Quantum dots Enhanced IMPACT Chip for Simple Pathogen Detection

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In recent years, the integration of high-aspect-ratio microstructures in diagnostic devices has been demonstrated to improve the overall detection performance. We previously developed a micropillars patterned microfluidic system and showed that the extended solid surface area can enhance the DNA probe binding[1]. Combining with a CRISPR-Cas12a assay, we show that this Integrated Micropillar Polydimethylsiloxane Accurate CRISPR deTection (IMPACT) chip can provide sensitive and low-background viral DNA sensing. In this work, we introduce an improved version of the IMPACT chip to further enhance the molecular binding capacity. Instead of using standard photolithography, laser micromachine was used to create microstructures with much higher aspect-to-ratio. To enable naked-eye readouts, the device was coupled with a Förster resonance energy transfer (FRET) assay and super-bright quantum dots (Qdots) as an indicator. Figure 1 depicts the detection process of the new IMPACT chip. Prior to the detection, the Polydimethylsiloxane (PDMS) surface of the microchannel was chemically treated and functionalized with Qdots. Next, the CRISPR-Cas12a reaction product was introduced into the microchannel. With the presence of the target, degraded probes cannot be conjugated on the chip, without interfering the bright fluorescence signal from the Qdots. On the other hand, without the target, the quenchers are captured onto the chip thus quenching the Qdots. Figure 2a shows the photograph of the chip, patterned with high-aspect ratio micropillars. Each micropillar has a diameter of 25 μm and a spacing of 122 μm between them (Figure 2b). To immobilize Qdots onto the surface of PDMS micropillars, we sequentially introduced (3- Aminopropyl)triethoxysilane, Glutaraldehyde, and streptavidin coated Qdots into the channel. As shown in Figure 2c, PDMS micropillars demonstrate a clear pink color, whereas the surrounding planar surface near the inlet and outlet has no color. A fluorescence image of the channel was taken to validate the visual readout, which shows clear circle spots with strong signals (Figure 2d).

[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).

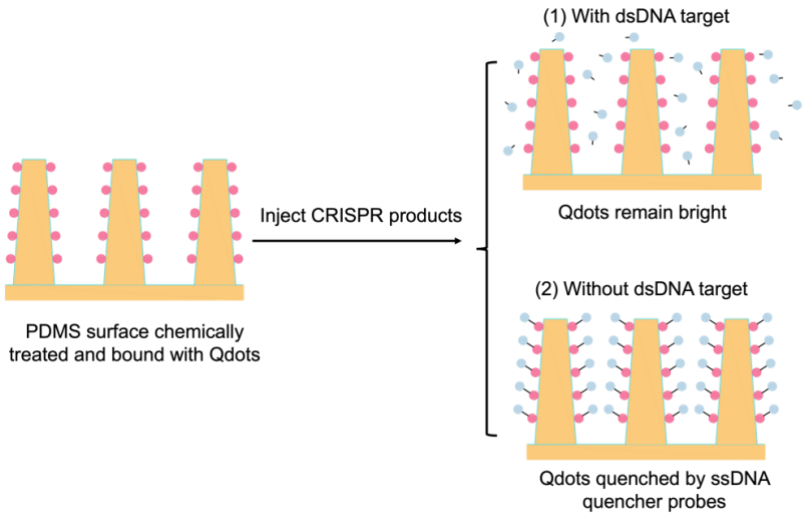

Figure 1: Schematic workflow of the CRISPR-Cas12a based detection. Prior to the detection, PDMS surface is chemically treated and functionalized with Qdots. With the presence of the dsDNA target, the CRISPR complex cleaves the quencher probes, thereby allowing Qdots to remain bright. On the other hand, in the absence of the target, quencher probes are conjugated to the Qdots and quench their fluorescence signals.

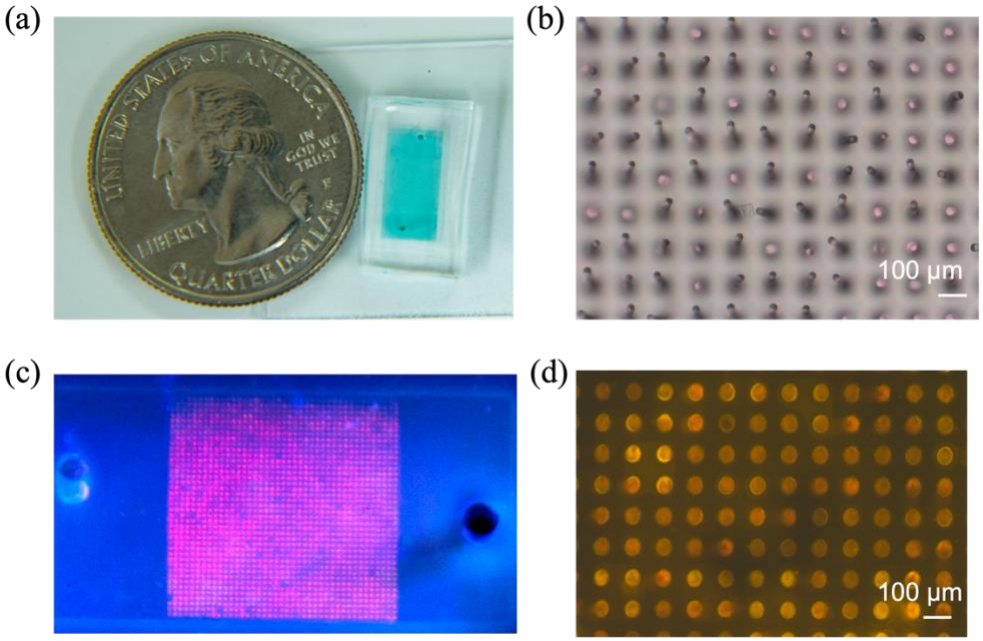

Figure 2: (a) Photograph of the IMPACT chip with food dye flowing through. (b) Microscope image of the micropillars (scale bar: 100 µm). (c) Photograph of the chip with chemical treatment and quantum dots binding. (d) Fluorescent image of the chip with the quantum dots conjugation (scale bar: 100 µm).