REAL-TIME ATP MONITORING IN HUMAN SERUM BY AN NANOFLUIDIC DEVICE INTEGRATED WITH AN APTAMER SENSOR

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This paper presents a novel biosensor capable of continuously monitoring specific molecules (*i.e.,* adenosine triphosphate - ATP) in the human serum by integrating the aptamer probes into a nanofluidic device. The advantage lies in its real-time signal regeneration of biosensor without the need of uploading the clean solutions for washing process since the ionic gate in the nanofluidic device could block or allow the target molecules flow through periodically.

Ion concentration polarization (ICP) is a transport phenomenon which is observed when an electric field is applied across the nanofluidic channels, for instance formed by using a nanoporous Nafion membrane. Once the ICP process is stabilized, an ion enrichment zone and a depletion zone are established at the both sides of the channels. These two effects have been utilized for a wide range of applications such as desalination [1], pre-concentration of target analyte species [2][3][4]. ATP is an important biomolecule found in the living cells (intra/extracellular) and it relates directly to many physiological and pathophysiological events. Current techniques of secreted biomarker detections were mainly based on ion conductivity measurement, so the correlation between the signals and disease progress was limited. There were several binding assays based platforms developed before, but the contradiction between portability and functionality of signal regeneration remained. For example, aptamer assay was demonstrated for continuous real-time small molecules measurement [5], but the washing process was required by uploading a large amount of clean buffer solutions by professional operators for aptamer signal regeneration, which is highly demanded for applications in domestic health monitoring.

In response, we have designed an aptamer based nanofluidic device for the real-time and continuous ATP detection in a small amount of human serum (100 µl for each cycle) (Figure 1). Our proposed nanofluidic device could allow the real-time monitoring individual patient's conditions so the proper therapy can be offered on time. The working mechanism of aptamer probes was studied in detail (Figure 2, 3). Unprecedentedly, we found that there is a strong relationship between the binding affinity of ligand-receptor and the detaching force caused by the hydrodynamic flow. The critical energy to detach the ATP from ATP-aptamer complex was calculated as 1.86 x 10^{-21} J and this energy can be used to settle the critical flowrate (*i.e.,* 1 µl/min; kinetic energy E = 1.99 x 10^{-21} J). We demonstrated that the device could produce the clean buffer solutions and endure five washing cycles to regenerate the aptamer without significant decrease of signal (Figure 4).

In conclusion, by converting patient samples (*i.e.,* human serum) to be clean solution for washing through applying electrical field to the device, the washing process could be approached without uploading buffer solutions. Accordingly, the dynamic signals of biomarkers could be measured in real time. With further miniaturization into a small wearable device for remote health condition monitoring, this nanofluidic device could improve current healthcare facilities significantly. The flexibility of this nanofluidic device could offer the possibility to integrate different receptors (*i.e.,* aptamers) to monitor other secreted biomarkers in sweat, saliva.

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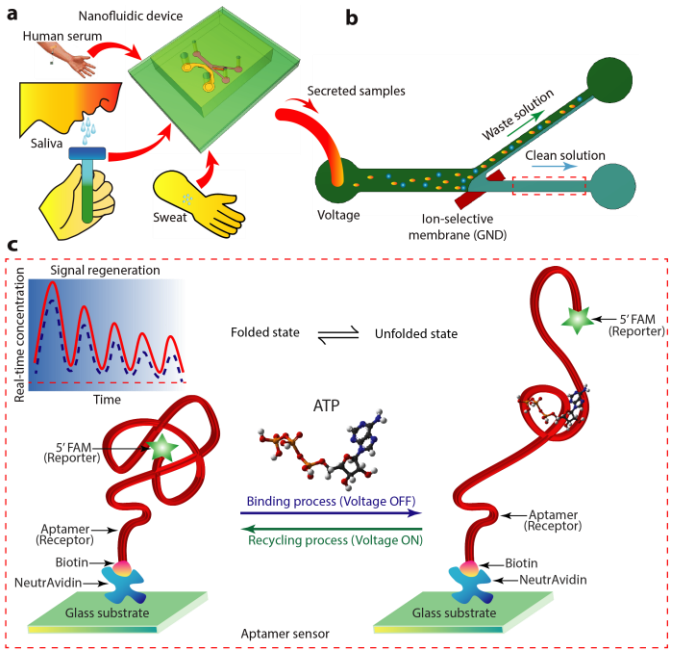

Fig. 1 The schematic of a portable nanofluidic device for real-time health monitoring. (a) The samples will be collected and transported to the inlet of device. (b) Samples will be converted to clean solution periodically by applying an electric field across the ion-selective membrane. (c) The binding and washing step of the target biomarker (ATP) to the aptamer immobilized on the glass surface. The conformation change induces a change in optical signal carried by the reporter (FAM). Regenerative signals will be monitored in a real-time manner (the inset).

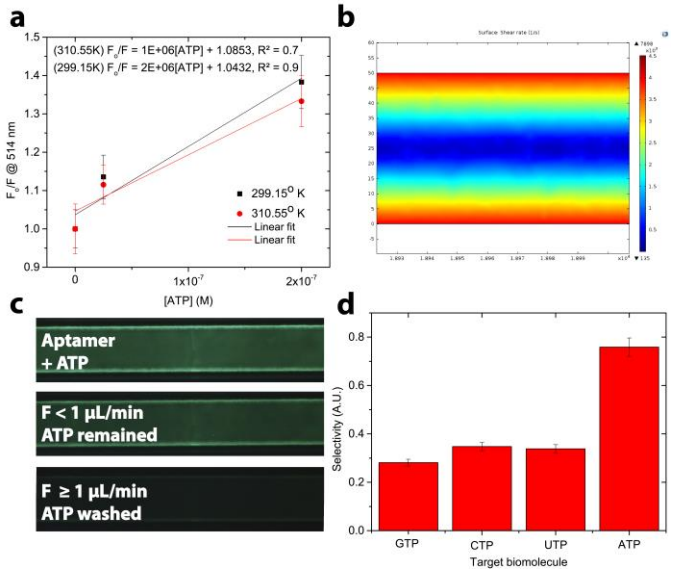

Fig. 3 Affinity and selectivity study of ATP-aptamer complex. (a) The Stern–Volmer plot (F_0/F vs. [ATP], where F_0 and F are the fluorescence intensities of aptamer in the absence and presence of ATP, respectively); the fluorescence was monitored at 514 nm. (b) The COMSOL simulation shows the shear rate γ at the fully-developed region immobilized with the aptamer probes. The kinetic energy $E = \gamma.\mu.A.d$ is derived via the relationship with dynamic viscosity μ, the channel width w, the immobilized distance of one line of aptamer d, where $A = w.d$ is the immobilized surface area of one line of aptamer perpendicular to the flow direction. (c) The fluorescence images show the effect of the critical washing flowrate. (d) The selectivity of the aptamer probes with different biomolecules.

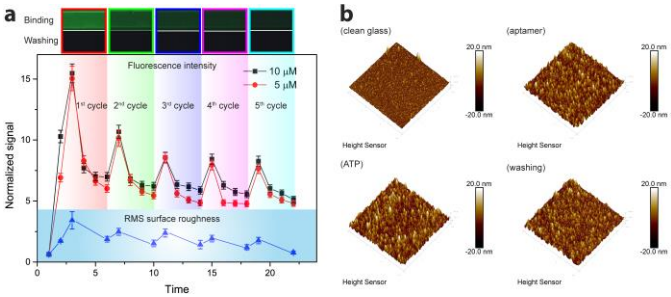

Fig. 2 (a) Five cycles washing with DI water by using ATP as a target molecule. (b) The AFM images show the change in surface roughness before and after ATP captured by aptamer probes.

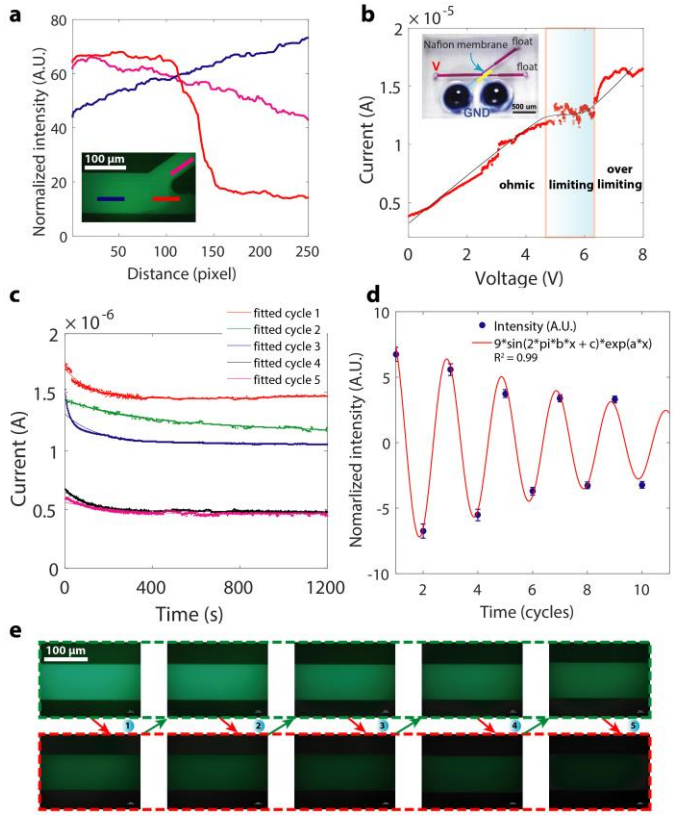

Fig. 4 Real-time monitoring of ATP in human serum and detailed characterization of ICP functionality. (a) The fluorescence intensity along the lines in input channel, across ICP boundary and output channel (line locations show in the inset) when the electric field is turned on, the nanofluidic component would block the biomolecules/ions in front of the ion-selective membrane. (b) Typical I-V response curves of ICP in the working device (the electrical connections show in the inset). (c) Current flowing through the nanojunction during five cycles of washing ATP by ICP. (d) Normalized fluorescence intensity shows the decay of regenerative signal by fitted with a damped sinusoidal wave function. (e) The optical image of aptamer immobilized channel with binding to ATP (top row) and without ATP due to the washing (bottom row) in five continuous cycles.