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Research paper

Surface immobilized hydrogels as versatile reagent reservoirs for microarrays

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ABSTRACT

The accuracy of antibody-based microarrays depends on eliminating or at least minimizing the effect of cross-reactive components. In this work, a method to create a versatile, compartmentalized storage system for antibodies and soluble microarray reagents is described. These containers are made of hydrogel plugs immobilized on a slide facing the corresponding sub-array on a printed surface.

The hydrogels were polymerized using a 4% w/v *N,N*-dimethylacrylamide and 3% w/v bisacrylamide solution, thus generating large pore sizes to allow the facile transfer of intact, functional biomolecules. The hydrogel microenvironments can be desiccated and rehydrated with a desired solution, and they can store reagents in a dry form.

These hydrogels were shown to provide limits of detection similar to those obtained by conventional incubation conditions in an assay for HIV p24 antigen. Moreover, they prevent cross-reactivity issues in the detection of *Staphylococcus aureus* enterotoxins A and B and promote the accurate quantification of interleukin 10 by a microarray-integrated calibration curve.

1. Introduction

In recent years, the use of DNA microarrays has become quite widespread due to their superior utility in molecular profiling of gene expression patterns for prognosis and diagnosis (Ewis et al., 2005). Accordingly, protein microarrays have been adopted to investigate protein expression patterns and the function of entire proteomes (Kung et al., 2009), to identify biomarkers in serum (Bohm et al., 2011) and to predict evolution of cancer disease (Carlsson et al., 2011). Despite their tremendous potential, protein and antibody microarrays still have not drastically changed human diagnostics because technical and operational challenges hinder their implementation in clinical settings (Ellington et al., 2010). Most of the issues in the development of protein microarrays stem from the complexity of protein-based interactions. Unlike the predictable sequence-specific hybridization chemistry of DNA arrays, proteins exhibit extraordinary diversity in their structures and affinities (Gaster et al., 2011). The selectivity of microarray-type immunoassays results from the specificity of the antigen–antibody reaction. In many cases, however, the specificity compromised the ability of other molecules with structurally similar or identical epitopes to bind to the same antibody. This competition is commonly referred to as cross-reactivity. The use of monoclonal antibodies has greatly increased the specificity of immunoassays; similarly, the use of two-site (or sandwich) assays that require two distinct epitopes on the antigen to be recognized greatly decreases cross-reactivity but does not fully eliminate this interference (Diamandis and Christopoulos, 1996). The accuracy of microarray-based immunoassays depends on the elimination or at least the minimization of antibody cross-reactivity.

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The ELISA format is the gold standard for multiplex protein profiling. In this type of assay, the dual binding of the target analytes to the pairs of capture and detection antibodies yields greater specificity than using singular analyte detection. However, when the detection antibodies are used in mixture, as in microarray based multiplex assays, cross-reactivity between non-matched pairs of antibodies is often observed. This effect produces background noise, which affects sensitivity and causes false positive results (Ellington et al., 2010). As a consequence, the development of multiplex immunoassays on a planar microarray format usually requires extensive validation and a time-consuming development process (Gonzalez et al., 2008).

Due to the vulnerability of multiplex sandwich assays to cross-reactivity (Pla-Roca et al., 2012), it was found that the number of required antibody pairs increases with the number of analytes, making the large-scale application of multiplex sandwich assays unfeasible.

To overcome this problem, assay components are physically separated in multiple bead-based assays (Schneiderhan-Marra et al., 2010) or by antibody colocalization microarrays that deliver detection antibodies specifically to the locations of the matched capture antibodies (Pla-Roca et al., 2012). However, multiplex sandwich assays so far have only been used simultaneously for tens of targets (Pla-Roca et al., 2012; Djoba Siawaya et al., 2008).

Recently, the SnapChip method was demonstrated (Li et al., 2012) to solve the problem of cross-reactivity in multiplex immunoassay. In this approach detection antibodies, spotted on a "transfer slide" are transferred at once by snapping on an "assay slide" with capture antibodies and antigens. Though interesting, this approach is limited by the spot volume in the amount of material to be transferred.

In this work, we present the use of hydrogels as a versatile method to create compartments for parallel analysis in microarrays. Hydrogel compartments of different formats can be polymerized on slides, desiccated and rehydrated with solutions containing microarray reagents. During the incubation step, hydrogel plugs are placed on the corresponding sub-array printed on the microarray slide to allow entrapped reagents to be exposed to the printed surface. Hydrogel compartments are versatile reservoirs that can be used to avoid cross-reactivity by separating single detection antibodies, to incorporate samples when low incubation volumes are needed or to contain calibration standards used for accurate quantification. Hydrogel compartments can also be used for long-term reagent storage in dry conditions. Examples of the applicability of hydrogel plugs in microarray technology are shown in several relevant applications. In particular, immunoassay for HIV p24 antigen has been chosen to demonstrate the equivalence in sensitivity when using either hydrogel plugs or standard incubation conditions due to the low limits of detection required by clinical assays for infectious disease diagnostics; interleukin 10 detection has been selected to demonstrate accuracy in quantification when using an integrated calibration curve since evaluation of cytokine expression levels is a critical step in biomarker discovery and development process; detection of *Staphylococcus aureus* enterotoxins A and B is used to show how hydrogel plugs prevent cross-reactivity issues since the detection antibodies selected in this work are good examples of cross-reacting reagents.

2. Materials and methods

2.1. Materials

Phosphate saline buffer (PBS), Trizma base (Tris), HCl, ethanolamine, NaCl, Tween 20, ammonium sulfate, *N,N*-dimethylacrylamide (DMA), 3-(trimethoxysilyl)propyl methacrylate (MAPS), ammonium persulfate (APS), tetramethylethylenediamine (TEMED) and *N,N′*-methylenebisacrylamide (Bis) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyanine 3 labeled goat anti-rabbit secondary IgG (anti-Rabbit IgG Cy3) and rabbit IgG were obtained from Jackson Immunoresearch (West Grove, PA, USA). Silicon oxide slides were bought from Silicon Valley Microelectronics (Santa Clara, CA, USA). Multiwell cell culture slides were purchased from Grace Bio-labs (Redmond, OR).

2.2. Coating of microarray slides with poly(DMA-co-NAS-co-MAPS)

Poly(DMA-co-NAS-co-MAPS)-coated silicon microarrays were fabricated as previously described (Cretich et al., 2004). Briefly, silicon slides were immersed for 30 min in a poly(DMA-co-NAS-co-MAPS) solution (1% w/v in 0.9 M $(NH_4)_2SO_4$). The chips were then rinsed with DI water, dried with nitrogen and finally cured under vacuum at 80 °C. A picture of the silicon slides used in this work is shown in the Supplementary information.

2.3. Fabrication of hydrogel plugs

Multiwell cell culture system slides (see Fig. 1) used to compartmentalize eight hydrogel plugs onto the same slide were pre-treated with oxygen plasma for 10 min. The silanization solution was prepared by dissolving MAPS in toluene at a final concentration of 10% v/v. Aliquots (30 μL) of this solution were added to each well. Slides were stored in a chamber saturated with toluene vapor for 30 min, washed with acetone, dried with nitrogen and cured in a vacuum oven at 80 °C for 30 min. A DMA-Bis solution (4% T, 3% C) was prepared by diluting a stock solution (20% T, 3% C) in deionized (DI) water (where % T is [grams(acrylamide + methylenebisacrylamide) / total volume] × 100 and % C is [grams(methylenebisacrylamide) / g(acrylamide + bis-acrylamide)] × 100). APS (40% w/v) and TEMED were added to give a final concentration of 5 μL/mL for both components. The monomer solution (30 μL) was immediately loaded into each well and polymerized for 30 min at room temperature. After polymerization the slides were washed twice with DI water (10 min each) and with a solution of 1% v/v glycerol in DI water (10 min) on a shaker. The excess water was removed by evaporation with nitrogen, and the hydrogel plugs were finally dried in a vacuum oven at room temperature. The hydrogels at the bottom of the wells were rehydrated simply by filling the wells with a volume of solution containing the protein of interest.

2.4. Microarray experiments: compartmentalization of secondary antibodies

Rabbit polyclonal antibodies against *Staphylococcus* enterotoxin A (SEA) and *Staphylococcus* enterotoxin B (SEB)