AUTONOMOUS CAPILLARY MICROFLUIDICS FOR RAPID NANORECEPTOR ASSEMBLY AND BIOSENSING

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ABSTRACT

We report an autonomous integrated microsystem comprising capillary microfluidics and impedimetric sensors for rapid nanoprobe assembly and antibody detection. Using open-channel microfluidics, *Tobacco mosaic* virus-like particles (VLPs) are autonomously delivered on the impedance sensor, forming a dense functional sensing layer due to enhanced surface evaporation-assisted assembly. The process utilizes 5μL of VLP solution (0.2mg/mL) and requires 6 minutes at room temperature compared to 3 hours in static environments. The functionalized impedance sensor is able to detect the presence of 1.2μg/mL target antibody within 10 minutes after functionalization. These results highlight the significant potential of the integrated system for rapid transducer functionalization and biosensing.

KEYWORDS

Capillary microfluidics, biosensing, sensing probe self-assembly

INTRODUCTION

In recent years, rapid pathogen mutations and infectious disease spreading have been escalated by the abuse of medicines and the fast growth of global transportation, increasing concerns for worldwide outbreaks of diseases such as SARS, influenza and Ebola [1]. Diagnostic technologies need to be adapted to achieve accurate pathogen detection with fast response in order to control the disease at the earliest stage. In parallel, there is a growing need for personalized sensors to better understand the body's function on a frequent basis. These requirements combined have created immense interest in next-generation portable and easy-to-use diagnostic biosensors.

Lab-on-a-chip (LOC) devices have been at the forefront of biosensor development in recent years. These devices can combine the benefits of high accuracy from immunoassays, precise sample control from microfluidics and sensitive readout from electrical or optical transducers [2], and are suitable for point-of-care applications. Most current LOC devices rely on external pumps and valves for fluid manipulation, which limits their expansion to point-of-care applications. At the same time, the quality of the functional layer in a LOC, which combines the density and affinity of the receptors, usually determines the sensitivity and selectivity of the sensor. Low receptor immobilization density or complex sensor surface treatment are common bottlenecks in the most widely used antibody receptor-based LOC sensors. Therefore, new sensing probes and sensor functionalization technologies that simplify the device-user interface need to be developed to facilitate the portability.

To address the functional layer limitations, we have employed virus-like-particles (VLPs), as bio-recognition elements. VLPs, direct derivatives of the *Tobacco mosaic* virus, are high surface area macromolecules possessing favorable features such as programmable affinity and self-assembly properties that make them very attractive as sensing probes for sensor functionalization [3]. They can be genetically modified to express a wide variety of programmable functional groups on their surface [4]. These include cysteines that facilitate self-assembly onto various substrates and FLAG-tag peptides with high affinity to target molecules. Previously, VLPs have been used as sensing probes in highly selective impedimetric and electrochemical sensors for bio/chemical detection [5, 6].

We further combine the benefits of VLPs as high density bioreceptors with autonomous passive pumps and valves in open-channel capillary microfluidics to accelerate the VLP self-assembly process, enhance the VLP assembly density, and eliminate the need for external equipment. It enables autonomous sample handling with minimal user involvement. Impedance microsensors are integrated with this platform to study the VLP sensing probe assembly process and detect antibody-antigen binding events.

METHODS

Impedance sensor integrated autonomous capillary microfluidic platform design

The proposed impedance microsensor and capillary microfluidics integrated microsystem is aimed at achieving autonomous liquid sample delivery, localized and accelerated sensing probe assembly, and real-time evaluation of antibody-antigen binding events. The microsystem is composed of three major components - impedance microsensors, open-channel capillary micropumps and passive microfluidic stop-valves. In this device, flow is driven by capillary action, where the surface energy difference between the outlet and inlet of the channel generate capillary forces that automatically put the liquid in the analysis area.

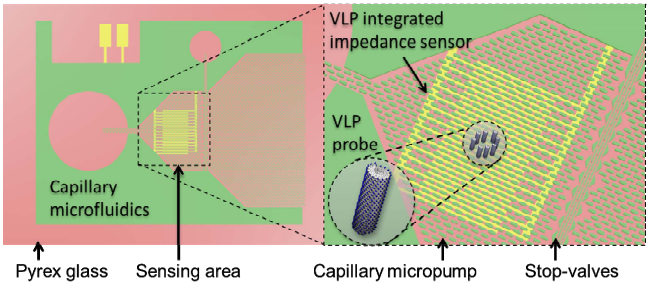

Figure 1: Schematic of the integrated microfluidic and sensor system

The three-dimensional schematic of the integrated microsystem is shown in Figure 1. It consists of two capillary micropumps, an array of passive stop-valves and impedance sensors on Pyrex glass substrate. The capillary micropump is formed by densely packed high-aspect-ratio elliptic micropillars whose major axes define the advancing direction of the flowing liquid. The passive stop-valve array prevents the liquid from getting into the right part of the platform due to a sharp change in the liquid-sidewall angle. The sensing area next to the sample reservoir contains a small capillary micropump and an impedance sensor with a total active area of $4mm^2$.

During the sensor surface functionalization, the solution that contains the VLP sensing probes is automatically delivered to the impedance sensors and rapidly evaporated at room temperature due to high-surface-area microstructures. This contributes to rapid self-assembly of VLPs with the maximized surface coverage on the impedance sensor. In the subsequent antibody binding study, a triggering fluid is introduced from the reservoir on the top in order to break the liquid-air interfacial energy at the stop-valves; thus, the antibodies can continuously flow through the functionalized impedance sensor area. The impedance is continuously monitored both during sensor functionalization and antibody binding to understand the dynamics of VLP assembly and detect the binding of antibodies to the VLP-functionalized sensor surface.

Microfabrication and surface treatments

The capillary micropumps are defined by high-aspect-ratio hydrophilic micropillar structures. A higher pillar aspect ratio ensures that the sidewall properties and shapes are the dominating factors for the flow control. KMPR 1050 negative photoresist was used as a structural material for the capillary microfluidics. KMPR was spin-coated at 3000 rpm for 30 seconds, forming a 60 µm thick layer on a 4 inch Pyrex glass wafer. The KMPR is soft-baked at 100^oC for 15 minutes before exposure at a dose of $990mJ/cm^2$ with UV light. The wafer was then post-baked at 100^oC for 3.5 minutes. The development process was completed in SU-8 developer for 3.5 minutes followed by rinsing in isopropyl alcohol.

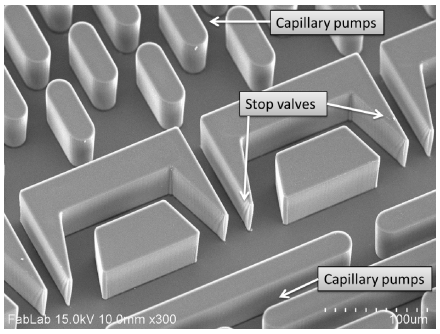

Figure 2: SEM image of the KMPR capillary microfluidic platform on a Pyrex substrate

In Figure 2, capillary micropumps defined by KMPR 1050 negative photoresist shows high-aspect-ratio pillar structures with smooth side-walls. The stop valves change the initial liquid-sidewall interface more than 150^o, forming sharp nozzle shapes. Another capillary micropump system shown on the lower right part of the image creates the triggering fluidic channel whose major flow direction is perpendicular to the first micropump. Thus, when the triggering flow is introduced, the stop-valve will be actuated sequentially, allowing the antibody-containing liquid samples to continuously flow from left to right.

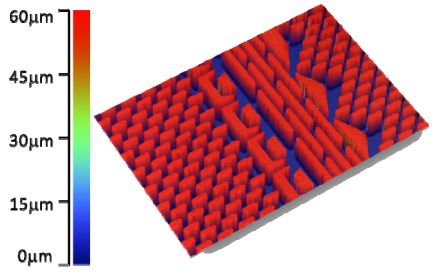

Figure 3: Optical profile of the microfluidic system

Figure 3 shows the optical profile of the developed microfluidic system obtained by a WYKO NT 1100 optical profilometer. The height of the micropillars is measured to be around 60µm, while the spacing is approximately 25µm, resulting in a 2.4 aspect ratio. Analytical calculations have shown that the contact-angle of the sidewall dominates over that of the Pyrex glass substrate if the aspect-ratio (depth/width) of the microfluidic channel is larger than 1.

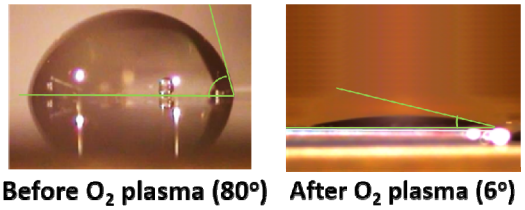

Figure 4: KMPR Contact angles before and after O2 plasma activation at 50W for 5 minutes

The KMPR sidewall needs to be hydrophilic in order to generate capillary forces that drive the liquid sample into the system. To alter the native hydrophobic surface to a hydrophilic surface, a 5 minutes O_2 plasma etching process (using an RF power of 50W) was performed on the fabricated capillary microfluidic devices to create nanoscale roughness and higher surface energy on the KMPR resist. After the O_2 plasma treatment, the KMPR contact angle was reduced from 80^o to 6^o (Figure 4), creating capillary forces that drive the liquid samples from the sample reservoir to the impedance sensors in the device.

VLP surface functionalization and immunoassays

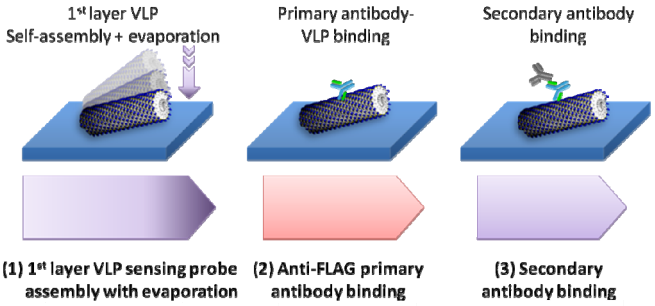

Figure 5: Experimental procedure for the VLP-FLAG surface functionalization and antibody sensing

Figure 5 shows the functionalization and biosensing procedure, which are continuously monitored through shifts in impedance. The VLP strain used here contains both a cysteine residue and a FLAG-tag peptide sequence (VLP-FLAG) to facilitate self-assembly and sensing of anti-FLAG antibody. The probes are assembled on the impedance sensor due to the gold/cysteine interaction and micropillar-assisted evaporation. The biosensing event includes the first two steps of an enzyme-linked immunosorbent assay, where 5µL of Tris-buffered saline (TBS) (1X) solution containing the target primary anti-FLAG antibody produced in rabbit (1.2µg/mL) and the amplifying secondary anti-rabbit IgG are introduced within 10 minutes. Due to the dielectric constant difference between the antibodies and water molecules, the substitution of water molecules by antibodies during the binding events lowers the effective dielectric constants of both the double-layer capacitance and the dielectric capacitance between electrodes.

RESULTS AND DISCUSSIONS

Enhanced VLP assembly on-chip

A 5mL of 0.1M sodium phosphate buffer solution that contains 0.2mg/mL of VLP-FLAG sensing probes was introduced in the left sample reservoir as a first step to functionalize the impedance sensor surface. This VLP solution was delivered by capillary force in the micropump and constrained by the stop-valve array to only the sensing area of the platform. The solution was kept stationary in the sensing area for a total time of 15 minutes to ensure it is fully evaporated at room temperature.

The scanning electron microscopy (SEM) images in Figure 6 show the surface morphology of the impedance sensor integrated in the capillary microfluidic platform after 15 minutes of VLP self-assembly. One-hour of palladium activation and a 4-minute nickel electroless deposition were performed to improve the visualization of the VLP structures in the SEM.

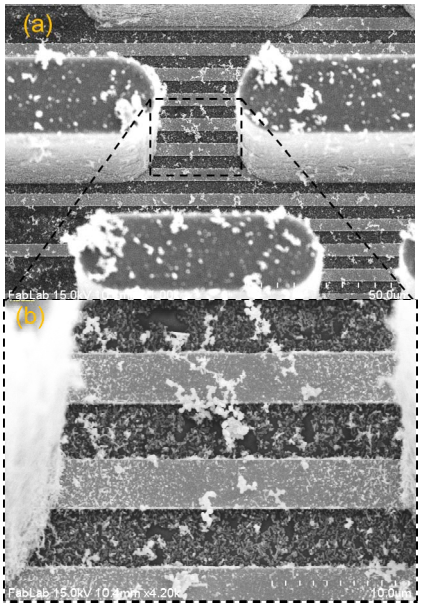

Figure 6: SEM images of the VLP-FLAG functional layer on the microfluidic channel and impedance sensor due to the enhanced evaporation process

A closer view of Figure 6b shows that the VLP-FLAG sensing probes form a dense functional layer on both the impedance sensor electrodes and the spaces in between. The original planar impedance sensor surface is then textured with uniformly coated nanostructured sensing probes. The density of the evaporation enhanced VLP assembly in the open microfluidic channel is much higher compared to that previously achieved in a closed chamber [6].

Real-time impedance monitoring during sensing probe self-assembly and antibody-antigen binding

The electrical impedance was continuously monitored to study the dynamics during the enhanced VLP sensing probe assembly and antibody-VLP probe interactions. An AC signal with 50mV amplitude at 100Hz was applied on an impedance sensor with 4µm electrode width and spacing. The change of the capacitive components between the impedance sensors was monitored and compared.

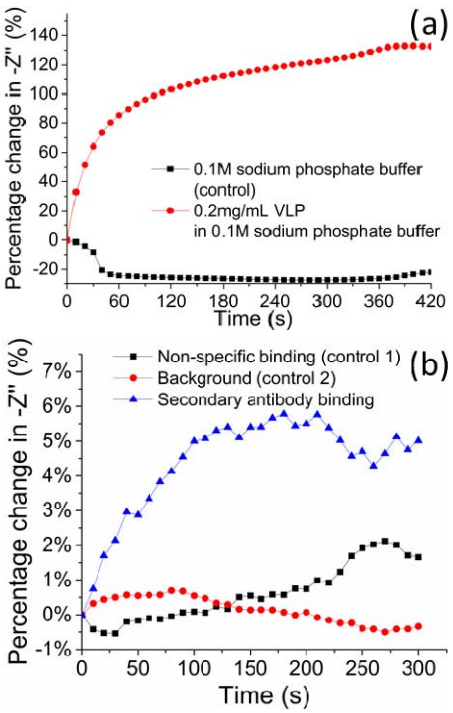

Figure 7: Normalized impedance response during the (a) enhanced VLP-FLAG assembly process in the open channel microfluidics and (b) the secondary antibody binding

The impedance change in Figure 7a shows that evaporation enhances VLP-FLAG assembly and rapidly saturates the sensor surface within 6 minutes, generating a 140% impedance shift due to a reduction in the effective dielectric constant. The system is able to detect 1.2µg/mL of the target primary anti-FLAG antibody in the TBS (1X) buffer through a 5.8% impedance shift during the secondary anti-rabbit antibody amplification (Figure 7b). In comparison, the noise-levels in the absence of primary antibodies (control 2) and the non-specific binding of anti-mouse secondary antibodies to the sensors (control 1) only generate about 0.7% and 2% impedance changes, respectively.

The experimental results demonstrate the feasibility of integrating open-channel microfluidics and impedimetric sensors to accelerate and monitor transducer functionalization and biosensing. This platform can potentially be utilized in rapid response and on-demand sensors for various field applications.

CONCLUSIONS

An autonomous capillary microfluidic platform was developed to achieve enhanced sensor functionalization using macromolecular VLP sensing probes. Impedance sensors were integrated into the platform to study the dynamics of biological sensing probe assembly on sensor surfaces and the impedimetric responses during antibody-antigen binding events. The evolution in impedance showed that, utilizing the developed open-channel capillary microfluidics, the VLP sensing probe can be rapidly assembled on the sensor as functional layer within 6 minutes, a significant reduction in time compared with the previous overnight process. The functionalized sensor was capable of identifying antibody-antigen interactions by applying primary and secondary antibodies within 10 minutes.

This work is the first demonstration of a capillary microfluidic platform developed and tailored toward accelerated, enhanced and controlled sensing. It shows the great potential of using capillary microfluidics in the enhanced sensor functionalization, and will contribute to the future development of programmable VLP-based fast response and on-site biosensors for applications in both public and personal health.

ACKNOWLEDGEMENTS

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