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DNA and protein microarray printing on silicon nitride waveguide surfaces

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Abstract

Sputtered silicon nitride optical waveguide surfaces were silanized and modified with a hetero-bifunctional crosslinker to facilitate thiol-reactive immobilization of contact-printed DNA probe oligonucleotides, streptavidin and murine anti-human interleukin-1β capture agents in microarray formats. X-ray photoelectron spectroscopy (XPS) was used to characterize each reaction sequence on the native silicon oxynitride surface. Thiol-terminated DNA probe oligonucleotides exhibited substantially higher surface printing immobilization and target hybridization efficiencies than non-thiolated DNA probe oligonucleotides: strong fluorescence signals from target DNA hybridization supported successful DNA oligonucleotide probe microarray fabrication and specific capture bioactivity. Analogously printed arrays of thiolated streptavidin and non-thiolated streptavidin did not exhibit noticeable differences in either surface immobilization or analyte capture assay signals. Non-thiolated anti-human interleukin-1β printed on modified silicon nitride surfaces reactive to thiol chemistry exhibited comparable performance for capturing human interleukin-1β analyte to commercial amine-reactive microarraying polymer surfaces in sandwich immunoassays, indicating substantial non-specific antibody-surface capture responsible for analyte capture signal.

Keywords: Immobilization; Hybridization; Sandwich immunoassay; Optical waveguide; Silicon nitride; Microarray

1. Introduction

Rapid detection and accurate monitoring of various trace biomolecular targets and interactions remain formidable bioanalytical challenges in developing assays for clinically relevant agents in physiological fluids, hazardous environmental agents, biological warfare agents and high-throughput drug screening. Combinations of optical analysis and direct immunosensing, frequently exploited in integrated optical waveguides (IOWs), represent an attractive sensing modality for these applications because of their high sensitivity and selectivity (Bradshaw et al., 2005; Brecht et al., 1998; Klainer et al., 1997; Ligler et al., 2002b; Misiakos and Kakabakos, 1998; Plowman et al., 1999; Prieto et al., 2003). First described by Marcuse (1974) as thin film dielectrics, IOWs currently comprise three major subclasses: (1) IOW-attenuated total reflection (ATR) spectrometry, (2) waveguide Raman spectroscopy (WRS) and (3) IOW-fluorosensing (IOW-FS) (Plowman et al., 1998). All have history and current interest in biosensing using immobilized capture agents, including nucleotides and antibodies (Clerc and Lukosz, 1997; Gao et al., 1995; Haron et al., 2003; Trummer et al., 2001). Frequently, however, IOW performance in bioassays is limited by surface capture performance, either poor surface immobilization of affinity capture reagents (e.g., antibodies), unacceptable maintenance of surface-bound bioactivity, or unacceptable non-specific non-analyte surface adsorption (noise).

Silicon-based oxides, nitrides and oxynitrides are common IOW materials (Sahu et al., 2000), more robust than polymer waveguides and easily fabricated using conventional silicon microfabrication techniques from bulk silicon substrates, allowing miniaturization and integration of multiplexed, active features within the same optically matched substrates (Valette, 1988). Refractive indices of these materi-

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als can be varied from 1.46 (SiO_2) to 2.2 (Si_3N_4) by altering the nitrogen mass fraction in the source compound during material deposition. Thus, optical waveguide mode size can be controlled without changing the waveguide geometry (Chelnokov et al., 1994). Also, the native silicon oxide surfaces on these nitrogenated materials facilitate organo–silane coupling, one popular initial step in device chemical surface modification to accommodate biomolecule immobilization (i.e., DNA, proteins) (Brennan et al., 1993; Cass and Ligler, 1998; Henke et al., 1997).

Immobilization of biosensing capture agents (e.g., DNA, antibodies, streptavidin) on silicon oxide IOWs is widely reported (Clerc and Lukosz, 1997; Gao et al., 1995; Haron et al., 2003; Trummer et al., 2001). Less is reported concerning analogous modification of potentially useful silicon nitride/oxynitride IOWs. Immunoassay using immobilized rabbit capture antibody and fluorescent-labeled anti-rabbit antibody (10 nM model analyte) on a silicon nitride optical waveguide was exploited to study a three-dimensional microfluidic confinement method (Hofmann et al., 2002). An integrated optical Mach–Zehnder interferometer has been developed for biosensing using a silicon oxynitride waveguide surface functionalized to bind to streptavidin and a biotinylated antibody (Busse et al., 1999). Immobilization of human IgG and goat anti-human IgG antibodies as well as their specific affinity reactions have also been studied using planar polarization interferometry comprising a silicon wafer with a silicon nitride layer sandwiched between two silicon oxide layers. Specific binding of 0.3 ng/ml human IgG model analyte was reported (Nabok et al., 2000).

To impart improved IOW sensing specificity and sensitivity, DNA and protein affinity capture agent immobilization on silicon oxide IOWs using silane coupling agents has also been widely investigated (Hermanson, 1995; Ligler and Rowe Taitt, 2002a). Additionally, nine hetero-bifunctional crosslinkers were investigated for their ability to immobilize active antibodies onto glass. Carbohydrate-reactive crosslinkers exhibited higher antibody immobilization activity than those with reactive succinimide esters but required a procedure that adversely affected antibody bioactivity (Shriver-Lake et al., 1997). The hetero-bifunctional *N*-hydroxysuccinimide (NHS)–maleimide coupling agent, succinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (SMCC), is a popular crosslinker used to immobilize DNA or proteins to silanized silicon-based substrates (Hermanson, 1995; Lateef et al., 2002; Rezania et al., 1999). The thiol-reactive maleimide group coupling by Michael addition is more stable than the amine-reactive succinimide group reacted by nucleophilic displacement, with the latter NHS displacement reaction very susceptible to non-specific hydrolysis even under high-humidity environments (Gong and Grainger, 2004; Hermanson, 1995; Tilstone, 2003). Using maleimide coupling for thiol-terminated DNA or thiolated proteins permits SMCC-activated substrates to be stored for relatively long periods before DNA/protein immobilization, avoiding considerable shelf-life stability issues known for NHS (Gong and Grainger, 2004; Metzger et al., 2002). Recently, DNA oligonucleotide printing onto 1,4-phenylene diisothiocyanate-activated silanized glass and silicon nitride in a printed microarray format has been reported (Manning et al., 2003; Manning and Redmond, 2005). Combinations of array-based sensing and IOWs are of interest for multiple-analyte screening in real-time assays using the IOW surface-capture detection (Bradshaw et al., 2005) and size-based analytical chemistry advantages (Ekins and Chu, 1991).

We report characterization of microarrayed bioassay formats on sputtered silicon nitride films suitable for IOW applications, use of SMCC–silane hetero-bifunctional coupling to immobilize standard nucleic acid and protein affinity capture agents in printed microarray formats, and their resulting target capture capabilities from simple solutions. Comparison of specific versus non-specific capture agent binding to silicon nitride, and subsequent analyte assay performance is described for DNA oligomers, streptavidin, and anti-cytokine antibodies. The intent is to optimize surface-printed microarray capture formats for silicon nitride IOW-based affinity sensing of analytes from solution.

2. Materials and methods

2.1. Materials

N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane (EDS) was obtained from Gelest (Morrisville, PA) and used as received. Sulfosuccinimidyl 4-[*N*-maleimidomethyl]-cyclohexane-1-carboxylate (sulfo-SMCC) was obtained from Pierce Biotechnology Inc. (Rockford, IL, USA). Dye-labeled thiol-terminated 5′-Cy3-DNAoligo1-SH-3′ (DNAoligo1 = CTGAACGGTAGCATCTTGAC-$(CH_2)_6$) and unlabeled 5′-DNAoligo1-SH-3′ were purchased from TriLink Biotechnologies (San Diego, CA). Analogous fluorescent dye-labeled non-thiol-modified DNA oligomers of identical sequence, 5′-Cy3-DNAoligo1-3′ and non-thiolated, unlabeled DNAoligo1, were both obtained from MWG Biotech (High Point, NC). Complementary matched DNAoligo2 (=5′-GTCAAGATGCTACCG TTCAG-3′) with a 3′-Cy5 dye tag was purchased from IDT (Coralville, IA). Murine anti-human interleukin-1β (anti-hIL-1β) and anti-hIL-1β-biotin were obtained from Antibody Solutions (Palo Alto, CA). Recombinant human interleukin-1β target and goat anti-mouse secondary antibody were purchased from Pierce Biotechnology Inc. Biotinylated goat anti-mouse antibody and streptavidin-Alexa Fluor® 647 conjugate were both obtained from Molecular Probes (Eugene, OR). Streptavidin was thiolated using a protein coupling kit from Pierce using their recommended protocols. Murine anti-Flag® M2-Cy3 conjugated antibody (Flag® octapeptide: Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) was obtained from Sigma (Milwaukee,