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Summary: we designed a miniature IFN-gamma aptasensor that was integrated into a microfluidic device and could be used to monitor in real-time cytokine secretion from human leukocytes captured in the device.

We report on the development of a microdevice for detecting interferon gamma (IFN-γ) release from primary human leukocytes. IFN-γ is an important inflammatory cytokine that provides a measure of the cell immune response to pathogens. The novelty of our microdevice lies in the use of miniature aptamer-modified electrodes integrated with microfluidics to monitor cellular production of IFN-γ. Aptamer recognition layer consisted of DNA hairpin molecules with thiol groups on the 3' end for self-assembly on gold electrodes and redox reporters (methylene blue (MB)) on the 5' end for electrochemical sensing. Binding of cytokine molecules resulted in conformational change of the aptamer hairpin causing the change in electrical signal measured at the gold electrode.

![Image](https://example.com/image.png)

Fig. 1. a) A microdevice consisted of aptamer-modified electrode arrays imbedded into a micropatterned poly(ethylene glycol) (PEG) so as to define cell attachment sites in the vicinity of each electrode. These attachment sites were modified with leukocyte-specific Abs (anti-CD45 or anti-CD4) to promote cell binding. Micropatterned glass substrates were then integrated with PDMS-based microfluidic channels and incubated with RBC-depleted human blood. Cells attached next to sensing electrodes inside the microfluidic channels and were mitogenically stimulated to induce cytokine production. IFN-γ released by cells was detected at the sensing electrodes using SWV measurements as early as 15 min post stimulation. b) Characterization of sensor response to IFN-γ. Voltammograms obtained with SWV showed that decrease in faradaic current was proportional to solution concentration of IFN-γ.

Binding of cytokine molecules resulted in conformational change of the aptamer hairpin causing the change in electrical signal. This aptasensor had excellent specificity and sensitivity for IFN-γ (60 pM detection limit), and responded to target analyte without additional washing or labeling steps. For cell sensing, electrode arrays on glass slides were packaged in poly(ethylene glycol) (PEG) hydrogel such as to expose glass regions adjacent to electrodes while protecting remainder of the surface with this non-fouling material.

These micropatterned substrates were integrated with PDMS microfluidic channels and incubated with leukocyte-specific antibodies (Abs) (anti-CD4 or anti-CD45) creating cell capture sites in the immediate vicinity of cytokine-sensing gold electrodes. Upon injection of blood, leukocytes became bound on Ab-modified glass regions next to aptasensors. Cytokine release form captured cells was triggered by mitogenic activation and was detected at the aptamer-modified electrodes using square wave voltammetry (SWV). Use of novel aptasensors allowed monitoring IFN-γ release in real-time with detectable signal appearing as early as 15 min post stimulation from as few as 50 cells. The work described in this paper will pave the way to future use of aptasensors in cell-based diagnostics.