Simultaneous Detection of Cell-Secreted TNF-α and IFN-γ Using Micropatterned Aptamer-Modified Electrodes

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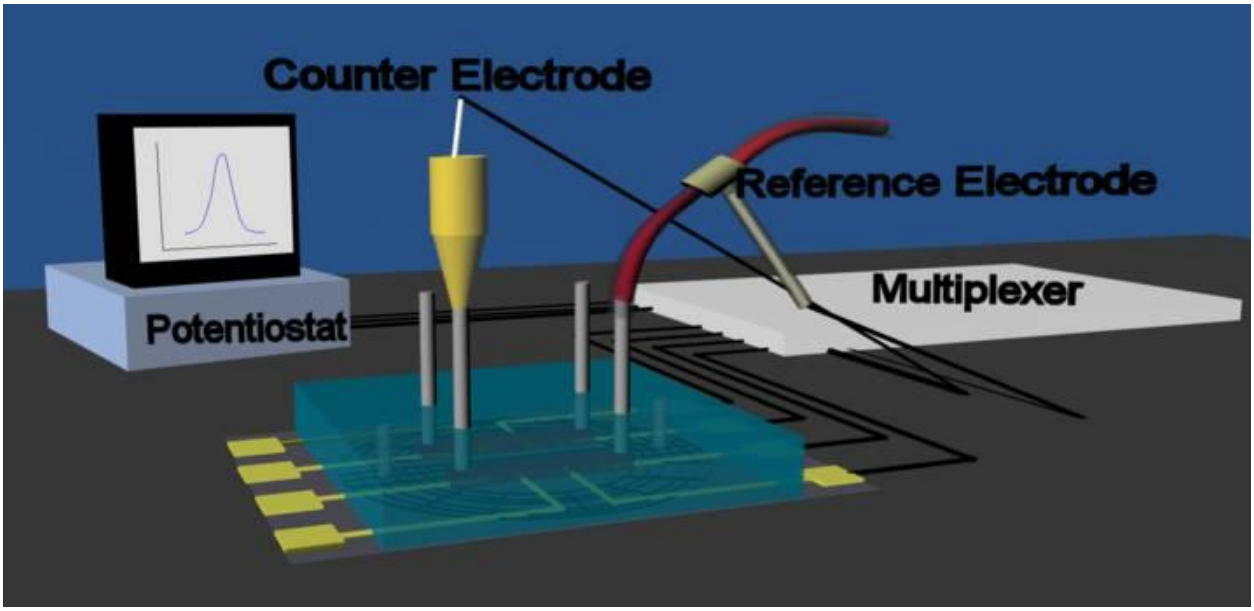
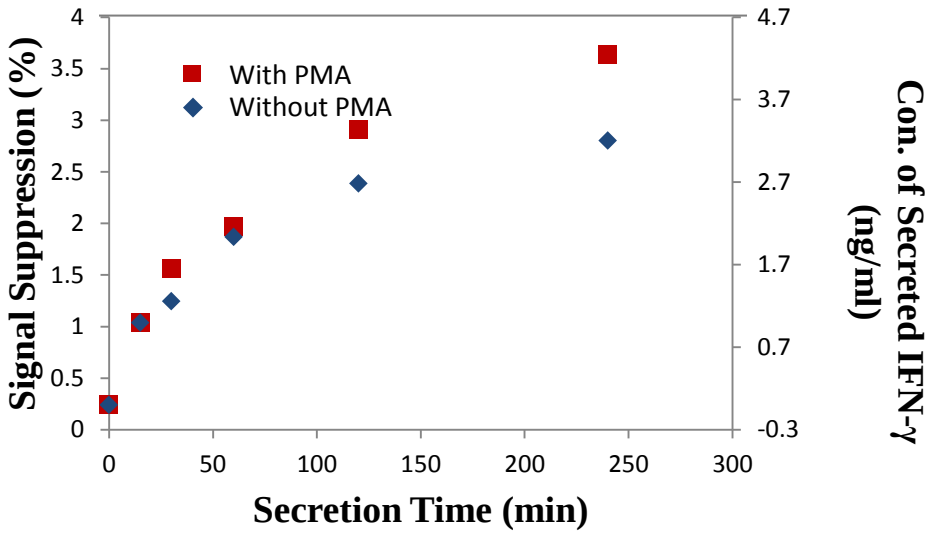


Figure S1: Layout of the experimental setup

IFN-γ/TNF-α Release from Molt-3 cells

IFN-γ secretion profile



TNF-α secretion profile

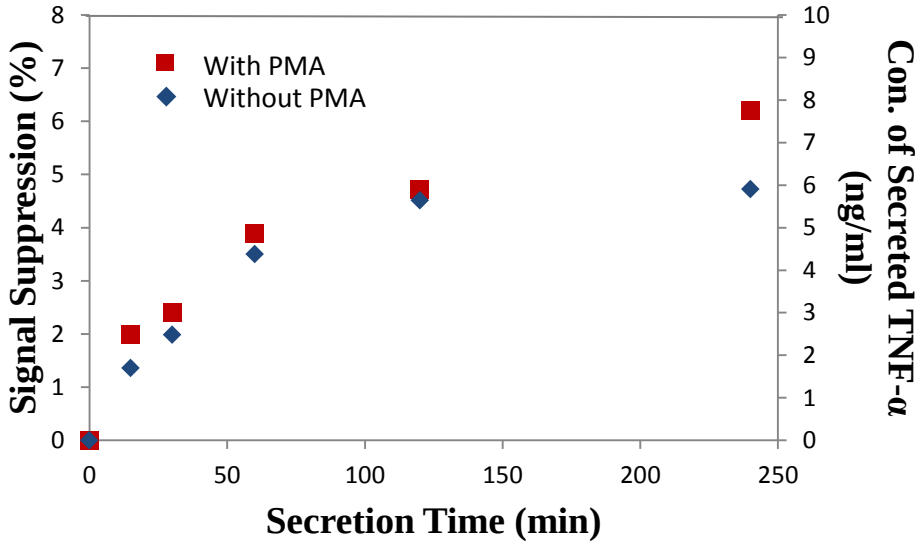


Figure S2 Continuous monitoring of IFN-γ/TNF-α release using dual cytokine binding aptamers modified electrodes from captured Molt-3 cells.

Part 4: Transport Considerations and the Determination of IFN-γ/ TNF-α Production Rates

Model Setup

Per cell IFN-γ and TNF-α production rates were calculated using COMSOL Multiphysics (COMSOL, Inc., Burlington, MA). A three-dimensional model was constructed based on a 3 x 10 x 0.1 mm microfluidic chamber. Four 7 um deep (depth estimated from SEM images) recessed wells were placed in the bottom of this chamber with a 300um inner diameter/400um outer diameter ring electrode in the center of each well. The size of these well correspond to the PEG-based microwells used in our experiments (300 um diameter). The area of the well surrounding the electrode was set to have an inward flux corresponding to the production of IFN-γ or TNF-α by cells captured in that area. The diffusivity of IFN-γ was estimated to be 1.45×10^{-6} and the diffusivity of TNF-α was estimated to be 9.9595×10^{-7} using methods published by Young et al. [Young, M. E., Carroad, P. A. and Bell, R. L. (1980), Estimation of diffusion coefficients of proteins. Biotechnology and Bioengineering, 22: 947–955].

Per Cell Production Rate Determination

In order to determine the per cell production rate, we iteratively simulated expected transient concentration profiles for a collection of IFN-γ and TNF-α production rates sequentially. Production of cytokines was assumed to begin at t = 0 and remain at a constant rate throughout the experiment. Time steps were taken freely as determined by the numerical solver and data was output at 15 minute time points up to 3 hours. The average profile of measured IFN-γ and TNF-α concentrations at each electrode were then fit against the predicted values from the numerical simulation. Data from the first 60 minutes of production was fit and a scaled least squares regression was used to determine the best fit production rate to 0.0001 pg/cell/hr precision.

Cell Production Rates at Short and Long Times

At time points earlier than 60 minutes, the fit begins to overestimate the amount of IFN-γ and TNF-α observed in our system. The over-estimation is due to the assumption of a constant rate of production over time. We expect that the cellular response to stimulation takes time to be fully realized as the cell produces cytokines for secretion. At longer time points, this rate then tapers off over time leading the reduction in observed cytokine production.

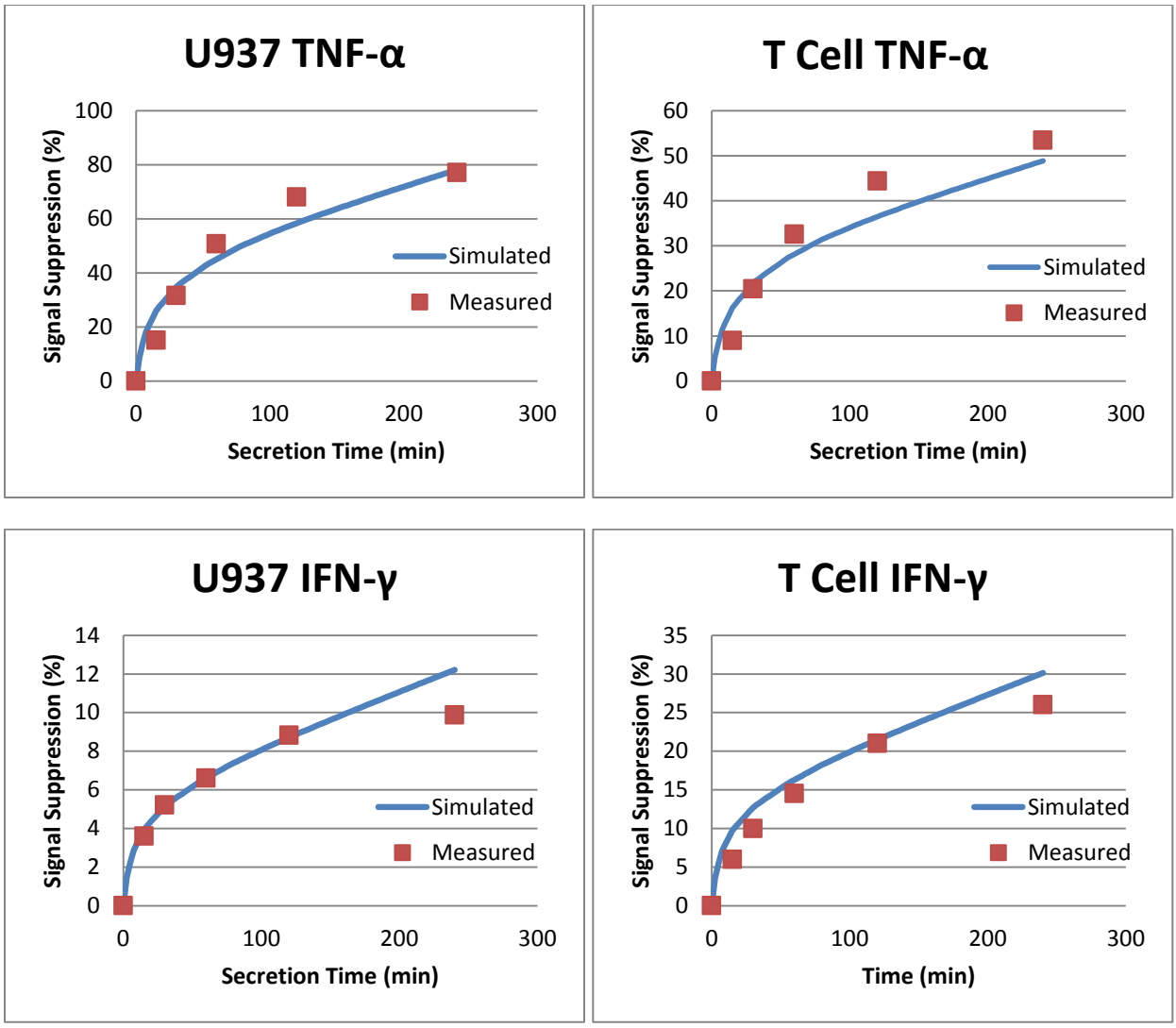


Figure S3. Data fitting for determination of IFN-γ and TNF-α per cell production rates. Lines represent simulated concentration profiles at the center point of each electrode. Squares represent mean data points averaged over 3 experiments. From this fit we estimate the production rate of TNF-α by activated human monocytes as 0.0160±0.0003 pg/cell/hr and IFN- γ is 0.0030±0.0001 pg/cell/hr; for primary T cells, the production rates of IFN-γ and TNF-α reported here are 0.0074±0.0002 pg/cell/hr and 0.0100±0.001 pg/cell/hr. Assumption of a constant production rate underestimates short-term and over-estimates the long-term cytokine production.