Quantum dot-based HIV detection and imaging in a microfluidic chip

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ABSTRACT

We developed an on-chip HIV capture and imaging method using quantum dots (Qdots) from microliter volume (10 μl) of HIV-infected patient whole blood using anti-gp120 antibody-immobilized microfluidic chip. Two Qdots (Qdot525 and Qdot655 streptavidin conjugates) were used to identify the captured HIV by simultaneous labeling the envelope gp120 glycoprotein and its high-mannose glycans. This double staining technique using Qdots provides a novel tool for accurate identification of HIV particles from whole blood. This on-chip HIV capture and imaging platform creates new avenues for point-of-care (POC) diagnostics and monitoring applications of infectious diseases.

KEYWORDS: Human immunodeficiency virus (HIV), Quantum dot (Qdot), Viral capture, Double-stain imaging

INTRODUCTION

Structure of the nanometer-sized HIV particle consists of a lipid membrane envelope nailed with envelop glycoproteins sustained by matrix proteins surrounding the two single-strand RNA and reverse transcriptase enclosed by p24 capsid proteins[1]. The envelop glycoprotein spike, gp120, directly mediate a recognition of CD4 receptors expressed on T-lymphocytes.[1, 2] Considering the HIV envelope structure, gp120 which is expressed with high-mannose glycans has been among the first targets of HIV vaccine development[3, 4] and is the most obvious target protein to capture and image the viruses in our current research. However, organic fluorescence-labeled anti-gp120 antibody is not appropriate for long time imaging of these small viral particles under a high magnification objective which generate high excitation light intensity.[5]

THEORY

The basic principle of HIV capture and detection using double-stain imaging *via* colocalized green and red Qdots is described in Figure 2. To capture the HIV particles from whole blood, we utilize two HIV-specific proteins for double staining the viruses: (i) anti-gp120 antibody that binds to the gp120 glycoprotein expressed on the outer membrane surface of HIV envelop and (ii) ConA lectin can bind to

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high-mannose glycan epitopes which are mainly expressed on the gp120. The lectin was used to avoid false-positive signal by only using HIV-1 gp120 antibody.

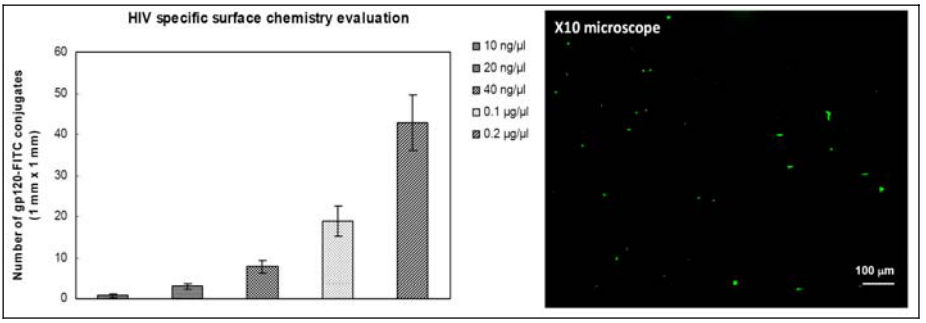

Figure 1. Validation of the surface chemistry through the direct capture of FITC-conjugate gp120 according to various concentrations (10, 20 and 40 ng/μl, 0.1 and 0.2 μg/μl) using anti-gp120 antibody-immobilized microfluidic device.

EXPERIMENTAL

To construct the microfluidic device surface to capture the HIV particles from whole blood, we derivatized the glass (SiO_2) surface by stepwise chemical treatment and finally coupled with anti-gp120 antibody onto the functionalized surface. The captured viruses were firstly recognized by biotinylated anti-gp120 antibody and Qdot525 streptavidin conjugate (Qdot525/anti-gp120 antibody). And then, they were also captured by biotinylated ConA and Qdot655 streptavidin conjugate (Qdot655/ConA). Using different fluorescent filters, we imaged the Qdots (green and red) and then merged the images obtained at the same position under a microscope.

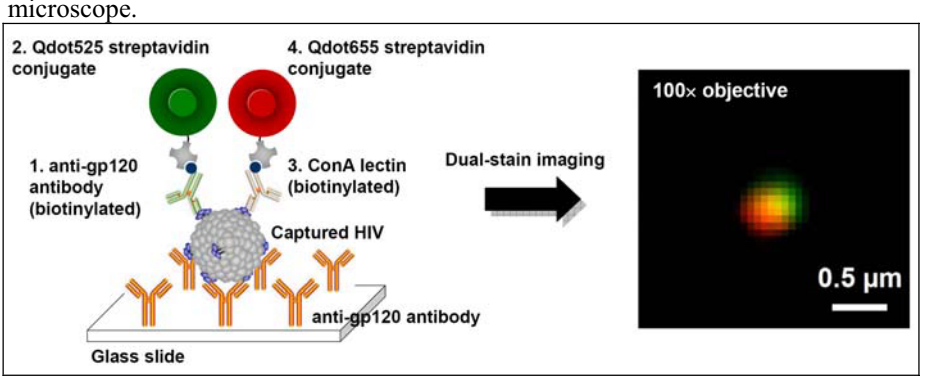

Figure 2. Schematic illustration of HIV capturing/imaging strategy using the dual-stain imaging technique and Fluorescent images 100× objective from the HIV particles using dual-stain imaging techniques, Qdot525/anti-gp120 antibody and Qdot655/ConA.

RESULTS AND DISCUSSION

To demonstrate the surface chemistry of microfluidic device for HIV capture, recombinant gp120-FITC conjugate into PBS was used at different concentrations (Figure 1), and the HIV envelope protein was captured by using the developed microfluidic device. Depending on the concentrations, the microfluidic device can evidently capture the target proteins and also sensitively recognize them below 20 ng/μl which is recommended as the lowest dilution. Using the microfluidic HIV-viral chip, we carried out HIV capturing from HIV-infected whole blood and imaging with Qdots and HIV-specific proteins. The HIV particles captured onto anti-gp120 antibody-coated surface were first recognized by Qdot525/anti-gp120 antibody. To verify the captured HIV viruses, ConA lectin solution was introduced and subsequently Qdot655 streptavidin conjugate was injected into the microfluidic device. The colocalized signals (yellow) were detected by both green and red probes binding to the HIV gp120 glycoprotein and its high-mannose oligosaccharides, respectively, in the merged 100× microscope images (Figure 2). The selected areas in the wide-field images are magnified to allow direct visualization of colocalized signals (yellow). As a facilitating point for our future goals to create a point-of-care device, the colocalized yellow Qdot signals were visible using 10x objective microscope.

CONCLUSIONS

In summary, we developed an integrated microfluidic device to rapidly capture and image HIV particles from a microliter volume of HIV-infected patient whole blood on a microfluidic chip that achieves sequentially: (i) selective HIV capture using anti-gp120 antibody on a microfluidic chip from whole blood (10 μl) within 10 minutes, (ii) sensitive detection and imaging of the captured HIV particles using colocalized Qdot signals *via* anti-gp120 antibody and ConA lectin.

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