A New Route for Exosome Detection Using All-Dielectric Metasurfaces

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Abstract:

Exosomes are extracellular vesicles (30-150 nm in diameter) that play critical roles in cellular processes such as immune modulation, signal transduction, and antigen response. Due to their ability to reflect the physiological state of their origin, exosomes hold promise as non-invasive biomarkers for disease diagnosis and prognosis. To harness this potential, exosomes must first be effectively isolated and detected. In this study, we present an advanced metasurface fluorescence (FL) biosensor, engineered with an all-dielectric metasurface substrate coupled to a six-channel microfluidic chip, designed for the high-sensitivity detection of exosomes. Our approach involved targeting both the exosomal surface marker CD63 and internal exosome proteins through fluorescence-tagged antibodies, specifically anti-CD63 and anti-EXOSC5. As a preliminary result, the developed sensor exhibited a remarkable limit of detection of 9.64 attomolar, corresponding to 5.804×10^3 exosomes/mL, positioning it among the most sensitive biosensors reported to date for exosome detection. These findings highlight the significant potential of this metasurface sensor to enhance the precision and efficacy of exosome-based diagnostics.

Summary of Research:

To detect extracellular exosomes, a metasurface fluorescence biosensor was constructed from an all-dielectric metasurface substrate and a six-channel microfluidic (MF) chip synthesized from transparent poly-dimethylsiloxane (PDMS). On the substrate are six sensors. Using nanolithography, the metasurface sensors were designed to be a periodic array of silicon nanopellets of diameter of 220 nm forming a square lattice. These nanopellets have strongly localized electric fields at the outermost surface and have localized strong magnetic fields inside allowing for a significant increase in detection sensitivity. The PDMS microfluidic chip is placed on top of the metasurface substrate and temporarily fuses together through adhesion.

The biosensor is placed in the microfluidic configuration for fluorescent imaging. It is placed on a microscope stage and tubes are connected to it allowing for fluids to run through the channels, over the sensors and out into a waste beaker. The movement of the fluids is powered by a rotary pump and flow of the fluids will be stopped when pictures are needed to be taken.

Experiment 1: Targeting CD63:

The first experiment centered on detecting CD63, a well-known transmembrane marker used for identifying exosomes. CD63 plays critical roles in cell-matrix adhesion, migration, and immune regulation, making it an essential target for exosome identification. In this experiment, the fluorophore HL555 was conjugated to an anti-CD63 antibody using the Hilyte Fluor 555 labeling kit, forming the detection antibody. Concurrently, biotin-labeled anti-CD63 antibodies were used as the capture mechanism. Human exosome standards were diluted into a range of concentrations (from 10^4 to 10^8 exosomes/mL) and incubated with both the HL555-labeled and biotin-labeled anti-CD63 antibodies, synthesizing the exosome complex.

The MF protocol follows a pattern of flowing necessary proteins or exosomes through the channels followed by phosphate-buffered saline (PBS) rinse, allowing the molecules time to adhere to the sensor and rid of any that are unbound. Cystatin-SA protein flowed through the channels to create a binding site for the biotin-labeled-anti-CD63 antibodies.

After immobilizing the exosomes on the cystatin-SA-coated nanopellets, the green excitation light is turned on and fluorescence images are taken of each channel. ImageJ software was used to measure net fluorescence intensity across the channels, providing a quantitative evaluation of exosome binding. Data analysis using the Hill equation revealed a negative correlation between fluorescence intensity and exosome concentration and a value less than one for the degree of cooperative reaction, suggesting anti-cooperative binding behavior between the antibodies and the target exosomes.

Experiment 2: Targeting Inner Exosome Proteins Using anti-EXOSC5:

The second experiment took a different approach by targeting the interior of exosomes through the protein EXOSC5, an exosome component involved in RNA degradation and processing. Studies have shown that EXOSC5 has similar properties to protein found within the extracellular exosomes, enough so, that anti-EXOSC5 can be used to target them. The same HL555 fluorophore conjugation procedure was employed to label the anti-EXOSC5 antibody for detection, while anti-CD63-biotin continued to serve as the capture mechanism.

In this experiment, exosome samples were prepared at concentrations ranging from 10^3 to 10^7 exosomes/mL. The samples were incubated with anti-CD63-biotin at room temperature to facilitate initial binding, followed by a second incubation with anti-EXOSC5-HL555 at 37°C (body temperature). Before the second incubation, anti-EXOSC5-HL555 was diluted in 0.1% PBS-T, which softens and partially cleaves the exosome membrane, allowing the labeled antibody to penetrate the vesicle. Once the exosome-antibody complexes were synthesized, they were introduced into the microfluidic system following a similarly modified MF protocol.

Fluorescence intensity measurements were once again obtained using ImageJ, and the results revealed a stronger positive correlation between fluorescence intensity and exosome concentration than observed in the first experiment. The Hill equation was applied to evaluate the binding interactions, showing a cooperative reaction with a degree of cooperative binding of 73.1425. The limit of detection (LOD) was determined to be 9.64 attomolar, equivalent to 5.804×10^3 exosomes/mL. This LOD is among the lowest reported for exosome biosensors, demonstrating the effectiveness of targeting internal exosome proteins in enhancing detection sensitivity.

These findings illustrate the superior sensitivity of the metasurface biosensor, particularly when targeting internal exosome proteins, and underscore its potential application in diagnostic platforms.

Conclusions and Future Steps:

This research highlights the efficacy of an all-dielectric metasurface biosensor in detecting extracellular exosomes, leveraging both external and internal protein markers. In the first experiment, targeting the transmembrane protein CD63 resulted in anti-cooperative binding and lower-than-anticipated fluorescence intensity, revealing the complexities of surface protein detection. However, the second experiment, focusing on internal proteins with anti-EXOSC5, demonstrated significantly improved outcomes, characterized by a strong cooperative binding response and an exceptionally low limit of detection. These findings underscore the potential of this biosensor technology in advancing diagnostic applications, particularly in diseases where exosome detection plays a pivotal role.

Future investigations should concentrate on refining both the binding and microfluidic protocols to enhance the efficiency and sensitivity of exosome detection. This includes optimizing antibody selection and incubation conditions to improve binding affinity and reduce variability. Additionally, expanding the biosensor's capabilities through multiplexing to simultaneously target multiple exosome markers could provide even greater diagnostic specificity. The results from this research affirm the rich potential of exosomes as next-generation biomarkers, offering significant promise for improving disease diagnosis and monitoring.

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