Microspheres Enhanced IMPACT Chip for Simple and Instrument-Free CRISPR Detection

M. Bao[1], K. Hass[1], Y. Chang[1], M. Park[2], K. Du[1, 3]

[1]*Department of Mechanical Engineering, Rochester Institute of Technology, Rochester, NY 14623, USA*
[2]*Department of Chemistry, Dong-A University, Busan, 49315, Korea*
[3]*Department of Microsystems Engineering, Rochester Institute of Technology, Rochester, NY 14623, USA*
kxdeme@rit.edu

Implementing molecular diagnosis with a simple and portable device is essential for point-of-care applications, particularly during disease outbreaks. In this work, we further improve the Integrated Micropillar Polydimethylsiloxane Accurate CRISPR detection (IMPACT) system[1] developed by us into a simple platform for naked-eye fluorescence detection. Rather than using the conventional organic dyes, the microchannel patterned with high-aspect-ratio micropillars is loaded with fluorescent microspheres with a strong fluorescence emission. This new system is coupled with CRISPR-Cas12a assay for target specific detection by hybridizing the target DNA with CRISPR complex. A Förster resonance energy transfer (FRET) assay is demonstrated by quenching the microspheres with single-stranded quencher probes. After target recognition and CRISPR-Cas12a medicated cleavage, the fluorescence signal of the microspheres is released and easily identified under a blue light excitation.

To fabricate the IMPACT chip, standard photolithography is used to create the microstructures on a 100 mm silicon wafer, followed by Polydimethylsiloxane (PDMS) molding via a standard soft lithography process. After oxygen plasma treatment, the PDMS replica is bonded with a glass slide to form a fully closed channel. Fig. 1 shows the process of channel surface modification and naked-eye nucleic acid detection. The PDMS surface of the microchannel is sequentially treated with (3- Aminopropyl)triethoxysilane (APTES), Glutaraldehyde, and streptavidin coated microspheres. Next, biotinylated probes linked with an organic quencher are coated on the microspheres to block the fluorescence signal. To perform CRISPR-Cas12a target recognition, Cas12a-crRNA complex is mixed with viral DNA target and injected into the microchannel. After incubation, the channel is washed to remove the released quenchers. Finally, the IMPACT channel is illuminated with a portable flashlight. We immobilized streptavidin magnetic microspheres with different concentrations onto the microchannel to demonstrate this novel platform and detection assay (Fig. 2).

---

[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).

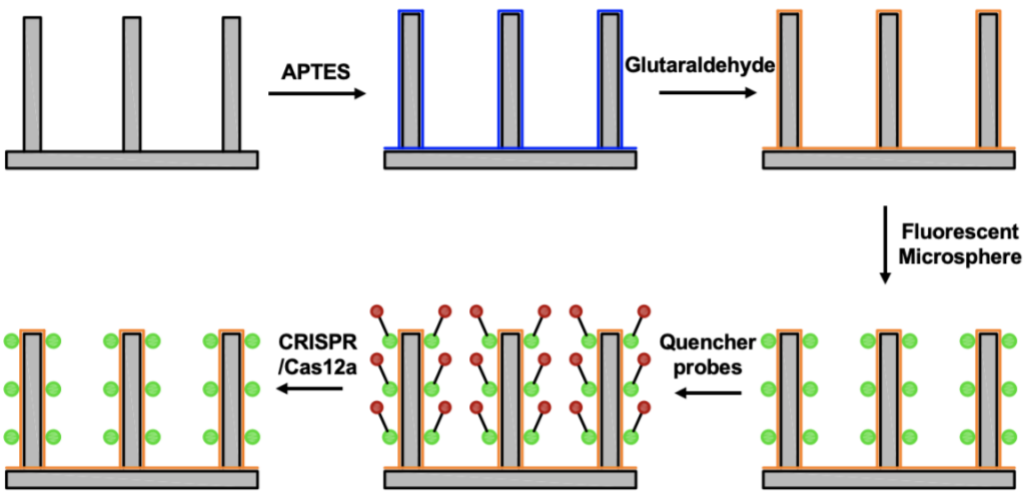

Figure 1: Schematic workflow of surface modification and the CRISPR-Cas12a based detection. PDMS surface is treated with APTES and glutaraldehyde for streptavidin-microsphere immobilization. Single-stranded DNA with quencher and biotin labels is conjugated on the microsphere coated surface. With the presence of the viral DNA, CRISPR complex cleaves the quencher probes and recovers the fluorescence signal.

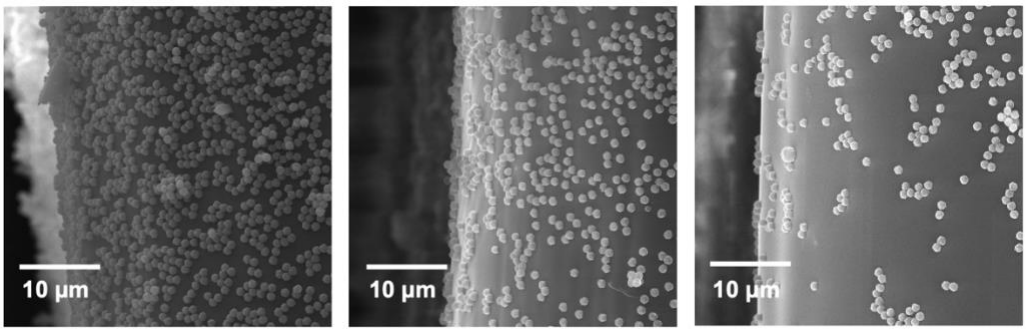

Figure 2: Scanning electron microscope images of streptavidin microspheres coated IMPACT chip with a concentration of: (i) 108 µg/µl; (ii) 36 µg/µl; (iii) 4 µg/µl streptavidin microspheres. Scale bars are 10 µm.