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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.

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 apexes 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.

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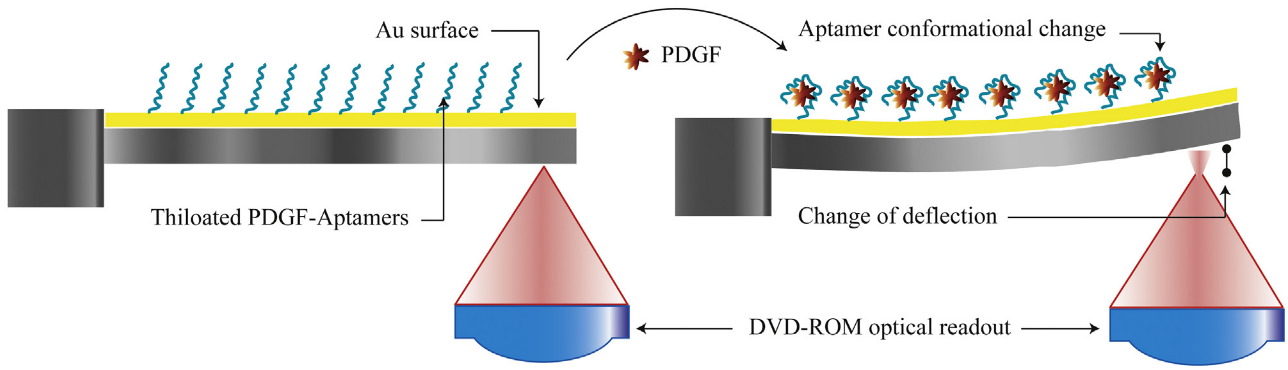

Fig. 1. Schematics of aptamer functionalization of Au coated cantilever beams. When PDGF binds to the aptamers, the conformational change of the aptamers induces a difference in surface stress between the two sides of the cantilever. This change in differential stress is translated into deflection of the mechanical beams according to Stoney's equation [18]. The change in the cantilevers bending is measured by the DVD-ROM pickup head unit.

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, $K_d \sim 100$ 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 Appartus, US) is connected to a six-nozzle injection system built into a circular PMMA

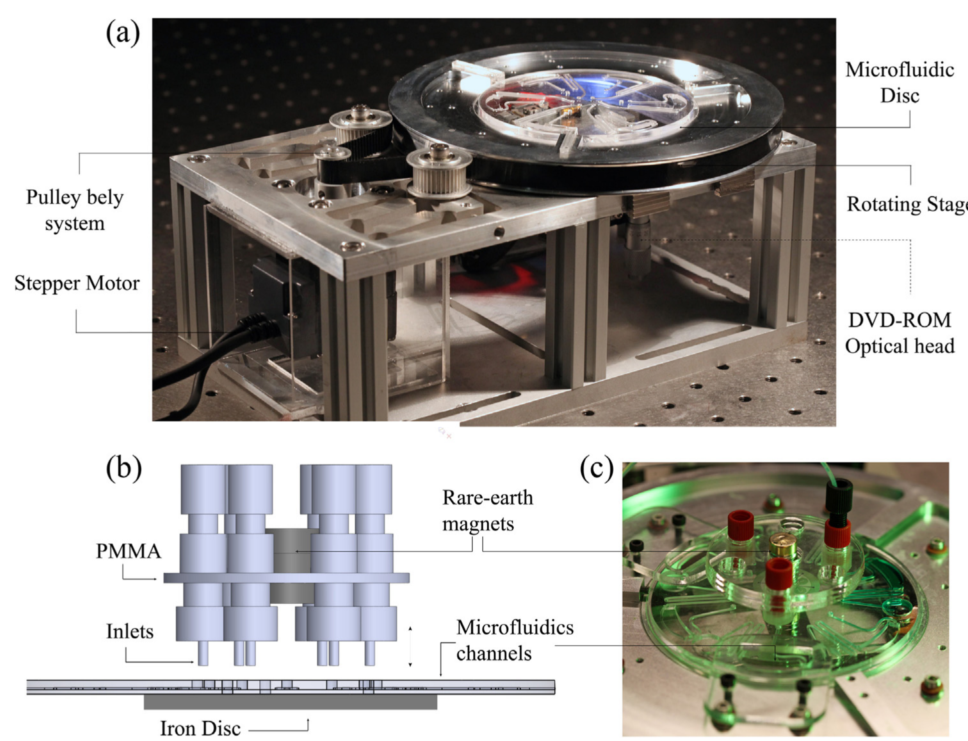

Fig. 2. (a) Picture of the complete setup. The polymer disc is mounted onto a rotating stage. (b) Schematic view of magnet-based microfluidic inlets assembly. The attractive force between the magnets and the iron plate below the disc keeps the six nozzles tightly connected to the corresponding injection points. (c) Picture of a polymer disc connected to 3 nozzles through 5 rare earth magnets.