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TOWARDS PICOMOLAR DETECTION WITH DVD-ROM OPTICAL TECHNOLOGY

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Soluble urokinase Plasminogen Activator Receptor (suPAR) is a protein which reveals information about inflammation in the human body. Several large scale studies have shown that the suPAR concentration reflects the activation state of the immune system from both viral, bacterial or parasitical infections and autoimmune diseases and cancer [1]. This makes it a good candidate as a potential clinical biomarker and triaging tool; an elevated suPAR concentration is associated with worse prognosis of the patients. suPAR concentrations in humans vary from tens (healthy) to hundreds (diseased) of picomolar, and the required sensing sensitivity is in the tens of picomolar. Currently, a diagnostics kit is on the market [2] for ELISA testing of suPAR.

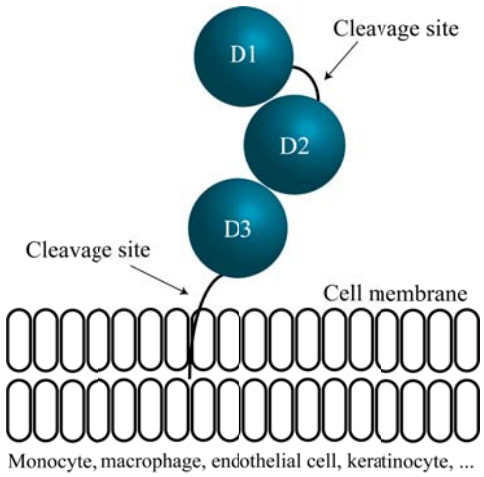

Figure 1: uPAR attached to a cell membrane. When cleaved from the membrane, it becomes suPAR. The domain D1 is usually also cleaved, leaving D2D3 as the interesting part of the protein.

The membrane bound molecule urokinase Plasminogen Activator Receptor (uPAR) consists of three domains (D1, D2 and D3) and is present on several kinds of cells as shown in Figure 1. When uPAR is cleaved from the membrane, soluble uPAR (suPAR) is formed. The receptor is likely to degrade - either before or quickly after cleavage from the membrane - into a two-domain protein as the D1 domain is cleaved from D2. This soluble two-doman protein $suPAR_{D2\text{-}D3}$ remains stable and is present in urine, blood (both plasma and serum) and cerebrospinal fluid [1].

The Multi Sensor DVD Platform (MUSE) framework [3] aims at providing a mainly reliable - but also cheap and rapid - tool for prognostics of inflammatory diseases via integration of micro- and nanotechnologies. In order to fulfill these needs, we propose a lab-on-a-chip device using multiple microcantilevers and a commercial DVD-ROM Optical Pickup Unit (OPU) for detection of surface stress changes in liquid [4] (see Figure 2). This technique allows high-throughput label-free sensing with statistical significant results and the radial symmetry enables very fast scanning of the sensors without applying torsional forces on the microcantilevers. Furthermore, the rotational platform facilitates high precision fluidic handling at the microliter scale.

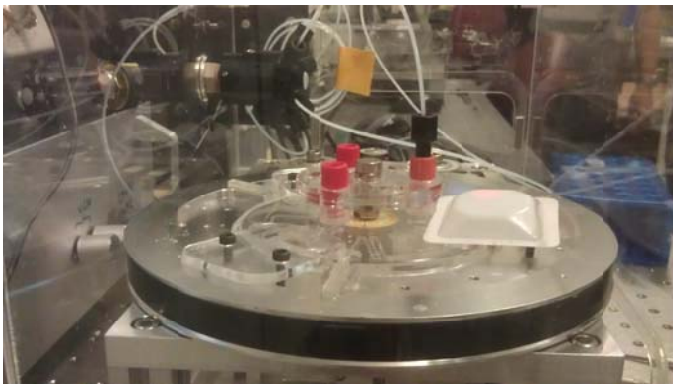

Figure 2: The DVD-ROM optical setup. A PMMA microfluidic discs contains the cantilevers. These are scanned by the DVD-ROM head which detects the deflection of the cantilevers.

Initial measurements have been conducted to pursue picomolar detection of suPAR with DVD-ROM technology for detection of cantilever deflection changes. Anti-D3 was functionalized on the cantilevers and PDGF

was used as reference protein. QCM-D measurements have been conducted to verify the functionalization strategy. From Figure 3 it can be deduced that it is possible to detect suPAR with the DVD-ROM technology down to below 10 nM. The sensitivity of the measurements is not yet sufficient to determine the concentration of suPAR below approx. 20 nM, neither the statistical significance. Since the relevant suPAR concentrations in the human body (serum) are in the range of tens to hundreds of picomolar, further optimization of the platform is needed both in terms of detection range but also in terms of sensitivity and statistical significance.

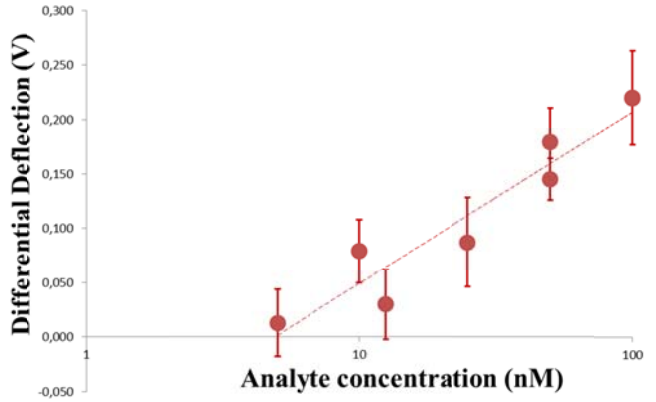

Figure 3: suPAR detection on cantilevers down to a concentration of 5 nM (logplot).The differential signal is the suPAR signal minus the PDGF signal.

In our pursuit to improve the required detection range, it is investigated which of two available antibodies yields the highest deflection of the microcantilevers. Anti-D3 and anti-D2 antibodies were each immobilized on 9 cantilever chips (IBM, CH); each containing 8 microcantilevers of dimensions 500 µm x 100 µm x 1 µm. The surface chemistry was optimized to ensure a uniform layer of antibody in contrast to the uneven layer attained in the previous experiments. As reference, 5 blank cantilever chips we used. Thus, the experiments were carried out on over 160 individual microcantilevers; reassuring statistical significant results (Note that data from a few cantilevers were lost due to i.a. defective cantilevers, misalignment or software malfunction). After 5 minutes of calibration with PBS buffer, 150 µl of suPAR solution was injected into the microfluidic system at a flow rate of 20 µl/min. The suPAR solution had a concentration of 200 nM.

The result of the experiments is summarized in Figure 4, where the absolute deflection signals (in mV) of the cantilevers with the two different antibodies and the reference cantilevers are illustrated. It is observed that both antibodies show a similar response to suPAR. This is not equivalent to concluding that the antibodies have equal binding properties to suPAR, since it is the generated surface stress that has the dominating influence on the deflection of the cantilevers. The surface stress will be affected by the amount of captured suPAR but also differences in antibody-suPAR configuration might change the generated surface stress. However, we notice that in our experimental configuration there seems to be no significant difference in using anti-D2 or anti-D3 antibodies.

New strategies will be employed to reach the clinical relevant concentrations of suPAR.

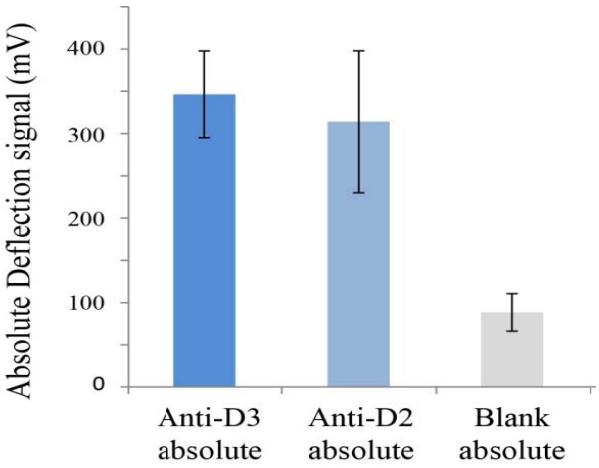

Figure 4: Antibody characterization in connection with cantilever measurements. The two antibodies anti-D3 and anti-D2 yield similar resulting deflection of the cantilevers.

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