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Acoustofluidic Focusing for High-Throughput and Sensitive Analysis of Shape-Encoded Hydrogel Microparticles in Multiplex Immunoassay

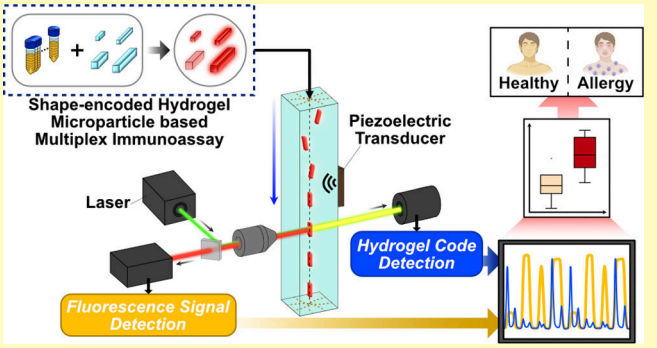
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ABSTRACT: Shape-encoded hydrogel microparticles (SHMPs) offer robust sensitivity and multiplexing capacity in immunoassays. However, their analysis has conventionally required labor-intensive imaging-based methods. Although flow-through analysis has been explored as an alternative approach, its throughput and sensitivity remain limited. In this study, we developed a high-throughput and highly sensitive multiplex immunoassay platform by integrating SHMPs with acoustofluidic focusing. Acoustofluidic focusing is a powerful technique that enables high-throughput analysis owing to its capability to densely align micro-objects. Since hydrogel materials are challenging to accurately align by acoustofluidic focusing, we optimized the flow rate as well as the amplitude and frequency of acoustic standing waves to achieve precise and dense particle alignment. The precise alignment of SHMPs in microchannels allowed reliable code recognition and fluorescence signal detection. Also, densely aligned SHMPs offered an 8-fold higher analysis throughput than previously studied flow-through methods, even at low flow rates. The synergistic effects of sufficient dwell time provided by acoustofluidic focusing at low flow rates and the high sensitivity of SHMPs achieved a 61.5-fold enhancement in the detection limit compared to commercial devices using flow-through analysis. The feasibility of multiplex detection was further validated through spike-in experiments, yielding recovery rates within 100 ± 20% for all four protein targets. Finally, we assayed cytokines in clinical samples from drug allergy and healthy groups and successfully identified significant differences in the cytokine levels.



KEYWORDS: *acoustofluidic focusing, shape-encoding, hydrogel microparticles, multiplex immunoassay, cytokine profiling*

Proteins are essential biomolecules that constitute our bodies and perform diverse physiological functions. In particular, they regulate cellular metabolism and play critical roles in activating immune cells and modulating immune responses through intercellular signaling.[1] Consequently, fluctuations in protein concentrations within the body are closely associated with disease onset and progression, placing them as pivotal biomarkers for diagnosing various medical conditions and monitoring therapeutic outcomes.[2,3] However, the analysis of a single target protein is often insufficient for accurate diagnosis since one protein may be involved in several immune responses.[4] For example, while interleukin (IL)−4 plays a key role in allergic responses, it also engages in anti-tumor activity.[5,6] Moreover, as numerous proteins such as interleukins often exist in very low concentrations in bodily fluids, assay sensitivity is crucial when dealing with clinical samples.[7] Thus, multiplex detection methods which simultaneously quantify multiple targets with high sensitivity are essential for precise immune assessment and clinical decision-making.

Enzyme-linked immunosorbent assay (ELISA) has been widely used for protein quantification. However, ELISA suffers from its single-analyte detection capability and relatively low sensitivity.[8] To overcome these limitations, several technologies have been developed, including protein microarrays and bead-based assays. Microarrays enable simultaneous detection of up to 10^4 distinct proteins, offering high-throughput multiplexing capabilities. However, their two-dimensional reaction format limits the amount of immobilized capture antibodies, which can reduce the detection sensitivity.[9] Compared to microarrays, bead-based assays are relatively more sensitive, as the suspended beads

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