a Type 45 rotor (Beckman). The precipitations were resuspended in 1 × PBS and dispersed extensively by whirling for 10 min. After that, PrV was incubated with 20 nM SA-QDs for 1 h on ice, and then purified by 30%–55% sucrose density gradient centrifugation at 26000 rpm for 2.5 h in an SW41 rotor. The SA-QDs labeled PrV (QDs-PrV) was collected and centrifuged at 25000 rpm for 3 h to remove sucrose. QDs-PrV was resuspended in 1 × PBS (pH 7.4) for further use.

2.5. Quantification of biotin

In our experiments, 2 mL of 5 nM FITC-SA (Sigma) was added to a stirred cuvette and allowed to equilibrate for 8 min at 25 °C. Then, biotin was gradually added into FITC-SA until the fluorescence intensity was steady. The fluorescence intensity at 515 nm in the system was recorded by Fluorolog-3 fluorescence spectrometer (HORIBA JOBIN YVON). Standard curve was established according to the amount of biotin and the corresponding fluorescence intensity increase. Afterwards, 40 μL of purified viruses were added into 2 mL of 5 nM FITC-SA under the same conditions. The fluorescence intensity at 515 nm was recorded before and after virus addition. Thus, the amount of biotin on B-PrV was quantified.