Electrochemical Analysis in Confined Spaces Involving Immunosorbant and DNA-Hybridization Assays

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Confining electrodes, modified surfaces, and sample volumes to small spaces can improve performance of electrochemical analysis. We are investigating such geometric configurations to develop immunosorbant and DNA-hybridization-based microelectrochemical sensors with superior detection limits and sensitivity. We have demonstrated a proof-of-concept device for detection of a model analyte, mouse IgG, and are now expanding the research for detection of microorganisms, such as *Cryptosporidium parvum* (*C. parvum*), and interfacing the sensors with microfluidics platforms.

Initial studies have been performed on a microcavity device containing three individually-addressable electrodes as integral components. The construction and initial characterization have been reported previously by us.[1] A microcavity consists of alternating layers of gold (with a chromium adhesion layer) and polyimide (an insulator) deposited and patterned on an oxidized silicon substrate. One of the electrodes is a recessed microdisk (RMD) electrode at the bottom of the cavity, a second, which is 4 μm away from the RMD, serves as a tubular nanoband (TNB) electrode along the inner wall, and the third (top layer), which is 4 μm away from the TNB and 8 μm away from the RMD, accesses the rim and top surface surrounding the cavity. Work was performed on microcavities that are 50-μm in diameter and 8 μm deep (geometric volume is 16 pL). Immunoassay or DNA-hybridization components are immobilized on the RMD (through self-assembled monolayers of alcohol or carboxylic acid groups) while the nearby TNB (working electrode) and top layer (pseudoreference/auxiliary electrode) perform the electrochemical detection. Non-specific adsorption to the surrounding material, polyimide, of the microcavity device was eliminated.

The fixed, close proximity between detector and modified surface makes low detection limits possible and reproducible, and does not require micromanipulators. The response is fast because of the short distance for enzymatically generated species to diffuse from the RMD to the TNB. Finally, separation of the modified surface from the detecting electrode has advantages over traditional electrochemical sensors where detecting electrodes are also the ones that are modified: (1) the stability of the modified surface is improved because there are no electron transfer events through or changes in potential in that layer, and (2) it allows for a large electrochemical signal because the detecting electrode is bare.

Mouse IgG was chosen as the model analyte for our system, and the general immunoassay procedure for it is based on that described by Heineman and coworkers.[2] This involves immobilization of the primary antibody (Ab, rat-anti mouse IgG), followed by exposure to a sequence of solutions containing the antigen (Ag, mouse IgG), the secondary antibody conjugated to an enzyme label (AP-Ab, rat anti mouse IgG and alkaline phosphatase), and *p*-aminophenyl phosphate (PAPP). The AP converts PAPP to *p*-aminophenol which is electrochemically reversible at potentials that do not interfere with reduction of oxygen and water at pH 9.0, where AP exhibits optimum activity. Detection limits for our microelectrochemical immunosensor for IgG, which have not yet been optimized, are 4.4 nM (6.4 ng/mL) or 880 fmol (129 pg) for PAP_R and 56 fM (9 pg/ml) or 56 zmol (9 fg) for IgG.[3] It takes less than 30 min for the assembly and incubation time. In addition, these results are for the smallest volume combination reported to date for an electrochemical immunosorbant assay (1 μL for the antigen, 1 μL for the secondary antibody-enzyme conjugate, and 200 nL for the electrochemically detected species).

The microcavity device is being modified to detect *C. parvum*. *C. parvum* is a waterborne pathogen which is responsible for deaths of 11,000 children each day and 5 million each year worldwide by invading the gastrointestinal systems of hosts.[4] It exists in an oocyst stage in environmental water systems and in feces from infected animals. The infective dose is only 1 to 132 ooycsts. The oocysts are not eliminated by ordinary water purification methods of drinking water utilities. Existing detection methods are inaccurate and unreliable, have high detection limits (>100 oocysts/L), require long analysis times (2-3 weeks), and cost $250-$500.[5] We will describe progress made toward achieving improved analysis time, cost, accuracy, and precision through the use of our microcavity devices. We are using two approaches, one involves detection of *C. parvum* oocysts through immunoassay chemistry, and the other involves hybridization of DNA probes to the hsp70 mRNA gene of *C. parvum*, which codes for a heat shock protein.

Finally, we will report preliminary work on confining these sensors within microfluidic devices. Such devices maintain better control over volume, delivery of solution, and evaporation. In addition, they offer a means to further improve detection limits and sensitivity.

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