DETECTION OF BIOMARKERS USING BIO-FUNCTIONALIZED MICROFLUIDIC CHANNELS

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We have developed a technique based on the use of protein functionalized microchannels for rapid electrical detection and quantification of any type of target protein biomarker in a sample. We successfully demonstrate detection of anti-hCG antibody, at a concentration of 1ng/ml and a dynamic range of three orders of magnitude, in less than one hour. We also demonstrate detection of CEA (Carcinoembryonic Antigen) in a complex mixture at a concentration of 100 pg/ml. Finally, we demonstrate the detection of DNA hybridization using our sensor.

Current microbiological techniques used for protein biomarker detection, involve time consuming methods based on sandwich immunoassays [1], requiring expensive labeling. In our assay for anti-hCG detection, useful for diagnosing infertility in women [2], 10um polystyrene beads (Fig. 1a) are coated with Anti-Rabbit IgG (Fig.1b), which has a specific affinity to anti-hCG antibody. The beads are then placed in the test solution for 40 minutes and the target biomarkers are captured if present (Fig. 1c). Presented in Fig. 1d is the biosensor, with anti-Rabbit IgG already immobilized on the surface of the channel. The beads are then injected into the micro-channel occluding the channel and increasing the resistance from the baseline value. They are allowed to come to rest for 1 minute. Beads that have captured the targeted biomarkers will attach to the receptors on the channel surface, forming a sandwich assay (Fig. 1e). We then flush the channel (50nl/min flow rate), removing unbound beads and reducing the channel's electrical resistance, which depends on the number of beads remaining (Fig. 1g). The number of attached beads is a function of the biomarker concentration. High concentration of biomarkers results in smaller resistance drop compared to low biomarker concentration. Thus, in addition to biomarker detection at low concentrations, this technique can also be used for biomarker quantification. The biochip (Fig. 2a) consists of microelectrodes on a glass substrate and a channel right above, formed in a PDMS cover. Electrodes were patterned using sputtering and lift-off processing (Fig 2b).

The assay was confirmed optically (Fig. 3a), where the beads in the channel were counted before and after washing. A dynamic range of three orders of magnitude and a repeatable detection limit of 1ng/ml are demonstrated. The percentage decrease in electrical resistance measured as a function of target biomarker concentration is shown in (Fig. 3b) confirming the optical results. Impedance sensitivity to the location of the beads between the electrodes results in a large standard error in electrical readout compared to the optical signal. Error can be reduced by using interdigitated electrodes which we are currently testing. We also performed a similar assay testing for the presence of CEA in BSA. We achieved detection at a concentration of 100 pg/ml against a background concentration of 1mg/ml BSA.

For DNA hybridization detection, the channel surface is functionalized with oligonucleotides (60 base) of a known sequence. Target DNA strands are immobilized on the surface of 22um polystyrene beads, which are injected into the micro-channel at a flow rate of less than 200nl/min. Hybridization of the DNA strands causes capture of beads resulting in an increase in the channel resistance (Fig 4c). The resistance is measured across the channel as function of time (Fig 4d). The resistance increases instantaneously as the beads passing through the channel are captured.

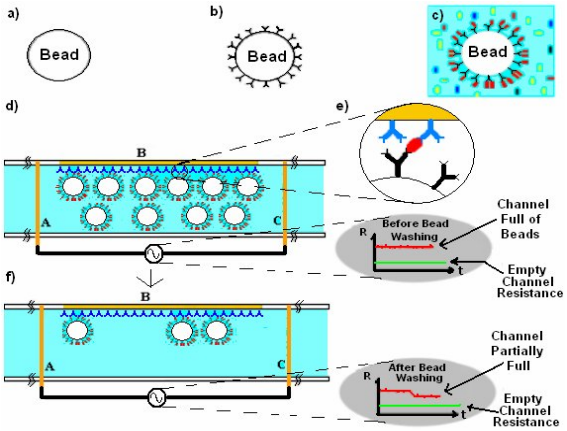

Figure 1: (a) Micron sized bead (b) Bead coated with receptors and then (c) immersed in a multi-analyte solution (d) The beads labeled with targeted biomarkers, in a Phosphate Buffer Saline (PBS) solution (138mM NaCl, 2.4mM KCl) with pH of 7.4, are loaded into the channel and allowed to bind to the secondary receptor molecules which are immobilized on the gold electrode forming a (e) (Top plot) sandwich assay at the channel surface. (Bottom plot) Prediction of resistance after injection of beads. (f) The channel is then flushed, causing the unbound beads to be removed from the channel. The magnitude of the resistance change is proportional to the target biomarker concentration.

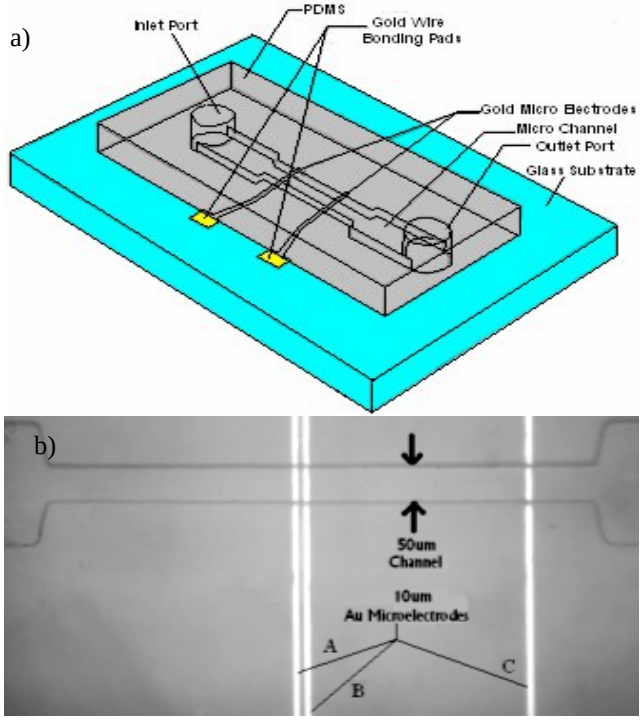

Figure 2: a) Schematic of microfluidic biochip. b) Optical image of top view of 50 um deep channel with three electrodes. Resistance is measured between electrodes A and C. Electrode B not used in this study.

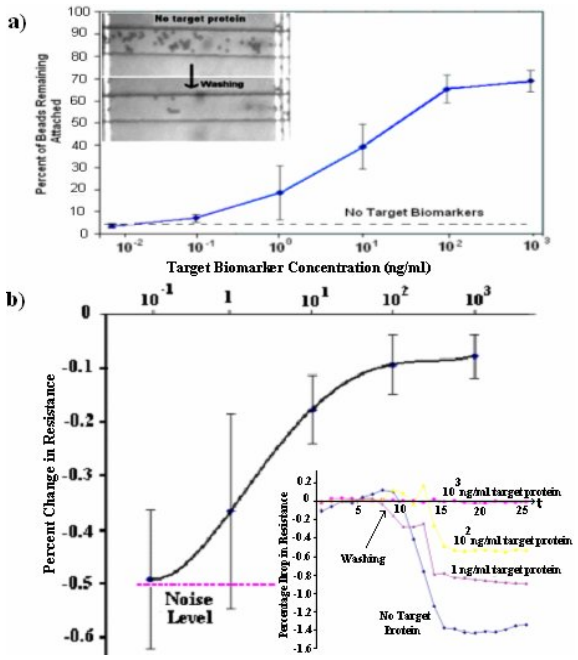

Figure 3: a) The percentage of beads remaining attached in the micro-channel after incubation, with different concentrations of target protein biomarker as measured optically, establishing dynamic range of 3 orders in magnitude. A detection limit of 1ng/ml has been demonstrated. Inset: Optical image of beads in channel before washing and after washing for the case where no target biomarker was present. b) The percentage decrease in ionic impedance across the channel as a function of protein biomarker concentration with standard error bars, confirming optical results. Inset: Percentage change in resistance as a function of time.

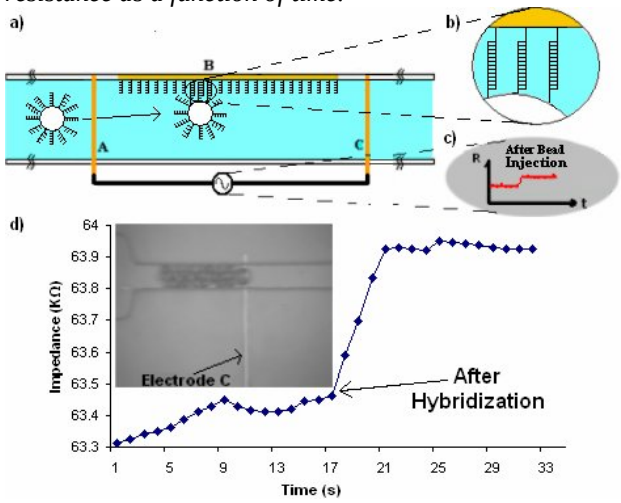

Fig. 4: a) Surface of channel activated with probe DNA. Target DNA is immobilized on the surface of polystyrene beads are injected into the micro-channel. b) DNA hybridization causes bead capture resulting in c) an increase in the channel resistance. d) The resistance is measured across electrodes A and C (electrode A not shown). The resistance increases at 17s as beads passing through the channel are trapped onto electrode C (inset).