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Antibody-modified microwell arrays and photobiotin patterning on hydrocarbon-free glass

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Abstract

Antibody-modified microwell arrays were fabricated using site-selective photobiotin chemistry and the biotin–avidin interaction. In one scheme, an aminopropyl-functionalized glass surface was the starting substrate and in the other an acid-treated glass surface was employed. In both schemes, the substrates were coated with the aryl azide photobiotin, and photoactivated with ultraviolet radiation to afford biotin-modified arrays before sequential reactions with ExtrAvidin, biotinylated-IgG, and dye-labeled anti-IgG. Antibody-modified microwell arrays were characterized using fluorescence microscopy. In both schemes, the biotin–avidin–biotin linkage was stable, and there was negligible non-specific binding (NSB) of photobiotin, ExtrAvidin, and dye-labeled anti-IgG to the modified substrates. However, negligible NSB of biotinylated-IgG was observed only with the acid-treated glass surface. To further characterize the nitrene reactions on these hydrocarbon-free glass surfaces, we developed a new protocol using a fluorescent-labeled aryl azide in place of photobiotin. Fluorescence microscopy characterizations of these modified surfaces revealed that detectable fluorescence was observed only from silanol-rich, acid-treated glasses. It is believed this work represents the first demonstration of photo-initiated reactions of photobiotin with reactive silanols on acid-treated glass surfaces.

Keywords: Protein arrays; Biochips; SAED; Luminescence imaging

1. Introduction

Current interest in antibody patterned surfaces is high due to their ability to selectively capture proteins and other important biolytes [1–4]. The diversity of novel antibody immobilization strategies is also high owing to the unique chemical and physical properties of the macromolecules involved, and the variety of chip substrates dictated by the chosen detection platform. In each strategy, the first goal is to stably localize a reproducible amount of functional antibody to the surface [5]. A second goal is to minimize non-specific binding (NSB) reactions [6–16], which can influence an immunoassay's sensitivity and precision and/or the definition and contrast of a patterned surface's features [17,18]. A partial list of pristine and coated substrates that have achieved success in immobilizing proteins includes: amine and aldehyde-silanized glasses [19,20], solid and porous gold [21,22], glassy carbon [23], native oxide silicon [24], aluminized plastic [25], xerogels [26], diamond films [27], dextran films [28], phospholipid bilayers [19,29], electro- and plasma-polymerized films [30,31], molecularly imprinted polymers [32], functionalized self-assembled monolayers [21,33–36], polypeptide-derivatized surfaces [37], and protein- and avidin-modified surfaces [38–41].

Recently, our laboratory demonstrated surface modification chemistry that permits an array of glass microwells to be partially filled with a thin layer of avidin across regions as wide as 1.5 mm (the diameter of the array) or as small as 22 μm (the width of a single microwell) [42]. The approach involved organosilane-functionalized glass, site-selective mask-less photobiotin patterning, and the biotin–avidin interaction. The first key feature of this approach is the biotin–avidin interaction. Avidin is a ~66 kDa egg-white protein with an extremely high affinity (K^{d} ~10^{-15} M) for vitamin H, biotin [43,44]. The biotin–avidin complex is practically unaffected by pH, temperature, organic solvents, and other denaturing agents. The tetravalency of avidin for biotin makes possible the formation of a number of biotin–avidin–biotin molecular complexes (Fig. 1) that permit biotinylated molecules to be attached to (i) covalently-derivatized biotinylated surfaces [45–48], or

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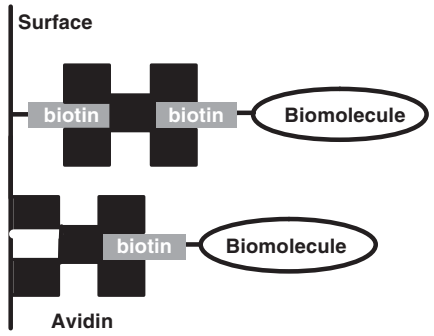
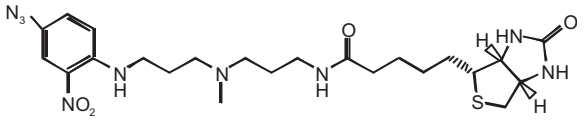


Fig. 1. Schematic diagram of biotinylated biomolecules immobilized to a surface via a biotin–avidin–biotin *{top}* or an avidin–biotin *{bottom}* molecular complex.

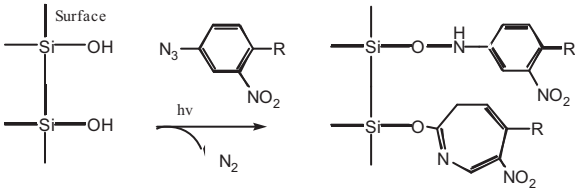
(ii) avidin-modified surfaces created by the direct adsorption or covalent attachment of avidin to the substrate [38,49–54]. Since countless biomolecules can be coupled to biotin (i.e., biotinylated) with negligible loss of activity, the avidin–biotin interaction has become the centerpiece for a variety of bioanalytical applications such as immunoassays, affinity chromatographies, enzyme-based sensing, DNA hybridization assays, and fluorescence activated cell sorting [55–63]. The second key feature of our avidin patterning approach is the use of photobiotin. Photobiotin is a photoactivatable derivative of biotin comprising the avidin-binding moiety, a ~1.6-nm long linker arm, and an aryl azide group. In brief, the exposure of aryl azides



to ultraviolet radiation generates nitrene radicals. Nitrene radicals are extremely reactive and can undergo a multitude of reactions such as insertion into C–H, N–H, and O–H bonds, addition to olefins, and in the case of aryl azides, rearrangement reactions that afford ring-expanded photoadducts [64–71].

There have been two prevalent biomolecule immobilizing strategies utilizing photobiotin. In the first, the starting substrate is an avidin-coated surface that serves to immobilize photobiotin. The resulting photobiotin-coated surface is irradiated in the presence of biomolecules to immobilize them [49,72–74]. An advantage of this approach is that biomolecules do not have to be biotinylated beforehand. In the second strategy, the starting substrate is a hydrocarbon-rich surface. This surface is coated with photobiotin and irradiated to yield a biotin-modified surface that is further reacted with avidin and biotinylated biomolecules. Hydrocarbon-rich surfaces have included polymers [75–77], carbon electrodes [18,77–80], cysteamine-modified gold [81], and organosilane-functionalized glasses and silicon wafers [42,77,80,82]. The advantage of this approach is that the size and position of surface-immobilized avidin domains can be tailored by controlling the size and position of the ultraviolet radiation directed onto the photobiotin-coated surface.

Herein, we present antibody-modified microwell array surfaces fabricated using our previously characterized avidin-modified arrays. Specifically, aminopropyl-modified glass surfaces were photoreacted with photobiotin before sequential reactions with ExtrAvidin, biotinylated-IgG, and dye-labeled anti-IgG. Fluorescence microscopy characterizations demonstrated stable antibody-to-surface binding but revealed that biotinylated-IgG was binding non-specifically to the aminopropyl-silanized surface regions that were not reacted with photobiotin. While coating the glass substrate with an alternative organosilane or polymer that would minimize the NSB could solve this problem, we began investigating the development of a new antibody-patterning scheme for glass that employed photobiotin without requiring a hydrocarbon-rich coating. The approach was partially motivated by a 1991 report where aryl azides were photografted onto bare quartz by nitrene radical reactions with surface silanols [83].



Herein, we also present: fluorescence microscopy characterizations of aryl azide reactions with surface silanols, the fabrication of biotin-modified surfaces afforded by the photo-initiated reactions of photobiotin on acid-treated borosilicate glass, and the use of these novel biotin-modified surfaces to fabricate antibody-modified microwell arrays. The resulting antibody-modified surfaces displayed stable antibody-to-surface binding, and more importantly, negligible NSB of all immobilization reagents including biotinylated IgG.

2. Experimental

2.1. Materials and solutions

3-aminopropyltriethoxysilane (APTS) was purchased from Aldrich Chemical (Milwaukee, WI). *N*-(4-azido-2-nitrophenyl)-*N*′-(3-biotinylaminopropyl)-*N*′-methyl-1,3-propanediamine (photobiotin) and ExtrAvidin™ were purchased from Sigma Chemical (St. Louis, MO). Goat anti-rabbit Texas Red labeled IgG (Texas Red anti-IgG) was purchased from Molecular Probes (Eugene, OR). Biotinylated rabbit IgG was purchased from Pierce Chemical (Rockford, IL). Sulfosuccinimidyl 2-(7-azido-4-methylcoumarin-3-acetamide) ethyl-1,3-dithiopropionate (SAED) was purchased from ProChem (Rockford, IL). Deionized water (18.3 MΩ-cm) was obtained using a Nanopure Infinity water purification system (Barnstead, Dubuque, IA). All other chemicals were from commercially available sources and were of the highest quality available. All chemicals were used as received without further purification.

Phosphate buffer solutions (PBSs; pH 7.4 and 8.5) were prepared by mixing appropriate proportions of a 10 mM dibasic sodium phosphate/200 mM KCl solution with a 10 mM monobasic sodium phosphate/200 mM KCl solution. The 15% (w/w) ammonium bifluoride (NH_4F:HF) etching solution was prepared by diluting a standardized 32.8% ammonium bifluoride solution (Columbus Chemical Industry, Columbus,