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Development of an aptasensor for electrochemical detection of exosomes

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ABSTRACT

Exosomes are small (50–100 nm in diameter) vesicles secreted from various mammalian cells. Exosomes have been correlated with tumor antigens and anti-tumor immune responses and may represent cancer biomarkers. Herein, we report on the development of an aptamer-based electrochemical biosensor for quantitative detection of exosomes. Aptamers specific to exosome transmembrane protein CD63 were immobilized onto gold electrode surfaces and incorporated into a microfluidic system. Probing strands pre-labeled with redox moieties were hybridized onto aptamer molecules anchored on the electrode surface. In the presence of exosomes these beacons released probing strands with redox reporters causing electrochemical signal to decrease. These biosensors could be used to detect as few as 1×10^6 particles/mL of exosomes, which represents 100-fold decrease in the limit of detection compared to commercial immunoassays relying on anti-CD63 antibodies. Given the importance of exosome-mediated signal transmission among cells, our study may represent an important step towards development of a simple biosensor that detects exosomes without washing or labeling steps in complex media.

1. Introduction

Membrane vesicle trafficking is carried out via the exosomes, which are small (50–100 nm in diameter) vesicles secreted from various mammalian cells [1,2]. The biogenesis of exosomes starts from the inward budding of endosome membranes, which generates multivesicular bodies (MVBs) and enclose exosomes inside. The MVBs then fuse with the cellular plasma membrane, resulting in the release and circulation of exosomes in various biofluids [3]. In recent decades, exosome shedding has been correlated with tumor antigens and anti-tumor immune response [4–7], and may have value for cancer diagnostics [8]. Moreover, exosomes carry molecular information of the parent cells, which offers a facile approach to observe and analyze the parental tumor cells without the need for biopsy [4].

Exosomes carry various membrane proteins, which are involved in membrane transport and fusion process, including heat shock proteins (HSPs), integrins, and tetraspanins (CD63, CD81 and CD82) [2]. Cell-surface proteins belonging to tetraspanin family typically contain four hydrophobic domains and are known to form complexes with integrins. CD63, a member of the tetraspanin family and a type III lysosomal membrane protein, is considered a classic marker for exosomes [9,10].

The essential first step of current exosome analysis is purification by ultracentrifugation [11,12]. Afterwards, exosomes may be analyzed using western blot [13], enzyme-linked immunosorbent assay [14] or flow cytometry [15]. Though robust and effective, these analytical methods are expensive, time-consuming and rely heavily on the sample handling skills. Recently, Im et al. reported a label-free exosome assay utilizing transmission surface plasmon resonance (SPR) through nanohole arrays functionalized with antibodies specific to exosome surface proteins [16]. Zhu et al. reported a mass-sensitive sensor which took advantage of the fact that exosomes are significantly larger than soluble proteins, thus are distinguishable from the biofluids in surface plasmon resonance imaging (SPRi) [17]. The goal of our study was to demonstrate the development of an aptamer-based biosensor for exosome detection.

In recent years, aptamers have emerged as an excellent alternative to antibodies as biorecognition elements in affinity biosensors [18–20]. Aptamers may be easily engineered to emit signal directly as a function of analyte binding, thus eliminating multiple washing steps associated with typical antibody-based assays. Furthermore, aptamers may be more chemically stable than antibodies and may

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be synthesized in animal- or cell-free manner. Our lab has a long-standing interest in developing aptamer-based biosensors and has incorporated aptasensors into microfluidic devices to construct miniaturized sensors for the detection of a variety of cell secreted molecules including interferon-γ (IFN-γ) [21,22], tumor necrosis factor-α (TNF-α) [23] and transforming growth factor-β1 (TGF-β1) [24].

In the present paper, we sought to develop an aptasensor with specificity to CD63 – a transmembrane protein commonly present in exosomes. We determined region of the aptamer responsible for binding to CD63 and designed an antisense strand masking this region. As shown in Fig. 1A, interaction of the aptamer-modified electrode with exosomes carrying CD63 resulted in displacement of the antisense strand and caused redox signal to decrease. Aptamer-functionalized electrodes were miniaturized by photolithography and integrated into microfluidic devices to show exosome detection from a small sample volume. Unlike antibody-based immunoassays, the aptasensor described here did not require handling or processing steps to generate the signal. In addition, to being much simpler than standard immunoassay our aptasensor was much more sensitive, with 100 times lower detection limit compared to commercial CD 63 antibody-based immunoassay.

2. Materials and methods

2.1. Chemicals and reagents

Poly(dimethylsilosane) (PDMS) and silicone elastomer curing agent were purchased from Dow Corning (Midland, MI). Positive photoresist (S1813) and developer solution (MF-319) were purchased from Shipley (Marlborough, MA). Chromium (CR-4S) and gold etchants (Au-5) were bought from Cyantek Corporation (Fremont, CA). 1× phosphate-buffered saline (PBS) without calcium and magnesium, dimethylformamide (DMF), 6-mercapto-1-hexanol (MCH), triton-X 100, bovine serum albumin (BSA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), sodium bicarbonate ($NaHCO_3$), collagen (Type I) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were bought from Sigma–Aldrich, USA. Methylene Blue (MB), carboxylic acid and succinimidyl ester (MB-NHS) were from Biosearch Technologies, Inc. (Novato, CA).

The 32 base pair CD63 Aptamer is developed by Base Pair Biotechnologies (cat. #ATW0056, Base Pair Biotechnologies, Pearland, TX), and Thiolated CD63 DNA aptamer with amine modification was synthesized in Integrated DNA Technologies (Coralville, Iowa). It has a loop structure with amine group at 5′ and thiol functionality at the 3′ end. The sequence of the aptamer is: 5′ 5AmMC6/CACCCCACCTCGCTCCCGTGACACTAATGCTA/iSpC3//3ThioMC3-D-3′. The stock solution (100 μM) was made by dissolving solid-state aptamer in 1× TE buffer, and then diluted to desired working concentration by HEPES buffer prior to use. The three probe strands are labeled with amine groups at the 5′ end. And the sequences of the three probe strands are as below:

5′-/5AmMC6/AGGTGGGGTG-3′ (Probe 1)
5′-/5AmMC6/CACGGGAGCG-3′ (Probe 2)
5′-/5AmMC6/GCATTAGTGT-3′ (Probe 3)

2.2. Surface plasmon resonance analysis of aptamer assembly and CD63 binding

The surface plasmon resonance (SPR) experiments were performed employing a two-channel SPR instrument from BI (Biosensing Instrument, AZ). All experiments were performed in HEPES buffer (10 mM HEPES, 150 mM NaCl) on bare gold chips purchased from BI, and flow rate was maintained constant at 20 μL min^{-1}. To create a non-fouling surface, a self-assembling monolayer (SAM) composed of polyethylene glycol carboxyl-terminated thiol (Thiol-PEG-COOH) was firstly constructed on top of the gold film, followed by 3 mM mercapto hexanol (MCH) to block the gold surface to prevent nonspecific absorption [25,26]. Then 0.1 M N-hydrosuccinimide (NHS) and 0.4 M N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide (EDC) were injected to activate the

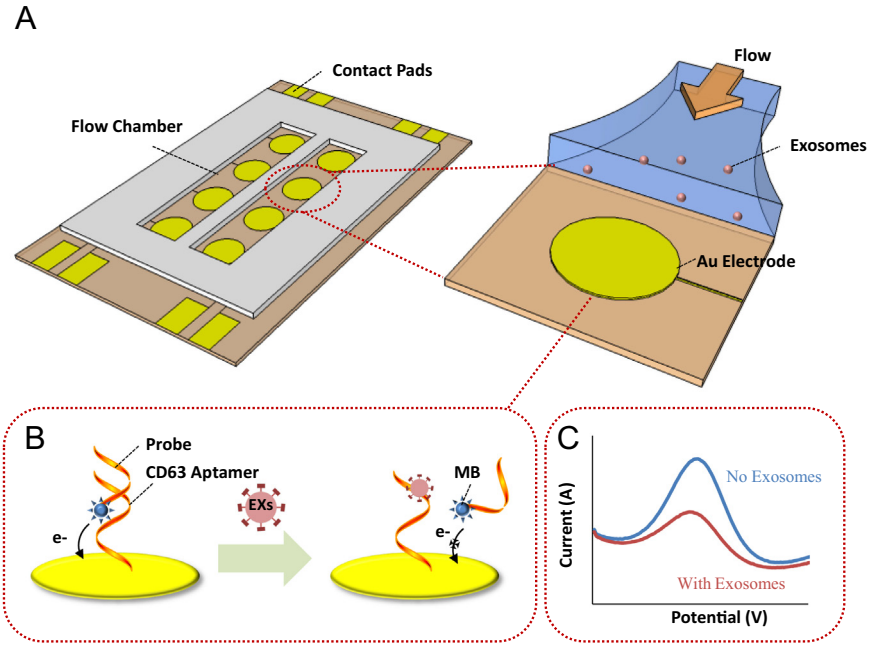

Fig. 1. Schematic illustration of aptamer-based exosome detection, (A) The device is composed of an Au electrode array patterned on a glass surface, along with a PDMS layer, which defines the flow chamber. Aptamers specific for CD63 were immobilized onto the Au electrodes prior to use. (B and C) MB-labeled probing strands hybridize with the aptamers anchored on the surface and emit an electrochemical signal (blue curve in Fig. C). Exosomes interact with DNA duplexes via CD63 proteins, displacing the antisense strand and causing electrochemical signal to decrease (C). The change in redox signal is proportional to the concentration of exosomes in solution.