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On-Chip Regeneration of Aptasensors for Monitoring Cell Secretion

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Abstract

We report on the use of reconfigurable microfluidics for on-chip regeneration of aptasensors used for continuous monitoring of cell-secreted products.

First discovered in the 1990s, aptamers are strands of DNA or RNA that selectively bind to specific targets[1-3]. Aptamers are considerably simpler in structure than antibodies and can be designed into beacons that emit optical or electrical signal directly upon binding with its target analyte[4, 5]. Because aptamer-based sensors do not require secondary labelling and washing, they may be used for rapid detection of particular analytes of interest[6, 7]. Notably, living cells communicate by producing signalling molecules—often proteins—that relay commands to neighbouring cells[8]. The importance of annotating cell-secreted molecules has been accepted for many years; however, the need to monitor dynamics of cell secretions is just emerging[9, 10]. While antibody-based assays integrated with microfluidic devices have been adapted for monitoring cell release over time, this was done by creating a complex microfluidic device requiring constant perfusion of media and reagents (including antibodies) into a gated channel where analytes would be separated via electrophoresis[11]. In contrast, aptamer beacons allow collecting multiple time points from the same set of affinity probes[12-14]. However, aptamer-based biosensors are also limited in that once the binding sites on the sensing surface are occupied, the sensor ceases to function. This is a significant limitation for applications where one may be interested in continuous on-chip monitoring of cell-secreted products.

Our lab has previously developed aptasensors for time-resolved detection of cell-secreted cytokines, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)[15, 16]. In the present study we sought to address the challenge of on-chip regeneration of aptamer-based

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biosensors to enable continuous monitoring of cells. While regeneration may easily be accomplished using denaturing buffers such as urea[17], it is incompatible with living cells. To remedy this we integrated cells and aptasensors into a reconfigurable microfluidic device. As shown in Figure 1A, this microdevice was comprised of a glass substrate with micropatterned Au electrodes and two layers of polydimethyl siloxane (PDMS). The first layer contained fluidic channels and semi-circular microcups while the second layer was used for pneumatic control. This reconfigurable microfluidic device functioned in two modes (Figure 1B): 1) raised microcups where cell-secreted proteins were allowed to diffuse toward the aptasensor and 2) lowered microcups where cells became physically separate from the sensing electrode. As shown in Figure 1B, with the device operating in mode 1, cell-secreted signals (IFN-γ) were detected and quantified at aptamer-modified electrodes using square wave voltammetry (SWV). Upon saturation of the aptasensor, the microdevice was reconfigured to protect the cells inside the microcups and then flushed with regeneration buffer. Afterwards the device was reconfigured once again to raise the microstructured roof and continue cell secretion monitoring at the aptamer-modified electrodes. To control the vertical motion of the cups, negative or positive pressure was applied in the control chamber, a typical technique for PDMS devices[18, 19].

Food dye experiments were used to highlight the effective separation of two types of solutions within the same microfluidic device. As seen from Figure 2A and 2B, the green dye entrapped within the cups remained unmixed with the red dye present in the fluidic channel containing the electrodes. (Lower magnification images showing multiple electrodes/cups in the same channel may be seen in Figure S1. Movie S1 and S2 show dye entrapment and release from the microcups upon actuation of the device.) Addinitional experiments were performed to elimiate the possibility that solution in the main channel may seep into the cups and thus affect cell function. Fluorescence microscopy was used to demonstrate that fluorescent solution infused into the main channel did not penetrate into the area protected by cups over the course of 3 hours (Figure S2). In another set of experiments cells were either enclosed inside the microcups or were left unprotected during regeneration process. Figure S3 and Movies S3, S4 demonstrate that unprotected cells were lysed rapidly whereas protected cells remained intact. Further proof of effective protection of cells from harsh solvents used in sensor regeneration was obtained by performing multiple cell-protection/urea-flush cycles. Lymphoblasts (U937 cells) were trapped inside the device and then exposed to cycles of 1) lowering the roof, 2) flushing device with urea, 3) rinsing away urea, and 4) raising the microcups. As demonstrated by LIVE/DEAD images in Figure 2C and 2D, the cells remained viable after introducing regeneration buffer into the microchannel. Importantly, cell viability was not affected after four regeneration cylces as shown in Figure S4. One may note presence of dead cells in Figure 2D. These are remnants of cells that were beneath the walls of PDMS microcups but not inside the cups and not protected from urea buffer.

In the present study we chose to prove the concept of on-chip regeneration using an IFN-γ aptasensor. It is presumed that the aptasensor functions through a conformational switch of its DNA hairpin structure upon binding of IFN-γ as shown in Figure S5A. Exposure to urea breaks secondary bonds between the aptamer and IFN-γ regenerating the sensor. When detected using square wave voltamttery (SWV) the binding of the target results in a drop of

the redox peak current whereas regeneration leads to recovery of this peak (Figure S5B). Decrese in the redox current correlates with the target analyte in a concentration-dependent fashion so that the concentration of cell-secreted signal (IFN-γ in this study) can be deterimend quantitatively. The limit of detection and linear range of the aptasensor as a function of regeneration cycle were characterized. Figure 3A indicates that the limit and range of detection (5ng/ml and 100 ng/ml respectively) remained unchanged after three cycles of regeneration. One should note that the change in signal due to analyte binding was presented in terms of signal suppression % - (initial SWV peak current - final peak current)/ initial peak current. The IFN-γ aptasensor became saturated at ∼100 ng/ml. The number of times a sensor could be regenerated was not limited to four. Figure S6A shows that ten regeneration cycles with minimal loss of sensitivity were possible.

To demonstrate utility of the reconfigurable microfluidics for on-chip aptasensor regeneration, microdevices were coated with anti-CD4 according to protocols established by us previously[14]. Subsequently, peripheral blood mononuclear cells (PBMCs) were infused into the flow channels to capture $CD4^+$ T cells. As shown in Figure 2A, a two channel microfluidic device was used in these experiments with one channel stimulated with mitogen solution (50ng/ml PMA and 2μM ionomycin) while the other remained unstimulated. Signals were acquired every 10 minutes using a potentiostat interfaced with a multiplexer (see SI). As seen from data in Figure 3B, IFN-γ from mitogenically activated T cells was detected as early as 10 min post-activation and was monitored continuously from then on. At ∼200 min post activation the sensor reaches saturation. To remedy this, the microdevice was reconfigured to protect the cells and the aptasensing electrode was flushed with regeneration buffer for 8 sec. This regeneration cycle resulted in recovery of the redox current and cell monitoring was resumed (Figure 3B, 1^{st} regeneration). When the aptasensor approached another saturation point at 370 min, the device was reconfigured and regenerated once again. As highlighted by our results, reconfigurable microfluidics and on-chip regeneration extended the life-time of the aptasensor which would have otherwise become unresponsive at the ∼200 min time point.

One can note that signal dynamics in Figure 3B change from first to third regeneration cycle. As evidenced by our results, microcups provided an excellent barrier against penetration of harsh buffers and cell viability was not affected over multiple regeneration cycles. The results in Figure S6A show that sensitivity of the aptasensor was not affected by the regeneration cycle. Furthermore, we performed an additional experiment, using surface plasmon resonance (SPR) to demonstrate that dynamics of aptamer-IFN-γ interactions are not affected by exposure to regeneration buffer (Figure S6B). This suggests that the difference in signal dynamics observed in Figure 3B is due to changes in cellular secretion rate over time. Yet another control experiment (Figure S7) shows that aptasensor signal is solely due to the presence of cells and that if the cells are removed/lysed the signal goes to zero and stays there. Solving diffusion-reaction equations in COMSOL revealed that secretion rates were 0.00144pg/cell/hour, 0.00048pg/cell/hour, 0.00040pg/cell/hour for first, second and third regeneration cycle respectively (Figure S8).

Detecting cell-secreted molecules is becoming increasingly important for applications in diagnostics and basic science[20-22]. With advances in microfabrication and sensing

technologies, it is conceivable to detect secreted proteins or other molecules at the level of single cells or small groups of cells[9, 23]. However, limited options are currently available for on-chip continuous detection of proteins secreted by the same small group of cells[24-26]. Culturing cells in microfluidic devices is attractive because of low cell and reagent consumption, as well as the possibility to precisely control cellular microenvironment.[27-31] Devising strategies for on-chip, continuous monitoring of cell function will make microfluidic devices more impactful. The approach of combining aptasensors and reconfigurable microfluidics holds considerable promise in this regard because it enables the collection of hundreds of time points from the same sensor during multi-hour or possibly multi-day experiments. While these experiments were carried with T-cells, the regeneration and sensing approach is broadly applicable to monitoring the function of anchorage-dependent or independent cells. Moreover, multi-analyte aptamer-based biosensors are being developed[15, 32] and may in the future be integrated with on-chip regeneration to allow for long-term monitoring of multiple cell-secreted molecules.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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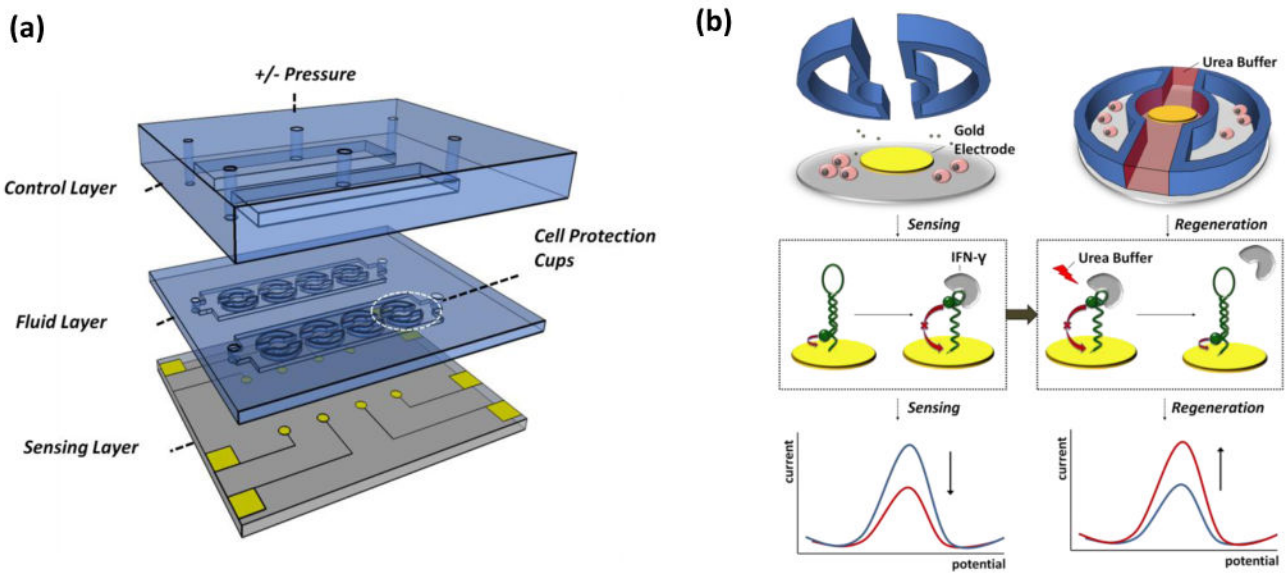

Fig.1.

(a) Layout of the device showing its three layer structure (b) (upper panel) Scheme indicating the principle of on-chip cytokine sensing and regeneration; (lower panel) square wave voltammetry signals during sensing (left) and regeneration steps (right).

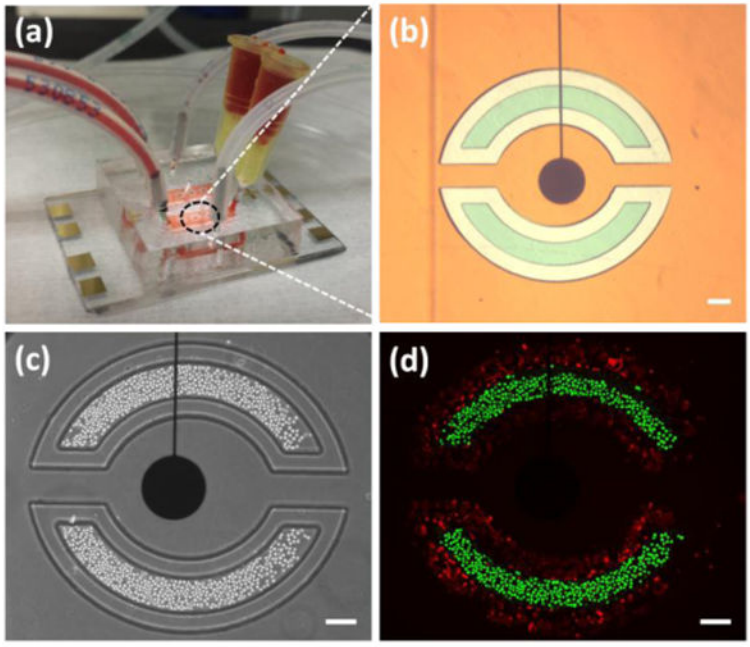

Fig.2.

(a) Picture of the sensing device containing micropatterned gold electrodes on the glass substrate and the reconfigurable double-layer PDMS top. (b) Enlarged view of the cups containing green food dye inside, surrounded by red food dye outside the chamber. (c) Bright-field image of the U937 cells captured inside the PDMS microcups next to sensing electrode in the center. (d) Live/dead staining of cells after aptasensor regeneration cycle involving urea. Viable cells were stained green while dead cells fluoresced in red. For all images the scale bar indicates 100 μm.

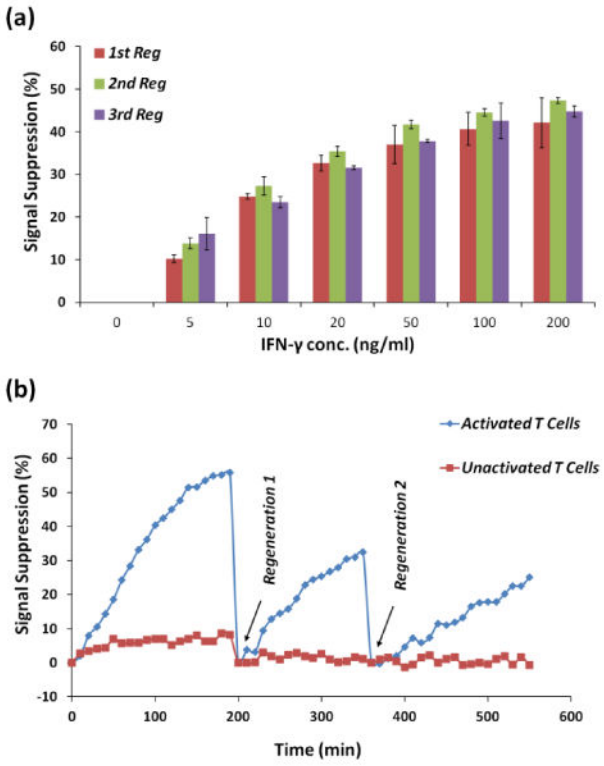

Fig.3.

(a) Response of aptasensors to various concentrations of IFN-γ as a function of regeneration cycle (b) Detection of cell-secreted IFN-γ in a regeneration device. This experiment shows three cycles of regeneration.