Quantum Dots Capped with Dengue Virus As Imaging Probes for Drug Screening

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Statement of Purpose: The emergence and spread of viral diseases worldwide, particularly HIV/AIDS, outbreaks of severe acute respiratory syndrome (SARS) virus, and the scares of pandemic avian influenza virus seriously raise the concern that any virus strain has the potential evolving into a life-threatening pathogen. In this regard, developing fast and efficient screening technology has its merits of identifying potential drugs against viral diseases that still lack of effective prevention or treatment. Dengue virus is the typical one representing an important emerging mosquito borne disease worldwide. It has spread from being endemic in just 9 countries in 1970 to 100 countries in 2002, according to the world health report. Apparently, global population growth, urbanization, and frequent modern transportation have contributed to the increased incidence and geographic spread of dengue viruses. These diseases occur in tropical and subtropical areas of the world, where 2.5 billion people are estimated at risk for dengue virus outbreaks. The pace of developing antiviral agents could be expedited by the availability of quick and efficient drug screening platforms. Fluorescent dye has been widely used for viral labeling experiments and improved our understanding of viral infection process. However, fluorophores are notorious for photobleaching and spectral overlaps, and therefore could affect the fluorescence imaging quality of dye-labeled virus since the observation time ranged from one to ten seconds before photobleach occurs. Obviously, a high fluorescence quantum yield and a large number of fluorescent photocycles before photobleach of the dye molecule occurs are the prerequisites for successfully detecting dye-labeled viral particles. Moreover, after labeling viral particles with fluorescent dyes, the number of viruses that can infect a cell most likely will be severely diminished. In this study, quantum dot (QD), an emerging probe for biological imaging and medical diagnostics, was employed to form complexes with virus and used as fluorescent imaging probes for exploring potential antiviral therapeutics.

Methods: 0.4 mg of CdSe/ZnS suspended in 5 mL of chloroform was added with 2 mg of alginate surfactant. The mixture was sonicated in a bath for 10 min, and then the chloroform was removed by a rotary evaporator at room temperature. At the end of the operation, the mixture was resuspended with PBS solution and filtered through a 0.22-m syringe filter to remove aggregates. The filtrate was freeze-dried to obtain the amphiphilic alginate coated quantum dots in the powder form. QDs encapsulated by amphiphilic alginate (AA-QDs) were thoroughly dispersed in aqueous solution by a sonicator for 10 min and then passed through a 0.22-μm syringe filter to collect non-aggregated AA-QDs which were analyzed for mean particle size and size distribution by the dynamic light scattering. The surface electric charge of AA-QDs, dengue virus, and QD-virus complexes was measured separately by the zeta potentiometer. UV–vis spectrophotometer and photoluminescence spectrophotometer were used to characterize AA-QDs. TEM specimens were made by evaporating one drop of quantum dots/virus complexes solution on carbon-coated copper grids. TEM micrographs were taken by a transmission electron microscope. BHK-21 cells were maintained at 37^{o}C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Dengue virus serotype-2 strain PL046 was propagated in mosquito C6/36 cell line maintained at 30^{o}C in DMEM, supplemented with 10% FBS, penicillin (200 U/mL) and streptomycin (100 μg/mL). Dengue virus-containing supernatant was first centrifuged at 10,000 rpm, and then ultracentrifuged at 100,000 × g at 4 ^{o}C for 3 h to purify dengue virions. The titer of the virus was evaluated using BHK21 cells by the plaque assay. In order to test if QD-virus complexes can be harnessed as diagnostic probes for fast screening potential anti-dengue therapeutic agents. Allophycocyanin with concentration of 6.25, 31.25, and 125 μg/mL was used to treat BHK-21 cells for one hour, and then rinsed away by PBS solution for three times. After allophycocyanin treatment, QD/virus complexes were added separately for 30, 60, and 120 min to examine the efficacy of drug screen assay proposed in this study.

Results: To demonstrate the formation of QD/virus complexes via colloidal clustering of negatively charged QD and dengue virus in cationic polybrene solution, TEM image of QD/virus complexes was taken and showed in Figure 1. In the image, the light-color particles with the size around 40-50 nm are dengue virus, and the dark-color particles tagged on the surface of viral particles are AA-QDs (about 10-15 nm). It is reasonable to speculate that, if any potential compound has anti-dengue viral feature, the intensity of green fluorescence emitted from QD-virus complexes internalized in cells should be drastically diminished. Allophycocyanin was selected as the model compound for exploring the efficacy of QD/virus imaging modality for screening anti-dengue viral therapeutic agents. The intensity of green images within BHK-21 cells was decreased along with the dosage increase of allophycocyanin provided one hour prior to the addition of QD-virus complexes which were constructed in the presence of 50 μg/mL polybrene. It should be noted that cells incubated with QD-virus complexes for only 30 min can give a fairly clear fluorescent intensity decrease with the concentration of allophyscocyanin up to 125 μg/mL.

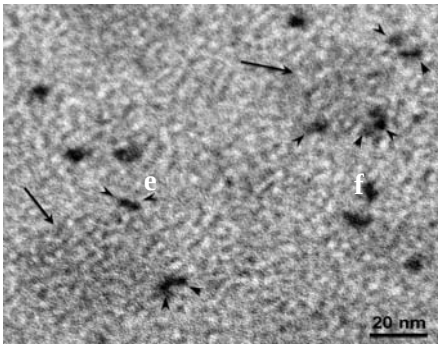

Figure 1. TEM image of the QD/virus complexes formed in the cationic polybrene solution. The light-color particles with the size around 40-50 nm are dengue virus (indicated by arrow). The dark-color particles tagged on the viral particles (indicated by arrowhead) are AA-QDs (about 10-15 nm).

Conclusions: Compared to other anti-viral agent screening assays, the cell-based QD/virus imaging modality exploited in this study indeed has fast and efficient features for screening antiviral therapeutics.