RAPID DIFFERENTIATION OF HOST AND PARASITE EXOSOME VESICLES USING PHOTONIC CRYSTAL BIOSENSOR

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ABSTRACT

The analysis of membranous extracellular vesicles, such as exosomes vesicles (EV) opens a new direction for the rapid disease diagnosis because EVs can carry molecular constituents of their originating cells [1]. Secreted by mammalian cells, the size of most membrane-bound phospholipid EVs ranges from 50 to 150 nm in diameter. Recent studies have demonstrated the potential of using EVs for cancer diagnosis and treatment monitoring [2-3]. To diagnose infectious diseases using EVs, the ability to discriminate EVs from host cells and parasites is key. Here, we report a rapid EV analysis assay that can discriminate EVs based on a host-specific transmembrane protein (CD63 antigen) using a label-free optical biosensor.

INTRODUCTION

Cancer cells secrete cytokines, chemokines and nucleic acids that have traditionally served as biomarkers for disease diagnosis and prognosis [4]. Profoundly, membranous extracellular vesicles, including nanoscale exosomes and other vesicles actively secreted from cancer cells, have also been found in the blood of cancer patients [5]. Exosome like vesicles (ELV) secreted from the helminth containing effector molecules including functional proteins and small RNAs. These membrane-bound phospholipid nanovesicles ranging from 50 to 150 nm in diameter actively secreted by mammalian cells [6].

Recently, label-free biosensors have been demonstrated for the analysis of exosomes. More recently, detection and molecular profiling of exosome based on surface plasmonic resonance demonstrate by Im *et al* [1]. Lee and Weissleder group at Massachusetts General Hospital have developed a miniaturized nuclear magnetic resonance-based platform (μNMR) to sensitively measure exosomes [7]. Wang *et al.* develop a nanotetrahedrom assisted aptasensor using electrochemical method for exosome detection [8]. In the paper, we detected the exosome vesicles from host and parasite captured on photonic crystal (PC) biosensor. Based on this method, we invent a method can rapid differential exosome vesicles from host and parasite using the host-specific antibody CD-63 based on the label-free optical biosensor. The PC-based biosensor offers the ability to tailor biological purification of exosomes using unique or atypical protein markers that would allow parasite exosomes to be differentiated from host exosomes based on parasite-specific proteins.

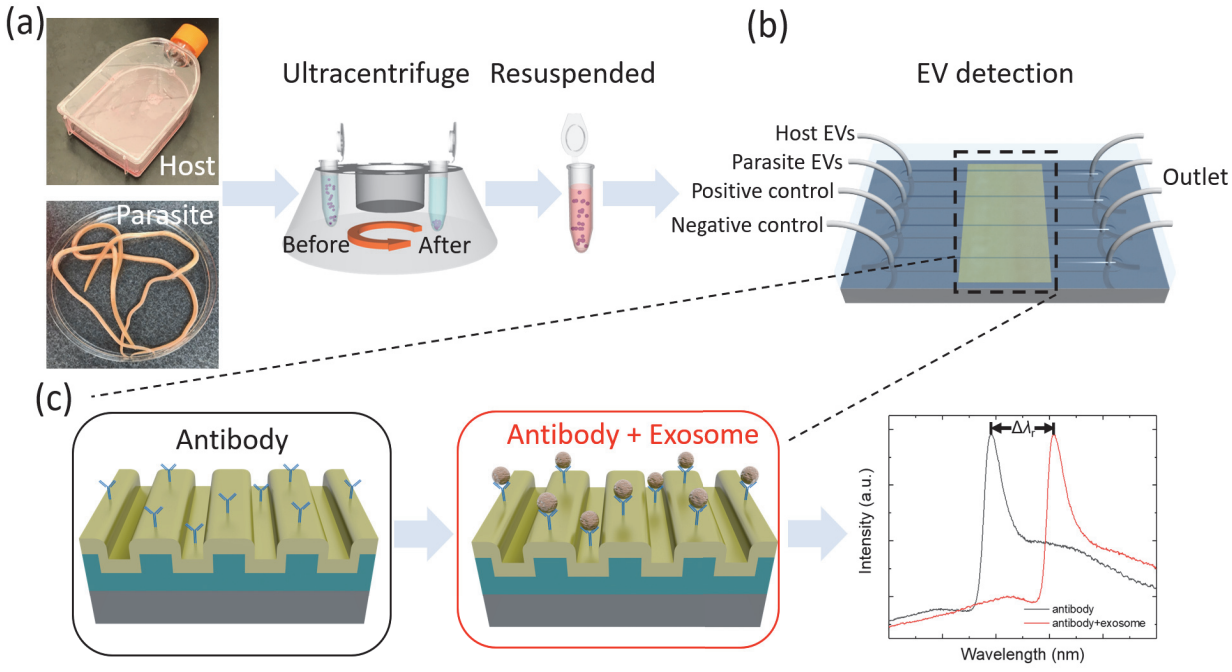

Figure 1: *(a) The process diagram of EV extraction for host and parasite cells. (b) PC biosensor schematic integrated with microfluidic channels. (c) Schematic of the PC biosensor functionalized with the antibody (black box), the exosome vesicles captured on the antibody (red box) illustrated by detecting the resonance wavelength shift Δλ..*

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RESULTS

Preparation of host and parasite EV samples

Female adult *Ascaris suum* was cultured in Ascaris ringer's solution. Murine macrophage cell line (J774) was cultured in DMEM containing 10% FBS, 10000 Units of penicillin, 10000 units of streptomycin, and L-glutamine. Culture media were filtered using 0.22-µm filters to remove the debris and then loaded on ultracentrifuge tube. After centrifuge at 120,000 g/min for 90 min, the EVs were washed by PBS and followed with a 90-min centrifuge at 120,000 g/min. Then the pellet was re-suspended in PBS and transferred into 1.5 ml Beckman ultracentrifuge tube. Then they were spun at 55,000 rpm/min for 2 hours and the pellet was re-suspended in PBS stored in -80 °C. All the centrifuge was processed at 4 °C. The contractions of EVs were measured by a dynamic light scattering method. The EVs extracted from J774 were diluted 100 times with PBS and loaded into the Nanoparticle Tracking Analysis System (NanoSight LM10, Malvern Instruments Inc.). Fig.1(a) shows the process diagram of EV extraction for host and parasite cells.

PC biosensor and readout instrument

The photonic crystal (PC) biosensor, as shown in fig. 1(b), was chosen to detect EVs by monitoring the change of PC reflectance. To facilitate the analysis of EV sample, the PC sensor was incorporated into microfluidics channels (fig. 1(b)). A broadband light (700–900 nm) was used as the excitation and the narrowband reflectance from the PC was recorded using a spectrometer. The spectral shift of the narrowband reflectance was defined as the sensor output. The schematics of the biosensor detection mechanisms is shown in Fig. 1(c), biomolecules (exosome vesicles) interaction on the biosensor surface (red box), was illustrated by detecting the resonance wavelength shift $\Delta\lambda_r$.

Surface functionalization for the PC biosensor

To capture the EVs, the PC sensor surface was functionalized using a four-step protocol. As shown in fig. 2(a), the PC biosensor surface was treated using polyvinyl amine (PVA) and glutaraldehyde (GA). Then, the anti-CD63 capture antibody was deposited on the GA-treated PC sensor at the concentration of at 0.1 mg mL^{-1}. Before performing the detection of EVs, we used bovine serum albumin (BSA) to block the GA-treated surface that was not conjugated with CD63 antibodies. During the label-free experiment, the sensor outputs were measured in real time. Each step of the assay protocol of the resonance wavelength shift was shown in Fig. 2 (b). The resonance wavelength measured and recorded every 2 seconds. Fig. 2(c) shows the resonance wavelength shift $\Delta\lambda_r$ for each step base on assay protocol.

Detection of EVs secreted by Murine macrophage

As a label-free detection method, the biosensor can monitor the analyte-ligand binding process by recording the sensor output in real time. The PC sensor outputs measured for the EV from the host, corresponding positive control, and negative control samples are shown in fig. 3(a). The detection of EVs secreted from the host cells generated an output of ~0.7 nm (black line). The host EVs concentration was 2×10^{11} particles/mL. For the positive control experiment, most host EVs were captured on the GA-treated PC surface and resulted in an output of 2 nm (red line). For the negative control experiment, the surface was coated using the BSA, rather than the anti-CD63 antibody. The sensor output is nearly zero for the negative control experiment (blue line).

Fig. 3(b) shows the time-dependent change of the sensor output when the host EVs flowed through the microfluidic channel. The association rate (k_a) and dissociation rate (k_d) can be calculated by fitting the sensor gram in fig. 3(b) using the Langmuir model. Based on fitting results, the association and dissociation rates are

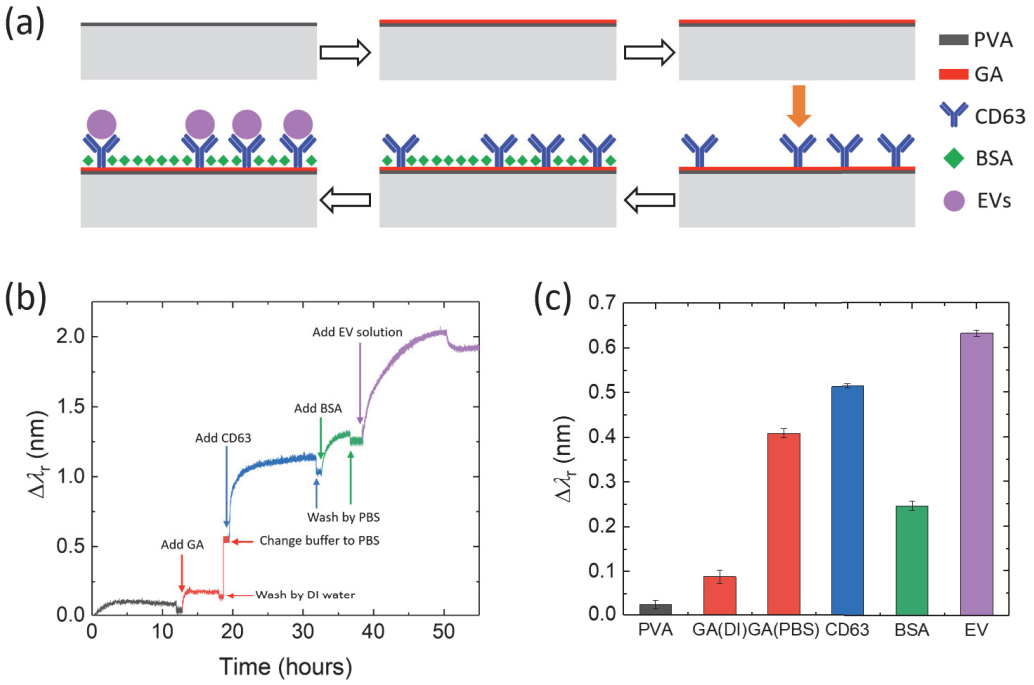

Figure 2: *(a) Schematics of the assay protocol for each step. (b) Measured kinetics binding for each step of the the functionalization assay protocol on PC biosensor surface. (c) Column plot of measured resonance wavelength shift of the assay protocol (PVA, GA with DI water, GA with PBS, antibody CD63, BSA, and EV).*

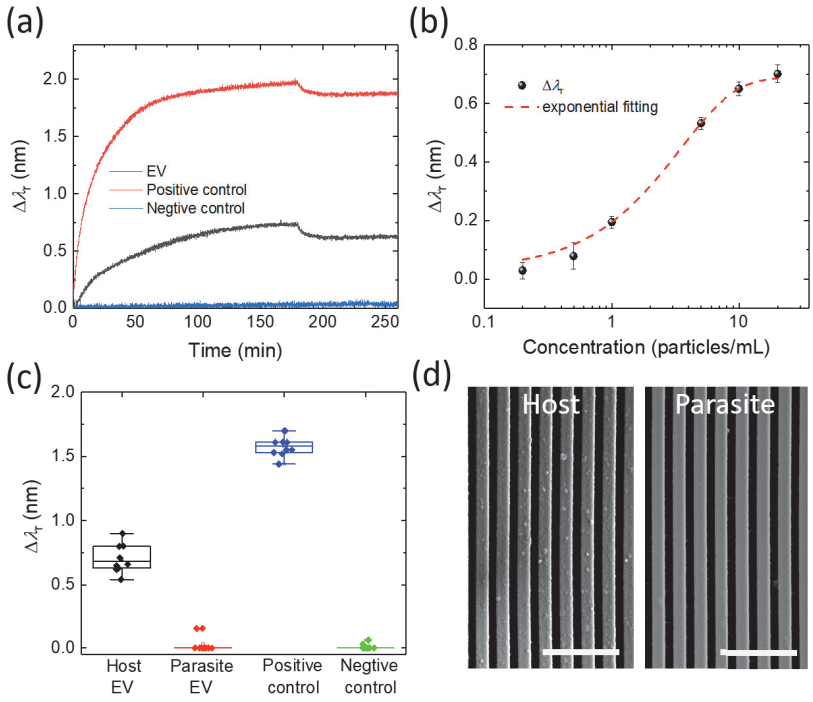

Figure 3: *(a) Kinetic binding of anti-CD63 and host EVs at the concentration of c =2×10^{11} particles/mL. (b) Measured resonance wavelength shifts as a function of 6 different concentrations of exosome vesicles from 2×10^{9} particles/mL to 2×10^{11} particles/mL. The experiment data is fitted (red dash line). (c) Box plot of the resonance wavelength shift for host and parasite exosome (c = 2×10^{11} EVs/mL) detection with the positive control and negative control. (d) SEM image of the host and parasite EVs immobilized on the PC surface at the concentrations of 2×10^{11} particles/mL. Scale bar: 2000 nm.*

k_a=1.6×10^9 particles mL^{-1} s^{-1}, and k_d=0.203 s^{-1}, respectively. The does-response curve was fitted as shown in fig. 4(b).

Differentiation of host and parasite EVs

The box plot in fig. 3(c) shows the resonance wavelength shift for host and parasite exosome detection with the positive control and negative control. Compared to the host EVs, the parasite EVs do not carry CD63 antigens. The detection of the parasite EVs using the anti-CD63 antibody coated PC sensor generated a nearly zero output. For the positive control experiment, the EVs were captured regardless of the type of transmembrane proteins. The positive control experiment shows a signal of 1.3 nm shift. The results in fig. 3(c) demonstrate the capability of discriminating EVs from host cells and parasites. The label-free assay can be utilized to diagnose infectious disease using EVs. Fig. 3(d) shows the scanning electron microscope (SEM) image of the host EVs and parasite EVs immobilized on the PC surface at the concentrations of 2×10^{11} particles/mL. The average size of the EVs is approximately 100 nm. Based on the label-free biosensor, we can rapid differentiation of exosome vesicles from host and parasite in 1 hours without any label.

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