**Materials Considerations in Developing Microelectrochemical Immuno- and DNA-Hybridization Assays**

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The materials that are chosen for electrochemical immuno- and DNA-hybridization assays need to take into consideration not only immobilization of assay components, but also nonspecific adsorption and the extent of passivation of the electrodes. Materials issues become especially important when the assays are miniaturized, involving smaller quantities of analyte and large ratios of surface area of the device to volume of the sample. We will report our recent results on this topic with respect to developing immuno- and DNA hybridization assays with materials that are often of interest in microfabricating analytical devices: gold, silicon, glass, polyimide, poly(dimethyl siloxane) (PDMS), and low temperature co-fired ceramics.

We have demonstrated a novel way to avoid gold electrode passivation by "prefouling" with self-assembled monolayers of organothiols of mercaptooctadecane (MOD).[1] In an electrochemical, sandwich-type immunoassay in a microfabricated cavity for a 1 µL sample of mouse IgG, MOD is used to passivate all gold electrode surfaces. When a surface is required for immobilization of immunocomponents or when detection at a bare electrode is needed, the appropriate gold surfaces are specifically cleaned under potential control.[1,2]

Not only does the MOD protect the electrode surfaces from further fouling, but it also conditions the polyimide material in the immunoassay device. A combination of MOD and bovine serum albumin coats the polyimide surface to prevent nonspecific adsorption of active immunocomponents.[1] This is especially important for reproducibile fabrication and assay response, as well as in developing small immunoassay arrays, where spatial separation of components is needed.

We are now exploring the effect of different materials in new assays for detection of *Cryptosporidium parvum*, a waterborne pathogen which is said to be responsible for 5 million deaths each year worldwide by invading the gastrointestinal systems of hosts.[3] 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 easily eliminated by ordinary water purification methods. 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.[4] We are developing electrochemical assays for *C. parvum* that improve analysis time, cost, accuracy, and precision. 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. The DNA-hybridization exhibits non-specific adsorption to $Si/SiO_2$ surfaces, but not to polyimide. Thus, it appears that down-sizing this assay to the microcavity devices as we have for the mouse IgG assay, where only polyimide and gold are present, is possible.

In order to handle small volumes for small-scale analyses, it is of interest to enclose the samples to avoid evaporation and to use microfluidics to automate analysis steps. Glass and PDMS are common materials for use in these applications. Studies we have preformed with these materials will be briefly addressed. A material that is still fairly new in its use in microfluidics is low temperature co-fired ceramic (LTCC). Three-dimensional devices may be constructed by stacking multiple sheets of the thin, flexible 100 µm-thick "green" (or unfired) material consisting of alumina, silica, and organic binder. The sheets can be easily machined or cut to achieve desired patterns. In addition, screen-printable ink of metals (e.g. gold or silver) can be patterned onto the sheets. Once assembled, the stack is laminated and fired at 800 °C to produce a hard, ceramic device that is durable under aqueous and nonaqueous conditions and may integrate electrical and channel features. We will show how well the gold ink serves as electrode material. In addition, the rough nature of the ceramic surfaces offers interesting possibilities for assay applications. We will present results using the mouse IgG assay. Entrapment, covalent immobilization, and nonspecific adsorption on this material will be discussed.

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