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Heavy metal ion detection using a capacitive micromechanical biosensor array for environmental monitoring☆

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ABSTRACT

Heavy metal ions are major contaminants in present day water supply. In this work the fabrication and evaluation of a DNAzyme functionalized capacitive micromechanical sensor array for the detection of lead ions is presented. The catalytic strand of a "8–17" DNAzyme can cleave a substrate DNA strand that has one ribonucleotide base, in the presence of Pb^{2+}, dissociating the complex into three fragments. The DNAzyme strand is laser printed and immobilized on the sensor surface and hybridized with the substrate strand. When self-cleaving occurs, the surface stress changes, which is then registered as change in the capacitance of the device. The sensor was able to detect 10 μM of Pb^{2+}, whilst in a reverse procedure the re-hybridization of the immobilized catalytic strand with the substrate strand was demonstrated. The reactions were validated by tagging the catalytic strand with FAM (6-carboxyfluorescein), while the substrate strand was tagged with Dabcyl, a quencher.

1. Introduction

Heavy metals (i.e. cadmium (Cd), lead (Pb), mercury (Hg) and manganese (Mg) amongst others) are some of the major environmental contaminants, found in air, soil as well as drinking water. Chronic exposure to these ions is related to such adverse health effects such as cancer [1] and neurodegenerative diseases [2,3] including Parkinsonism – a disease with Parkinson's resembling symptoms [4]. Lead, in particular is toxic even at low concentrations [5]. Accumulation of Pb^{2+} in the human body has diverse detrimental effects on human health [6] as it can induce renal tumors, increased blood pressure as well as neurological and behavioral effects [7]. Lead has even more severe effects in children during their developmental stage, as it leads to reduced intelligence and other neuropsychological defects [8]. Given the prevalence and severe toxicity of Pb^{2+}, developing methods that are selective, sensitive, and cost-effective for Pb^{2+} detection has received great attention both in the past as well as today. Toward this goal, a number of Pb^{2+} sensing techniques have been developed.

While spectroscopic techniques, such as atomic absorption spectroscopy and inductively coupled plasma atomic emission spectroscopy, can offer high sensitivity for Pb^{2+} detection [9] the need for accurate, on-site and real time measurements has led to the development of sensors based on biomolecules that fulfill these requirements without compromising neither on selectivity nor sensitivity. Among them, DNAzymes have emerged recently as a promising class of molecules to build sensors. DNAzymes that can catalyze a broad range of chemical and biological reactions such as RNA cleavage have been isolated in test tubes [10]. Most require certain metal ions as cofactors for function, and many DNAzymes show high metal-binding affinity and specificity [11]. They are selected through SELEX (systematic evolution of ligands by exponential enrichment), a process which presents the target analyte to a library of short oligonucleotide sequences and selects for the nucleic acids that bind selectively to the former. For practical applications, DNAzymes are low-cost to produce, much more stable than protein- or RNA-based enzymes and can be denatured and regenerated many times without losing their catalytic activity or the ability to bind to their target analyte [12].

Many new detection techniques have been developed using the DNAzyme specific for lead ions, termed "8–17" DNAzyme, which can cleave a substrate DNA strand that has one ribonucleotide base [13]. Typical examples include colorimetric [14,15], fluorescent [16] and electrochemical biosensors [17]. Although colorimetry

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and fluorescence-based sensors are simple and low-cost, the linear range of colorimetry is narrow and the selectivity of fluorescence sensors is low, while electrochemistry is sensitive but the operation is complex [18]. Moreover, most of them require labels and reporter molecules which increase the cost [19]. Although label-free detection of Pb^{2+} can be implemented using abasic-specific dyes, the weak affinity of these dyes may limit the sensitivity [20]. More recently, DNAzyme sensors utilizing advanced materials such as graphene [21] and quantum dots [22] or novel techniques such as dynamic light scattering and rolling circle amplification [23,24] have been reported. These techniques too, however, have limitations like, for example, their operational complexity. Hence, developing methods that are sensitive, label free, and simple to use for Pb^{2+} detection is still an issue that needs to be addressed.

Recent reports have shown that mechanical biosensors present a number of advantages including high mass resolution (in the zeptogram range while operating in vacuum and nanogram in liquid environment), can resolve forces down to ~10 pN, and fast response times which enable real time monitoring of biological reactions with time-resolved measurements [25]. On the other hand, one of their major disadvantages is that micromechanical biosensors are usually read out using cumbersome optical setups, which hinder the construction of small, low weight, portable instruments which can be used in point-of-care applications. Recently, however, micromechanical sensors relying on electrical detection have been proposed as an alternative method for sensor readout [26,27].

For example, Yoshikawa et al. have presented a membrane-type surface stress sensor (MSS), which is based on piezoresistive read-out integrated in the sensor chip. The MSS consists of a membrane suspended by four piezoresistive beams connected in a full Wheatstone bridge configuration. The operation of the MSS then relies on the analyte-induced isotropic surface stress on the membrane being efficiently transduced to the piezoresistive beams as an amplified uniaxial stress [28]. Similar efforts to fabricate capacitive surface stress based sensors have been presented by Satyanarayana et al. [29] where a parylene micromembrane surface stress sensor was presented and its response demonstrated in chemical sensing. The achievement of more effective chemical and potentially biological sensing through a membrane closer to its substrate was developed [37], where electret membranes were exploited.

A polydimethylsiloxane (PDMS) membrane biosensor has been presented by [30]. The sensor readout was capacitive and their sensing element was a PDMS membrane of 2–4 μm thickness. The authors demonstrated detection of DNA hybridization and protein recognition by aptamers. In another application, a capacitive biosensor element made of a silicon membrane has been presented for the detection of the biotin–streptavidin interaction [31]. Using an array of similar surface stress biosensors the detection down to 9 nM of the CD19 mutation in beta-thalassemia following the polymerase chain reaction amplification of a DNA fragment bearing the mutation has been presented [32].

In this work, the fabrication and evaluation of a DNAzyme functionalized capacitive type micromechanical sensor array for the detection of Pb^{2+} ions is presented. Each DNAzyme consists of a catalytic strand and a substrate strand. To construct the sensor, the catalytic strand is covalently immobilized onto the surface of the capacitive micromembranes by printing of the biomaterial with high spatial resolution using the Laser Induced Forward Transfer Technique (LIFT) and hybridized afterwards. Upon addition of Pb^{2+}, self-cleaving occurs at a specific site of the substrate strand, resulting in a change of surface stress and thus the capacitance of the device (Fig. 1). In order to validate the hybridization of the substrate strand to its immobilized complementary DNA sequence (catalytic strand), the latter was tagged with FAM (6-carboxyfluorescein), while the former with Dabcyl, which quenches the fluorescence emitted by FAM when found in close proximity. In the presence

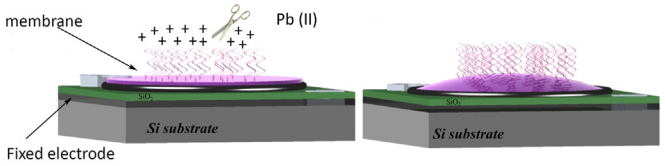

Fig. 1. Schematic representation of the capacitive sensing elements (a) before and (b) after the hybridization.

of lead ions, the cleavage of the substrate strand and its dissociation into two smaller fragments results in the restoration of the fluorescence emitted by the tagged with FAM immobilized strand (Fig. 2).

2. Materials and methods

2.1. Materials

All reagents were obtained from Aldrich Chemical. All solutions were made with deionized water (18 MΩ cm resistivity) from a Millipore MilliQ system. DNA oligonucleotides were purchased from Eurofins MWG Operon (Ebersberg, Germany). The sequence of the catalytic strand was 5′-FAM-TTTTTCATCTCTTCT-CCGAGCCGGTCGAAATAGTGAGT-SH-3 and was labeled with FAM at its 5′ end and a 3′-thiol C5 linker. The sequence of the substrate strand was chimeric 5′-ACTCACTAT rA GGAAGAGATG-Dabcyl-3′, bearing a ribonucleotide at the 10th position of the DNA strand, while the 3′ end was labeled with Dabcyl, a FAM fluorescence quencher. 6-Mercapto-1-hexanol (MCH) was used in a 1.0 mM solution in deionized water. A 2% 3-glycidoxypropyltrimethoxysilane (GOPTS) in 95% ethanol was used for the functionalization of the surfaces. DNA deposition was performed from 1 M potassium phosphate buffer (0.5 M KH_2PO_4, and 0.5 M K_2HPO_4, pH 8) (Buffer 1). Hybridization of the substrate strand with the immobilized catalytic strand was performed from a 1 M NaCl and 50 mM Tris–acetate–EDTA (TAE) buffer (pH 7.2) (Buffer 2). Unbound catalytic or substrate strands were removed by washing with Buffer 2. Lead nitrate ($Pb(NO_3)_2$) and magnesium chloride ($MgCl_2$) was dissolved in a 50 mM NaCl and 50 mM Tris–acetate–EDTA (TAE) buffer (pH 7.2) (Buffer 3). Low temperature silicon dioxide (LTO) on silicon (Si) substrates were used for the initial investigation into the optimal concentrations for the catalytic and substrate strands.

2.2. Investigation into the concentrations of the catalytic and substrate strands

The LTO/Si substrates were functionalized with GOPTS according to a method described previously [33]. Briefly, the substrates

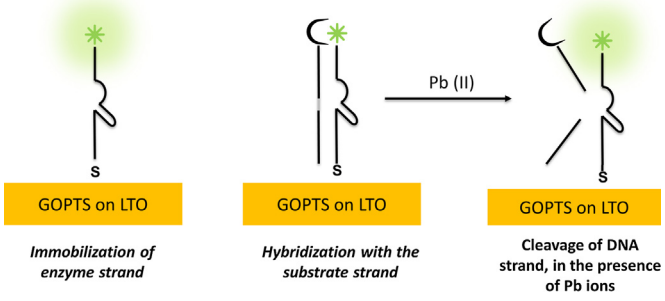

Fig. 2. Schematic representation of the modified sensor surfaces. The immobilized FAM-tagged catalytic strand emits a fluorescence signal which is quenched upon hybridization with the Dabcyl-modified substrate strand. Recognition of Pb^{2+} results in the self-cleavage of the complex and the restoration of fluorescence.