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Micromechanical PDGF recognition via lab-on-a-disc aptasensor arrays

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Abstract

A plug-and-play CD-like platform is used to perform a statistical detection of platelet derived growth factor (PDGF) proteins through aptamer-based surface functionalization of multiple microcantilever arrays. When PDGF proteins bind to aptamer coatings, the cantilevers deflect. The deflection response is monitored by optical read-out units from a commercial DVD-ROM device. We report on the use of an improved sensing platform which facilitates measurements under continuous liquid flow and with temperature control. Also, the mechanical wobbling of the DVD-ROM platform has been minimized and the scanning system has been optimized in order to detect cantilever deflections in liquid with nanometer scale resolution. The capability of the sensing platform is demonstrated by detection of clinically relevant concentrations of PDGF proteins. We present statistical measurements on 100 microcantilevers at different concentrations of PDGF, ranging from 10 nM to 400 nM. Hereby it is possible to reliably characterize the averaged mechanical response of cantilevers as a function of protein concentration.

Keywords

Platelet derived growth factor; Biosensing; Cantilevers; DVD-ROM; Lab-on-a-disc; Protein detection

1. Introduction

Aptamers are single stranded oligonucleotides selectively isolated from large pools of random DNA or RNA sequences, a process called systematic evolution of ligands by exponential enrichment (SELEX) [1]. Due to their capability of specific binding to target molecules with high affinity, aptamers are widely employed as bio-recognition elements [2]. They have a series of advantages compared to antibodies, including higher affinity, robustness and stability [3]. These characteristics are making them increasingly interesting to apply in biological detection processes [4]. Platelet derived growth factor (PDGF) is a major mitogen which regulates growth and division of a variety of cells such as fibroblasts, smooth muscle cells and tumor cells. Pathologically, PDGF is involved as a protein biomarker in many diseases. For examples, PDGF stimulates autocrine growth of different types of tumor cells (e.g. breast cancer, prostate carcinoma, leukemia etc.) and is

increasingly expressed in atherosclerosis and fibrosis [5]. Because of its physiological and clinical importance, different methods for PDGF detection using aptamers have been reported. Field effect transistor (FET) based [6], electrochemical [7], colorimetric [8] and fluorescence-based [9] aptasensing approaches have been demonstrated elsewhere.

In this paper we present the use of a plug-and-play CD-like platform for statistical detection of PDGF proteins through aptamer-based surface functionalization of microcantilever arrays [10,11]. Micrometer and even nanometer sized cantilever-based sensors have since the mid-1990s emerged as a promising label free detection technique, which has been used for high precision mass detection and bio-molecular recognition [12,13]. Microcantilevers are thus well established tools for biomolecular sensing at the nM scale concentration [14,15]. However, this technology often lacks the possibility of generating sufficient data to evaluate the statistical significance of the measured responses. The platform employed in the PDGF detection was designed to specifically overcome such challenges. The working principle of this platform was previously reported through preliminary sensing measurements in air [16,17]. We here demonstrate a further developed system which facilitates measurements in continuous liquid flow and with improved measurement resolution.

As sensing platform we use a CD-like polymer disc fabricated with micromilling technology. This disc, consisting of microfluidic channels and sensing reservoirs, is designed in order to keep the cantilever apices precisely aligned on sensing “tracks” that are scanned by a DVD-ROM laser system. At present, the rotating disc platform can hold up to 144 independent microcantilevers (6 sensing chambers, each with up to 24 microcantilevers) which can be read sequentially. Furthermore, a magnetic sealing of the microfluidic system has been implemented, allowing easy replacement of the individual cantilever chips and fast preparation of experiments.

Gold coated silicon cantilevers (500 μm long, 100 μm wide and 1 μm thick) have been functionalized with thiolated aptamers. The used aptamer has a three-way helix junction in its secondary structure, which specifically binds to PDGF with high affinity, $K_d \sim 100 \text{ pM}$ [19]. As illustrated in Fig. 1, when PDGFs are captured by aptamers, surface stress is generated on one side of the cantilever, resulting in a deflection of the cantilever [20,21]. We show that the deflection of the free end of the cantilever is dependent on the concentration of PDGF.

2. Sensing platform

The sensing platform consists of a polymer-based CD-shaped microfluidic disc loaded on a DVD-ROM based optical readout system. Fig. 2 shows the complete readout system and the polymer disc mounted on the DVD-ROM optical unit (OPU). The disc is loaded onto a rotating stage, where a DVD-ROM OPU provides nanometer resolution readout of cantilever deflection [22,23]. A laser scans from the bottom, passes through the bottom of the disc and focuses on the cantilever surfaces. The deflection profiles are measured using the astigmatic detection method embedded in commercial OPUs [24]. The laser spot diameter is only 565 nm (Full Width Half Maximum), making it possible to measure several thousand points across each cantilever width [25].

The spinning of the disc was optimized through the use of a pulley belt system in connection with a high-precision rotating bearing (Reali-slim Type X, Kaydon Corporation Inc, US). This design minimizes mechanical wobbling, making it possible to measure cantilevers mounted across the whole surface of the disc. The rotating bearing is composed by steel spheres that allow the structure to float above the rotating ring, minimizing vibrations induced by the rotational stepper motor (see Fig. 2a).

A syringe pump (PhD 2000, Harvard Apparatus, US) is connected to a six-nozzle injection system built into a circular PMMA frame. A magnets-assisted clamping of the nozzles to the disc is used to tighten the injection points to microfluidic inlets. The working principle is illustrated in Fig. 2b. Six injection points (tip diameter 1.8 mm) are encapsulated in a circular PMMA structure. The disc is positioned above a 500 μm thick iron disc, clamped to the rotational bearing. Rare earth magnets (Supermagnete, Webcraft GmbH Corp, DE) are attached to the center of the PMMA frame, and the magnetic force generated from the iron disc is used to seal the inlets. Several tests were performed at different liquid pressures without observing leakage problems. This simple magnet-assisted clamping method was found to be an extremely efficient way of building a plug-and-play injection system. To avoid entanglement of connecting tubes, the disc is constantly spun clock-wise and counter-clock-wise at each sensing revolution.

Cantilevers chips are mounted on titanium holders specifically designed for precise aligning of the cantilevers free-ends along the laser path on the disc (see Fig. 3a). The holders were fabricated by electrical discharge machining (EDM). Titanium-made flexible structures (500 μm thick) act as clamping springs and provide an efficient fixation of the chips. The holders are inserted into sensing reservoir, which are connected to inlet and outlet microfluidic channels. In order to operate within an easy to open and close device, a magnet-assisted microfluidic sealing was implemented.

As illustrated in Fig. 3b, the polymer disc was fabricated via bonding of two 1.5 mm thick PMMA plates. One plate was patterned with channels and chambers; the second was used as channel sealing layer. On this plate, a 1 mm deep square pool was patterned around the sensing reservoir. This pool was then filled with PDMS which acts as gasket which prevents leakage out of the reservoir. Magnets were encapsulated in the top side of the PMMA disc, and the attractive force toward the iron disc placed below the glass wafer was used to seal the whole structure. Fig. 3a schematically illustrates the PDMS “O-ring” structure.

3. Materials and methods

PDGF Aptamer (5'-CACAGGCTACGGCACGTAGAGCATCACCATGATCCTGTG-3'T) was obtained from IDT DNA. The following chemicals were purchased from Sigma Aldrich: PDGF-BB (M.W. = 25 kDa); Water (DNA grade) (CAS Number: 7732-18-5); PBS buffer (100 mM); BSA proteins solution (M.W. = 67 kDa); HCl (M.W. = 36.5) (37%) (density = 1.2 g/mL); BSA proteins solution (M.W. = 67 kDa); UREA 98% (M.W. = 60.06); EDTA (M.W. = 292.24). For optimal aptamer-ligand binding condition, the entire system was kept at a constant temperature of 37 °C by using a closed-loop air flow controller.

3.1. PDGF sample preparation

PDGF was reconstituted in 4 mM HCL containing 0.1% BSA. The stock solution was serially diluted and prepared in PBSM buffer (10.1 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 137 mM NaCl, 2.7 mM KCl, and 1 mM MgCl_2 , pH 7.4) at different concentrations. Initial tests were performed at 400 nM and 100 nM concentration. Further investigation involved samples at concentrations of 50 nM, 10 nM and 2 nM.

3.2. Aptamer functionalization

Au-coated cantilever chips were thoroughly cleaned in distilled water and absolute ethanol immediately before aptamer immobilization [26]. Chips were incubated in 5 nM HS-aptamer solution overnight, then rinsed by 4 M Urea + 15 mM EDTA in Milli-Q water for 1 min and washed by water twice.

3.3. PDGF detection

Each chamber contains 8–40 functionalized cantilevers as well as untreated reference cantilevers. MMT500 chips were used for the experiments. In order to prevent nonspecific adsorption of PDGF in the system, the disc and connecting tubes were flushed with 1% BSA solution for 30 min at 20 $\mu\text{l}/\text{min}$ followed by rinsing with PBS buffer. The cantilevers were then placed in the sensing chamber and a buffer solution was injected in the system while recording the cantilever baselines during the first 5 min. While the cantilever signals were recorded continuously, a valve injected 100 μl of PDGF sample solution for 15 additional minutes at a continuous flow rate of 15 $\mu\text{l}/\text{min}$.

4. Experimental results

PDGF concentrations of 10, 50, 100 and 400 nM were measured in order to characterize the stress transduction from the aptamer layers to the microcantilevers. The initially tested PDGF concentrations were chosen to be relatively high, in order to have preliminary indications of the detecting capability of the system combined with the aptasensors. Fig. 4 illustrates averaged measurements of functionalized (dark and light blue) and reference (black and grey) cantilevers for 400 nM and 100 nM of PDGF concentration during 20 min of exposure to the sample flow.

The averaged differential signal (functionalized cantilevers minus reference cantilevers) is shown in Fig. 4b. Each chamber was loaded with two functionalized and one reference chip (24 cantilevers in total). It can be noticed that, while the reference cantilevers give a signal of around 180 mV independently of the PDGF concentration, the functionalized cantilevers show a significantly higher response. After these preliminary tests, responses to PDGF concentrations of 50 nM and 10 nM were investigated.

Fig. 5 presents the results obtained measuring PDGF solution at 50 nM concentration. Again the functionalized cantilevers experience a change of bending clearly higher than the reference ones. The signal from the two functionalized chips are plotted separately (Fig. 5a) and they are seen to differ approximately 100 mV in the final averaged detection. This could be due to functionalization processes or to small misalignments of the cantilever apices on the radial track read by the OPU. The averaged differential signal is shown in Fig. 5b. An average deflection signal of $255 \text{ mV} \pm 80 \text{ mV}$ is obtained. Fig. 6 shows the result of the detection at the lowest concentration of PDGF (10 nM). 16 cantilevers have been monitored (8 aptamer functionalized and 8 blank) for 18 min after the exposure to the PDGF sample flow.

The average change of bending for the functionalized cantilevers compared to the reference ones is significant, and the obtained average differential deflection signal is around 175 mV. The collective data obtained from 100 independent cantilevers (52 aptamer-functionalized, 48 reference) at different PDGF concentrations can be seen in Fig. 7. A clear indication that the average deflection value increases with protein concentration can be observed.

5. Conclusions

We have reported on the detection of PDGF proteins at different concentrations employing a high-throughput cantilever sensing detection system in connection with aptamer-based surface functionalization. In total, 100 independent cantilevers were employed in the experiments, and their statistical response as a function of analyte concentration has been investigated. A monotonic dependence of the averaged acquired signal based on the protein concentration was observed during the experimental processes.

The use of the DVD-based readout method offers a number of advantages compared with standard cantilever sensing technologies. It provides large amount of data for implementation of statistical data analysis and sensing reliability evaluation. Compared to traditional optical readout systems, the nanometer sized laser spot of the OPU facilitates multiple measurements along individual cantilevers profiles, enhancing the amount of statistical data acquired on each sensor. A specifically designed polymer disc substrate for measurements in liquid flow was fabricated and employed in the experiments. This plug-and-play device embeds a microfluidic system which can easily and repeatedly be opened and closed thereby allowing easy manipulation and replacement of cantilevers.

We envision the future use of this fast, cheap and reliable sensing technique in parallelized detection processes of sub-nanomolar concentrations of multiple biomolecules.

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Fig. 1.

Schematics of aptamer functionalization of Au coated cantilever beams. When PDGF binds to the aptamers, the conformational change of the aptamers induces a difference in surface stress between the two sides of the cantilever. This change in differential stress is translated into deflection of the mechanical beams according to Stoney's equation [18]. The change in the cantilevers bending is measured by the DVD-ROM pickup head unit.

Fig. 2.

(a) Picture of the complete setup. The polymer disc is mounted onto a rotating stage. (b) Schematic view of magnet-based microfluidic inlets assembly. The attractive force between the magnets and the iron plate below the disc keeps the six nozzles tightly connected to the corresponding injection points. (c) Picture of a polymer disc connected to 3 nozzles through 5 rare earth magnets.

Fig. 3.

(a) The chips are placed on the bulk Titanium holder and then clamped through specially designed springs. (b) Cross-section scheme of Disc V 3-B. The substrate is composed by two thermally bonded PMMA plates. A PDMS ring is used for sealing of the chamber and magnets are used to apply force between the PMMA substrate and the Pyrex wafer. Typical channel section dimensions are $1\text{ mm} \times 500\text{ }\mu\text{m}$.

Fig. 4.

(a) Measured cantilever deflections in continuous flow for functionalized and reference cantilevers at PDGF concentrations of 400 nM and 100 nM. (b) Differential signals obtained at 100 nM (black) and 400 nM (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Fig. 5.
 (a) Bending results in continuous flow for 13 functionalized and 8 reference cantilevers at 50 nM PDGF flow. (b) Differential signal.

Fig. 6.

(a) Bending results in continuous flow for 8 functionalized and 8 reference cantilevers at 10 nM PDGF flow. (b) Differential signal.

Fig. 7. Averaged saturated cantilever deflections as a function of PDGF concentration measured over in total 52 functionalized cantilevers and 48 reference cantilevers.