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Flow-through immunosensors using antibody-immobilized polymer monoliths

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ABSTRACT

High-sensitivity and rapid flow-through immunosensors based on photopolymerized surface-reactive polymer monoliths are investigated. The porous monoliths were synthesized within silica capillaries from glycidyl methacrylate and ethoxylated trimethylolpropane triacrylate precursors, providing a tortuous pore structure with high surface area for the immobilization of antibodies or other biosensing ligands. The unique morphology of the monolith ensures efficient mass transport and interactions between solvated analyte molecules and covalently immobilize antibodies anchored to the monolith surface, resulting in rapid immunorecognition. The efficacy of this approach is demonstrated through a direct immunoassay model using anti-IgG as a monolith-bound capture antibody and fluorescein-labeled IgG as an antigen. *In situ* antigen measurements exhibited a linear response over a concentration range between 0.1 and 50 ng/mL with 5 min assay times, while controllable injection of 1 μL volumes of antigen through the monolith elements yielded a mass detection limit of 100 pg (~700 amol). These results suggest that porous monolith supports represent a flexible and promising material for the fabrication of rapid and sensitive immunosensors suitable for integration into capillary or microfluidic devices.

1. Introduction

Biosensors represent an expansive family of detection systems that utilize biological molecules as sensing elements to probe variations in selected physicochemical properties of analyte molecules (Borisov and Wolfbeis, 2008). In comparison to conventional instrumental analysis techniques such as chromatography and spectroscopy, biosensors are generally highly sensitive, selective, compact, and adaptable to on-site or in-field applications. As an important subset of affinity biosensors, immunosensors exploit non-covalent antibody–antigen interactions to detect and quantify target analytes. Due to the unique recognition process and strong affinity of antibody–antigen interactions, immunosensors are highly selective and sensitive, and capable of identifying low abundance species from complex sample matrixes in competitive and noncompetitive assays. In a competitive assay, the sample is mixed with a labeled form of the antigen of interest, resulting in a signal intensity is inversely proportional to the concentration of the unlabeled antigen within the sample. In a noncompetitive assay, unbound sample components are removed from the antibody capture surface after establishing antibody–antigen interactions, followed by quantification of the bound antigen. In a direct immunoassay, captured antigen is measured directly, e.g. by prelabeling the antigen with a fluorescent probe. In contrast, sandwich assays employ antigens with at least two epitopes which can bind to the capture antibodies immobilized on the immunosensor surface as well as a labeled secondary antibody for enhanced specificity and signal amplification (Bange et al., 2005; Borisov and Wolfbeis, 2008). Immunosensors based on these various formats have been widely employed to detect toxins (Ionescu et al., 2004; Konry et al., 2003; Parker et al., 2009), explosives (Bakaltcheva et al., 1999; Van Bergen et al., 2000), pesticides (Kim et al., 2006; Szekacs et al., 2003; Valera et al., 2007), drugs (Anderson and Miller, 1988; Benito-Pena et al., 2005), proteins (Alvarez et al., 2009; Lepesheva et al., 2000), cancer markers (Dai et al., 2003; Munge et al., 2009; Yu et al., 2006), virus (Heinze et al., 2009; Ionescu et al., 2007; Konry et al., 2005; Zuo et al., 2004) and bacteria (Bae et al., 2004; Wang et al., 2008; Yang et al., 2004).

Regardless of the assay type, immobilization of antibodies on a solid support is a key requirement for all immunoassays. In a traditional immunoassay, primary antibodies are adsorbed onto the polymer surfaces of titer plate wells. Alternately, using materials including glass, silicon, quartz, polymers and metals that allow anchoring of antibodies through appropriate surface modifications, a variety of alternative antibody support topologies have been developed, including planar films (Kurita et al., 2006; Rowe et al., 1999; Sai et al., 2006), porous membranes (Tang et al., 2008), optical fibers (McCormack et al., 1997; Narang et al., 1997), nanowires (Bangar et al., 2009; Wang et al., 2008) and microbeads (Biagini et al., 2004; Heinze et al., 2009; Matsunaga et al., 2007). Of par-

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ticular interest are flow-through immunosensor designs, in which sample is hydrodynamically driven past one or more sites with immobilized primary antibodies. Flow-through devices comprising an open-flow path with antibodies bound to the sidewalls have been reported using both silica capillaries (Mastichiadis et al., 2002; Narang et al., 1998) and microfluidic channels (Dong et al., 2007; Gervais and Delamarche, 2009). While flow-through designs can enhance antigen–antibody interactions by increasing mass transport due to the superposition of convective flow on top of simple diffusion, open-flow systems retain the same basic topology as simple planar sensor surfaces, with probe density limited by the two-dimensional nature of the capture surface. One approach to improving the density of primary antibodies within the detection volume is to employ a porous anchoring medium in the flow path. Various porous media have been explored for this purpose in large-scale flow-through systems, including agarose gel (Gonzalez-Martinez et al., 1997) and polymer frits or membranes (Abdel-Hamid et al., 1999; Alvarez et al., 2009; Charles et al., 2000; Jain et al., 2004). These porous materials provide larger surface areas than planar supports for the immobilization of capture species, thereby increasing detection sensitivity, while also providing smaller characteristic diffusion times for antigens or secondary antibodies infused through the micron-scale pores. However, seamless integration of suitable porous media into capillary or microfluidic immunoassays remains a challenge. For example, while packed microbeads have been used to anchor antibodies inside microfluidic channels (Kim et al., 2009), weirs or frits are required to retain packed the beads within the flow path, and defining discrete sensing regions within complex microchannel networks is challenging.

Here we propose an alternative solid support for microscale flow-through immunoassays based on polymer monoliths. Monoliths are highly porous inorganic or organic materials (Svec and Huber, 2006) commonly used as chromatographic stationary phases (Guiochon, 2007) and solid-phase extraction elements (Yu et al., 2001) for analytical separation, sample processing and purification. More recently, monoliths have been applied to a range of microfluidic systems including proteolytic bioreactors (Peterson et al., 2002), mixers (Rohr et al., 2001), valves (Chen et al., 2008) and electrospray emitters (Koerner et al., 2004). Monolith preparation is achieved by a range of polymerization techniques, with free radical polymerization the most commonly used method. Both heat and UV radiation can initiate the polymerization process, with the latter approach suitable for fabrication of monoliths with well defined geometries at specific locations. Monoliths with different porosity, surface area and flow resistance may be synthesized by tuning relative concentrations of monomers, organic solvents and free radical initiator. By incorporating monomers with desired functionalities in the polymerization, reactive moieties are introduced to the monolith surface, thus facilitating covalent immobilization of antibodies and enzymes to the surface. This method has been employed in applications including preparative proteolytic bioreactors (Peterson et al., 2002) and affinity chromatography (Mallik and Hage, 2006) where the digestion of samples and capture of target analytes are performed in monoliths and the products are monitored with downstream detectors in releasing processes. Although originally developed for large-scale sample preparation, the fabrication techniques and surface chemistries of surface-reactive monoliths can be directly adapted to the development of microscale immunosensors.

To demonstrate the utility of monoliths as porous supports for immunoassays, *in situ* photopolymerized epoxide polymethacrylate monoliths have been fabricated within silica capillaries. A multi-step reaction process involving thiol treatment and succinimidyl ester grafting was utilized to activate the monolith, followed by the covalent immobilization of antibodies. The performance of the system was verified through a direct immunoassay test, using mouse IgG as a capture antibody and fluorescein-labeled anti-mouse IgG as a target antigen. Our initial results reveal that monoliths can serve as a novel and effective solid support for the development of rapid, sensitive, versatile immunosensors. The fabrication process is also suitable for further integration of monolithic immunosensor elements into disposable micro-total analysis systems.

2. Experiment

2.1. Materials

Glycidyl methacrylate (GMA), cyclohexanol, 2,2′-dimethoxy-2-phenylacetophenone (DMPA), sodium chloride, potassium chloride, sodium phosphate monobasic, sodium phosphate dibasic, hydrochloric acid (HCl), trimethoxysilylpropyl methacrylate (TPM), N-γ-maleimidobutyryloxy succinimide ester (GMBS), bovine serum albumin (BSA), fluorescein isothiocyanate (FITC)-labeled rabbit IgG (MW ~ 140 kDa) were purchased from Sigma–Aldrich (St. Louis, MO). Goat anti-rabbit IgG, HPLC water, dimethylformamide (DMF), methanol, ethanol and acetone was obtained from Thermo Fisher Scientific (Rockford, IL). Ethoxylated trimethylolpropane triacrylate (SR454) was received as a free sample from Sartomer (Warrington, PA). Polyimide coated silica capillary with 360 μm O.D. and 100 μm I.D. was procured from Polymicro (Phoenix, AZ).

2.2. Monolith preparation

Before monolith preparation, the silica capillary inner surface was treated with TPM for anchoring the monolith. Briefly, two sets of MicroTight fittings and unions (Upchurch Scientific, Oak Harbor, WA) were connected to both ends of a 50 cm long capillary. Acetone, HPLC water, and 0.1 M HCl were then injected using a syringe connected to one of the unions to rinse the capillary. After rinsing, the two unions were capped with gauge plugs (Upchurch Scientific) to seal 0.1 M HCl solution in the capillary, and then the capillary assembly was kept in an oven set at 105 °C for at least 12 h to condition the capillary surface. The conditioned capillary was cooled to room temperature and its polymer coating removed with a lighter. After displacing the HCl solution with HPLC water, a 30% (v/v) TPM ethanol solution was filled in the capillary. Silanization of the capillary surface was allowed to proceed in dark for 24 h. The TPM treated capillary was rinsed with absolute ethanol and dried with nitrogen before use.

To synthesize a monolith section, first, a pre-monolith solution containing 24% (w/w) GMA, 16% (w/w) SR454, 50% (w/w) cyclohexanol, and 10% (w/w) methanol was prepared. Photoinitiator (DMPA) was added to the pre-monolith solution at 1% (w/w) of the combined weight of GMA and SR454. After filled with the pre-monolith, the outer surface of the capillary was coated with an opaque liquid rubber coating (Plasti Dip International, Blaine, MN) except for a 3 mm long section. The masked capillary was exposed to a UV source (PRX-1000; Tamarack Scientific, Corona, CA) with an incident power of 22.0 mW/cm^2 for 360 s, forming a monolith segment within the exposed capillary region. The monolith section was thoroughly rinsed with absolute methanol then 20% (v/v) methanol aqueous solution before further treatment.

2.3. Antibody immobilization

The surface of the GMA-SR454 monolith was modified through a multi-step reaction process. In the first step, a fresh 2 M sodium hydrosulfide solution was prepared by dissolving the compound in a methanol–0.1 M aqueous sodium phosphate dibasic mixture