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Immobilized Hydrogels for Screening of Molecular Interactions

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Spatially arrayed, high-density microarrays enable the rapid assessment of biological recognition events, and this information is of widespread interest for those working in basic research laboratories as well as in the clinic. Today, one can find DNA, protein, or small molecule arrays. Limitations with these systems include covalent modification of the target complement to the array substrate, array- and target-dependent setup conditions, multiple steps, and loss of hydration at the surface. To overcome these limitations, we have designed, prepared, and evaluated immobilized hydrogels as general screening chambers for small molecule−protein, protein−protein, and nucleic acid−nucleic acid interactions. This biomaterial-based approach is facile, rapid, requires only one setup protocol, and physically entraps the target complement within the polymer network and thus offers advantages over the conventional chips.

Recent advances in our ability to access and analyze genomic and proteomic information has resulted in a significant increase in the rate at which scientific and medical advances are made. These advances range from improved understanding of fundamental biological processes to the evaluation of new drug leads. Rapid assessment of biological information is essential and relies heavily on high-throughput screening of molecular−molecular or molecular−cellular interactions.[1−10] One area of screening that has greatly enhanced our understanding of molecular and cellular processes is microarray technology, which acquires biological information in a spatially arrayed, high-density format. Many of the current formats focus primarily on covalent attachment chemistry of one component to a solid support.[6,11−31] This approach can affect molecular and macromolecular properties as well as create unwanted molecular interactions at the surface, influencing assay outcome and results. Eliminating the necessity for covalent attachment or chemical modification prior to screening may provide a means to explore a greater diversity of interactions. Such strategies have been explored using conventional polyacrylamide chemistry, fluorous-tagged carbohydrate substrates, and self-assembled fibers.[32−34] Herein, we report the preparation and evaluation of immobilized hydrogels as general screening chambers for molecular interactions. Specifically, we are printing molecules and macromolecules without prior chemical modification within three-dimensional hydrogels formed in situ using a one-step process. Using this screening platform, we demonstrate small molecule−protein, protein−protein, and nucleic acid−nucleic acid molecular recognition.

For efficient formation of individual hydrogel chambers on a aldehyde-modified glass surface, we evaluated two-component

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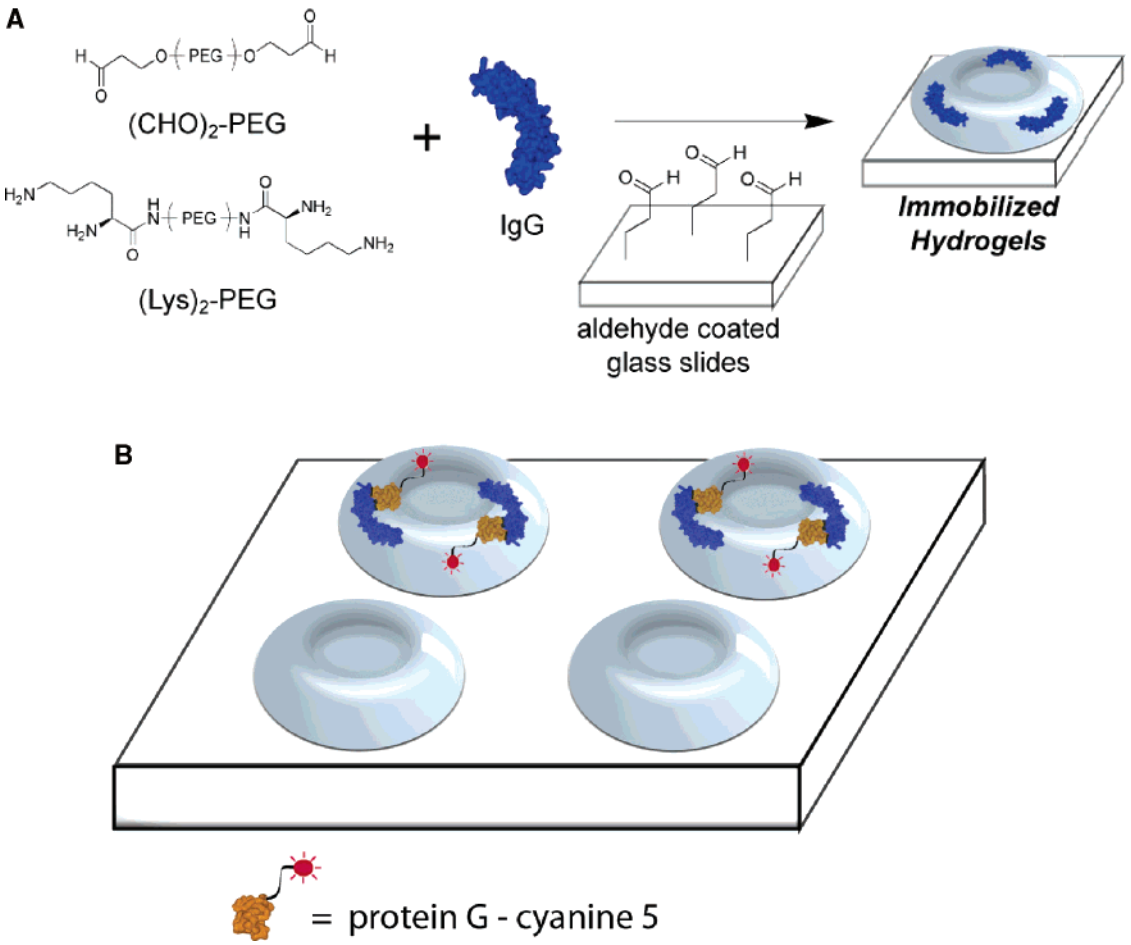

Figure 1. (A) Construction of IgG-immobilized hydrogels on aldehyde-coated slides using $(CHO)_2$-PEG and $(Lys\text{-}NH_2)_2$-PEG. (B) Hybridization of cyanine 5-labeled protein G to hydrogels containing IgG.

systems consisting of tetra- or octaamine functionalized peptide-based branched or dendritic macromolecules[35−38] including [G1]-Lys-NH_2, [G2]-Lys-NH_2, $(Lys\text{-}NH_2)_2$-PEG, or $((Lys\text{-}NH_2)_2)_2$-PEG (see Figure SI, Supporting Information) and a linear poly(ethylene glycol) dialdehyde, $(CHO)_2$-PEG (1:1 reactive functionalities). The $(CHO)_2$-PEG reacts with the terminal amines of the peptide-based branched macromolecules affording a Schiff-base linkage and a highly cross-linked hydrogel network. We found the $(Lys)_2$-PEG and $(CHO)_2$-PEG system to be optimal after a preliminary screen, since it formed a hydrogel chamber for all substrates tested (see Figure SI). Hydrogels possess several favorable properties including a high water content and controlled physical properties as well as have precedent as a medium that supports biological reactions and cellular responses.[39,40] The aldehyde-modified surface is required for formation of an immobilized hydrogel. Specifically, we used an OmniGrid Accent microarraying robot equipped with a Stealth Printhead containing Stealth Micro Spotting Pins to dispense 1-nL volumes of the hydrogel precursors (aqueous solution; pH 7.4; 17% w/w polymer) plus the printing molecule/macromolecule on the aldehyde-coated glass slides. Using this mechanical printing technique, one can print ~50 000 torroid-like hydrogel chambers on an 18 × 72 mm glass slide (Figure 1). After printing, the slides were washed with 1% (w/w) BSA in PBS (pH 7.4) to block the remaining surface aldehydes. Figure 2 shows a photograph of the hydrogel chambers after printing and washing, confirming that the hydrogels remained attached to the glass slide. For the following screens, we used Cy5 red fluorescent dyes instead of Cy3 green dyes since the hydrogel chambers emitted a green background fluorescence signal.

To apply this method where the hydrogel chamber and the printing of the molecule or macromolecule occur simultaneously, we prepared arrays suitable for various screens of molecular interactions. As a prototypical screen for small molecule−protein interactions, we prepared hydrogel chambers containing biotin

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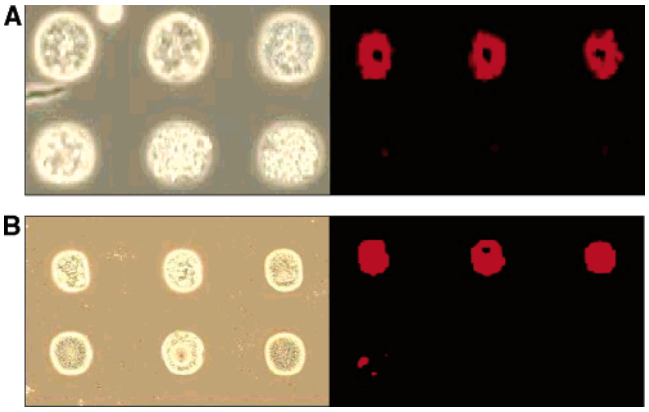

Figure 2. (A) Optical (left) and fluorescent (right) images of hydrogel chambers containing biotin (top row) and controls (without biotin) (bottom row) after probing with Cy5−streptavidin. (B) Optical (left) and fluorescent (right) images of gels containing goat IgG (top row) and controls (without IgG) (bottom row) after probing with Cy5−protein G. Each chamber is ∼200 μm in diameter.

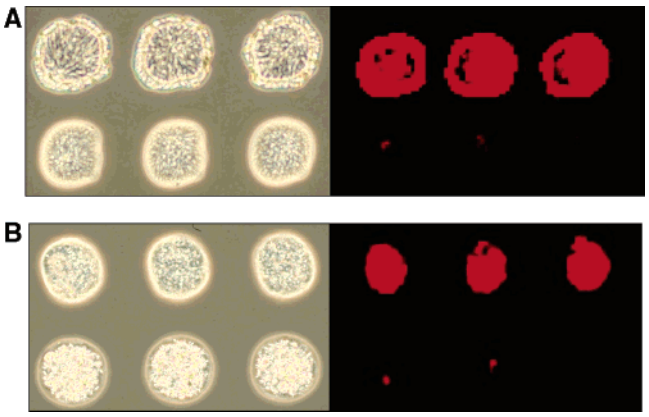

Figure 3. (A) Optical (left) and fluorescent (right) images of gels containing aRNA (250−5000 nt) (top row) and controls (without aRNA) (bottom row) after probing with Cy5−aRNA (60−200 nt). (B) Optical (left) and fluorescence (right) image of gels containing a DNA 20-mer after probing with the complimentary Cy5−DNA 20-mer (top row) and controls (without DNA) (bottom row).

and probed the chambers with Cy5-labeled streptavidin (Figure 2A). The slides were incubated for 1 h at room temperature in a hybridization cassette with buffer to prevent dehydration in air. Cy5−streptavidin was successfully incorporated into the hydrogel chambers containing the entrapped biotin and was shown to be specific, based on a 2-fold increase in red fluorescence relative to controls (hydrogel chambers without biotin). We then assayed for protein−protein interactions by printing goat IgG in the hydrogel chambers and subsequently probing with Cy5-labeled protein G (Figure 2B). After printing the IgG, the slide was hybridized with Cy5−protein G for 1 h at room temperature in buffer. Again, a greater than a 2-fold increase in red fluorescence was observed for hydrogel chambers containing IgG relative to controls (hydrogel chambers without IgG) with a P value 10^{-5}. Two additional control experiments were performed to confirm that the increase in fluorescence was the result of specific protein−protein recognition and not physical entrapment of the complementary protein in the hydrogel chamber during the assay. A hydrogel chamber containing IgG was probed with Cy5−streptavidin, and a second hydrogel chamber containing BSA was probed with Cy5−protein G. In both cases, no significant increase in fluorescence (less than 18%) was observed indicating that hybridization had not occurred and that the noncomplementary protein did not get significantly trapped within the hydrogel chambers.

Finally, we tested for detection of nucleic acid−nucleic acid recognition. Hydrogel chamber containing fragmented antisense RNA (aRNA) was probed with Cy5-labeled aRNA (Figure 3A). The aRNA used in these screens was produced from total RNA isolated from *Saccharomyces cerevisiae* using the RNeasy kit. Cy5−aRNA was then heated at 80 °C for 2 min, quickly spun, and then heated for a few seconds before being applied to the slide. Slides were incubated at 48 °C for 5 h, then washed in a solution of buffer, rinsed with NanoPure water, and dried. The aRNA screen proved to be the most specific, as the fluorescence intensities for hydrogel chambers containing aRNA were ∼8-fold greater than controls ($P = 10^{-12}$). Probing the aRNA with noncomplementary Cy5-labeled aRNA afforded no increase in fluorescence, indicating that hybridization had not occurred and that the noncomplementary Cy5-labeled aRNA did not get trapped within the hydrogel chambers. With this success, we next prepared arrays containing a 20-mer DNA (5′-TGAGTCTTCTAAGCTCTCCG-3′) and probed with the Cy5-labeled complement (5′-Cy5-CGGAGAGCTTAGAA-GACTCA-3′) (Figure 3B). The slide was hybridized for 16 h at 48 °C in a hybridization cassette with buffer, rinsed with NanoPure water, and dried. An increase in red fluorescence of 5-fold was observed for hydrogel chambers containing duplex DNA ($P = 0.01$). Probing the DNA with noncomplementary RNA afforded no increase in fluorescence, indicating that hybridization had not occurred and that the RNA was not trapped within the hydrogel chambers.

In summary, a screening methodology is described that utilizes a hydrogel as a site-isolated hydrogel chamber for biological assays. Monitoring of specific molecular recognition events can be achieved in an unbiased facile manner without modification or chemical attachment of the entities prior to use. Moreover, the hydrogel chambers are amenable to the preparation of arrays for high-throughput screening and to a variety of screening formats from small molecules to proteins and nucleic acids. One potential limitation of this technique is the background green fluorescence of the hydrogel chambers; however, by using other colored fluorescent dyes, this can be easily rectified. Moreover, the hydrogel chambers are amenable to the preparation of arrays for high-throughput screening. This general strategy with hydrogel screening chambers provides new opportunities to study fundamental biological processes as well as a means to rapidly screen therapeutics for a range of molecular targets associated with specific diseases.

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SUPPORTING INFORMATION AVAILABLE

Experimental details and additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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