Integrated Nanoplasmonic Sensing for Cellular Functional Immunoanalysis Using Human Blood

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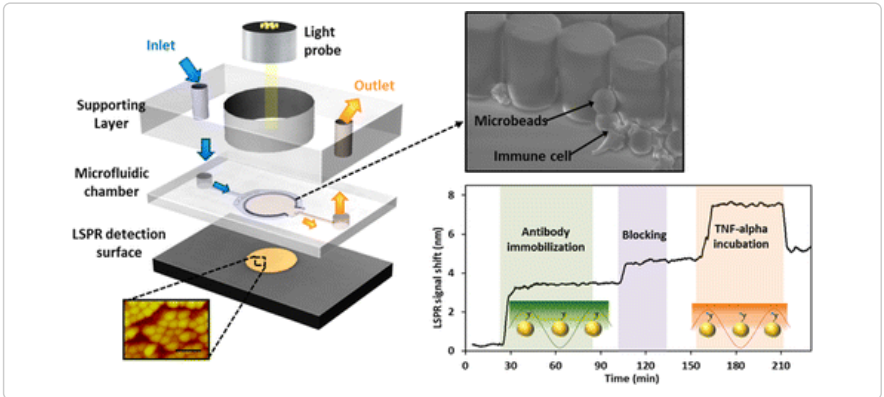

Localized surface plasmon resonance (LSPR) nanoplasmonic effects allow for label-free, real-time detection of biomolecule binding events on a nanostructured metallic surface with simple optics and sensing tunability. Despite numerous reports on LSPR bionanosensing in the past,

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no study thus far has applied the technique for a cytokine secretion assay using clinically relevant immune cells from human blood. Cytokine secretion assays, a technique to quantify intercellular-signaling proteins secreted by blood immune cells, allow determination of the functional response of the donor's immune cells, thus providing valuable information about the immune status of the donor. However, implementation of LSPR bionanosensing in cellular functional immunoanalysis based on a cytokine secretion assay poses major challenges primarily owing to its limited sensitivity and a lack of sufficient sample handling capability. In this paper, we have developed a label-free LSPR biosensing technique to detect cell-secreted tumor necrosis factor (TNF)-α cytokines in clinical blood samples. Our approach integrates LSPR bionanosensors in an optofluidic platform that permits trapping and stimulation of target immune cells in a microfluidic chamber with optical access for subsequent cytokine detection. The on-chip spatial confinement of the cells is the key to rapidly increasing a cytokine concentration high enough for detection by the LSPR setup, thereby allowing the assay time and sample volume to be significantly reduced. We have successfully applied this approach first to THP-1 cells and then later to CD45 cells isolated directly from human blood. Our LSPR optofluidics device allows for detection of TNF-α secreted from cells as few as 1000, which translates into a nearly 100 times decrease in sample volume than conventional cytokine secretion assay techniques require. We achieved cellular functional immunoanalysis with a minimal blood sample volume (3 μL) and a total assay time 3 times shorter than that of the conventional enzyme-linked immunosorbent assay (ELISA).

Keywords: localized surface plasmon resonance (LSPR); **nanoplasmonic sensing**; **optofluidics**; **cellular immunoanalysis**; **tumor necrosis factor alpha (TNF-α)**

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