Quantum dots Enhanced IMPACT Chip for Viral Nucleic Acid Detection

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During the past two decades, different types of viruses have been identified. Some of which are highly contagious and have a significant negative impact on human lives. Due to the fact that it takes a long time to produce effective vaccines and they are typically unavailable at the onset of pandemics, identifying infected patients quickly is the only method available to prevent further transmission among the general population. Traditional detection strategy relies on quantitative polymerase chain reaction (qPCR), which amplifies low copies of nucleic acid targets and measures the generated fluorescence intensities. The entire procedure requires a thermocycling machine and a detector. Recent advancements in CRISPR-Cas technology offer an alternative detection method requiring fewer electrical driven components. Originating in bacteria, it is widely employed in in-vitro diagnostics. Compared to qPCR, the CRISPR-Cas method is simple and can be performed in isothermal conditions. Traditional CRISPR-Cas detection utilizes Cas12a or Cas13 enzyme to identify target nucleic acids and then cleaves non-target single-stranded probes to amplify detectable signals. We previously utilized a microfluidics chip with a high-aspect-ratio micropillar to enhance CRISPR detection. The single-stranded probes are pre-immobilized on the surface; consequently, the reaction has lower background signals[1].

To eliminate end-point signal measurement and achieve visualized readout, we coupled quantum dots (Qdots), single-stranded DNA, and gold nanoparticles (AuNPs) to form a Förster resonance energy transfer (FRET) assay in this work. We previously used an organic quencher to quench the fluorescence of Qdots, but the quenching efficiency was quite limited and endpoint measurements were still necessary. Now, we switched from organic quenchers to AuNPs in an effort to obtain straightforward visual readouts. Figure 1 illustrates the detection process. Prior to detection, the chip surface was chemically treated and functionalized with Qdots and DNA probes. Next, the CRISPR-Cas12a reaction product and AuNPs were introduced sequentially into the microchannel. Without the target, the AuNPs are closely conjugated to Qdots via the DNA probes in between, thereby quenching their fluorescence. Alternatively, with the presence of the target, AuNPs are free to float in the microchannel allowing Qdots to maintain high fluorescence signals. Figure 2a are the SEM images of the

[1] K. N. Hass, M. Bao, Q. He, L. Liu, J. He, M. Park, P. Qin, and K. Du, *Integrated Micropillar Polydimethylsiloxane Accurate CRISPR Detection System for Viral DNA Sensing*, ACS Omega **5**, 27433 (2020).

micropillars. The purpose of designing micropillars with a taper angle is to improve the imaging process. Figure 2b shows the quenching effect of AuNPs on Qdots. The fluorescence intensities of Qdots decreased significantly with the addition of AnNPs to the solution, indicating that AnNPs are the ideal quencher for Qdots and laying the foundation for our assay design.

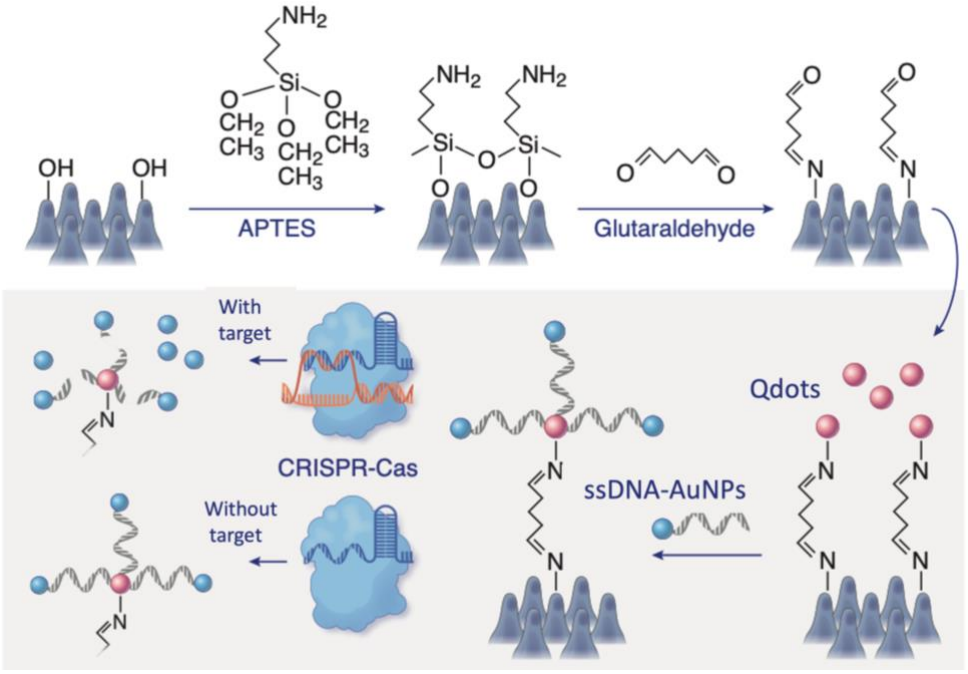

Figure 1: Schematic workflow of the FRET and CRISPR-Cas12a-based detection.

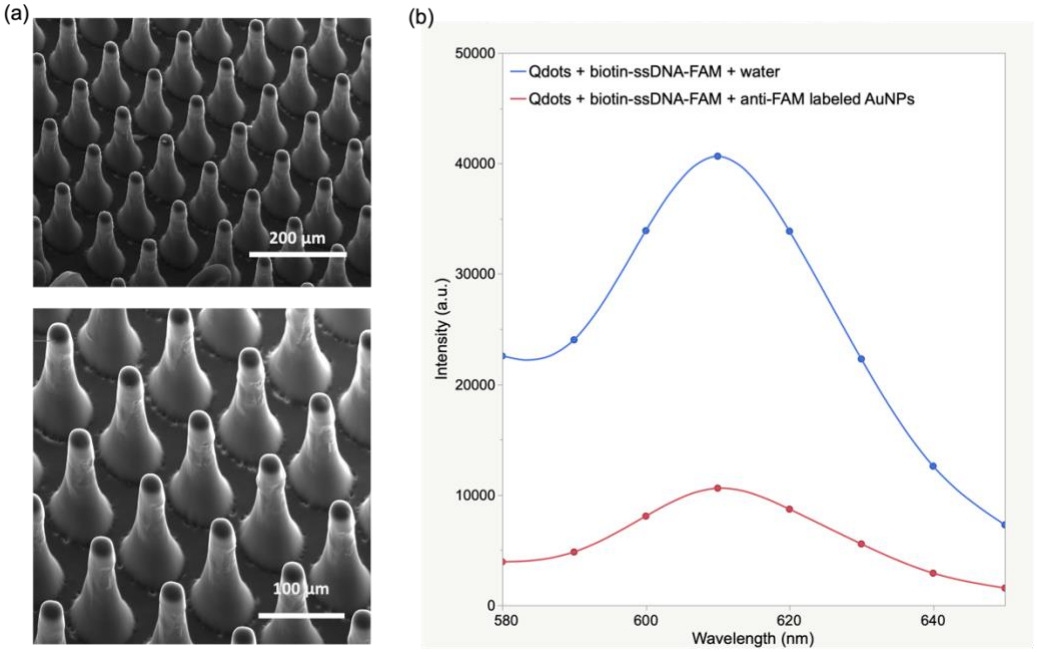

Figure 2: (a) SEM image of micropillars. Top: scale bar is 200 µm. Bottom: scale bar is 100 µm. (b) Fluorescent intensity of Qdots with and without AuNPs conjugation.