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Microelectrode array biosensor for studying carbohydrate-mediated interactions

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ABSTRACT

Carbohydrate-mediated host-pathogen interactions are essential to bacterial and viral pathogenesis, and represent an attractive target for the development of antiadhesives to prevent infection. We present a versatile microelectrode array-based platform to investigate carbohydrate-mediated protein and bacterial binding, with the objective of developing a generalizable method for screening inhibitors of host-microbe interactions. Microelectrode arrays are well suited for interrogating biological binding events, including proteins and whole-cells, and are amenable to electrochemical derivitization, facilitating rapid deposition of biomolecules. In this study, we achieve microelectrode functionalization with carbohydrates via controlled polymerization of pyrrole to individual microelectrodes, followed by physisorption of neoglycoconjugates to the polypyrrole-coated electrodes. Bioactivity of the immobilized carbohydrates was confirmed with carbohydrate-binding proteins (lectins) detected by both fluorescent and electrochemical means. The platform's ability to analyze whole-cell binding was demonstrated using strains of *Escherichia coli* and *Salmonella enterica*, and the dose-dependent inhibition of *S. enterica* by a soluble carbohydrate antiadhesive.

1. Introduction and background

Increasing demand for biosensors in fields such as medical diagnostics, basic research, drug design, environmental monitoring, and food safety has led to a rapid expansion in biosensing technologies. Out of the various biosensor architectures, those based on microelectrode arrays stand out as one of the most versatile and promising for studying binding interactions (Jones et al., 2011; Mannoor et al., 2010). Part of this versatility stems from the fact that microelectrode array biosensors can be fabricated using well-established techniques such as complementary metal oxide semiconductor (CMOS) processing, which enables the production of sensitive, compact, and affordable biosensors. Electrode-based arrays are compatible with a diverse range of detection modalities including fluorescence, electrochemical, and label-free, adding further to their versatility. Fluorescent detection is expedient for platform development and research purposes because of the simple readout and amenability to existing fluorescent microarray infrastructure. Additionally, the electrically-active nature of the platform facilitates the development of compact and portable instrumentation based on electrochemical (ECD) and label-free detection. When coupled with microfluidics and portable computers, microelectrode biosensors are readily adapted for distributed point of care (POC) applications (Mannoor et al., 2010; Suehiro et al., 2006).

Immobilized nucleic acid (Schena et al., 1995), protein (MacBeath and Schreiber, 2000), and carbohydrate microarrays (Houseman and Mrksich, 2002; Ratner et al., 2004; Wang et al., 2002) have become instrumental research tools for conducting high-throughput studies of binding interactions. In addition to the ability to conduct multiplexed analysis, microarrays can be fabricated using small volumes of reagent (pl to nl volumes per spot) and the 2-dimensional presentation of binding ligands mimics the cellular surface (Whitesides et al., 1998). While DNA and protein microarrays have been and will continue to be valuable research tools, glycobiology may stand to benefit the most from microarray technology: the structural complexity of carbohydrates and the difficulties involved with their synthesis necessitate high-throughput and low-volume reagent consumption, respectively (Feizi et al., 2003; Kiessling and Cairo, 2002; Liu et al., 2009).

Carbohydrates are involved in a wide variety of biological processes (Taylor and Drickamer, 2003; Varki, 1993) including development (Karlsson, 1999; Kumari et al., 2007), cancer metastasis (Hakomori and Zhang, 1997), and pathogen adhesion to host

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tissues (Karlsson, 1995; Kumari et al., 2007; Whitesides et al., 1998). The ubiquity of carbohydrate-mediated interactions is not surprising given that nearly all secreted and membrane-associated proteins are glycosylated, and a survey of the makeup of the cellular surface reveals that it is abundant in carbohydrate-modified species (i.e. glycolipids, glycoproteins, and glycosaminoglycans) (Voet et al., 2008). Glycoconjugates on the gastrointestinal mucosa are common targets for viral and bacterial adhesion, frequently the first step of pathogenesis (Bavington and Page, 2005; Klemm et al., 2010; Ofek et al., 2003; Sharon, 2006); we are interested in gaining an improved understanding of pathogen-host adhesion because of the potential for immediate and profound impacts on healthcare. Diarrheal diseases, often a result of infection by enteric pathogens, cause 1.8 million deaths worldwide (Morens et al., 2004), a disproportionate number of which are infants and children. Severe diarrhea alone accounts for 18% of worldwide deaths in children under the age of five (WHO, 2005). Moreover, each year in the United States, there are 9.4 million reported cases of foodborne illness, leading to 56,000 hospitalizations and 1,400 deaths, costing more than $23 billion (Cheng et al., 2005; Garthright et al., 1988; Scallan et al., 2011).

Improved methods are needed to identify and further characterize carbohydrate-mediated pathogen binding to host tissues, such as those in the gastrointestinal tract. A platform capable of screening these interactions would facilitate the development of binding inhibitors to disrupt adhesion and thereby prevent pathogenesis. Specifically, soluble carbohydrates have been suggested for bacterial anti-adhesive prophylaxis and therapy (Angstrom et al., 1998; Ofek et al., 2003; Sharon and Ofek, 2000), where they act as competitive inhibitors to the tissue-expressed glycan receptors. The inhibitors decrease bacterial binding or prevent it altogether while minimizing the selective pressure exerted on the pathogen, thus addressing the growing problem of microbial resistance to antibiotics. The effectiveness of this approach has been demonstrated *in vitro* (Barghouthi et al., 1996; Bouckaert et al., 2005), in animal models (Aronson et al., 1979; Ashkenazi, 1994; IdanpaanHeikkila et al., 1997), and in the protection of infants from diarrheal disease by the naturally-occurring glycans present in human breast milk (Morrow et al., 2004; Newburg et al., 2001; Sharon and Ofek, 2000).

In this study we bring together the microelectrode biosensor and the carbohydrate microarray using a highly multiplexed, CMOS microelectrode array to study carbohydrate-mediated ligand-receptor interactions using lectins (carbohydrate-binding proteins) and bacteria. Glycans are covalently linked to bovine serum albumin (BSA) and adsorbed on polypyrrole (PPy) coated electrodes. We have previously demonstrated this approach for immobilizing antibodies and DNA onto the CustomArray (Bothell, WA) microelectrode array (Cooper et al., 2010; Maurer et al., 2010). Herein we describe an extension of this methodology for the functionalization of microelectrodes with glycoconjugates for applications in glycomics research. PPy is deposited via electropolymerization on designated electrodes, and BSA glycoconjugates are adsorbed on the PPy immediately thereafter. We validate carbohydrate functionalization by showing specific binding of lectins to BSA-sugar conjugates using both fluorescence and ECD methods. We subsequently confirm specific bacterial binding with the mannose-binding K12 strain of *Escherichia coli* via fluorescent detection and scanning electron microscopy (SEM). Finally, we demonstrate the utility of this platform for studying carbohydrate bacterial binding inhibitors through the inhibition of mannose-binding *Salmonella enterica* with methyl-α-D-mannopyranoside (αMM). This technology could play a critical role in the development of anti-adhesive prophylactics by indentifying bacteria-carbohydrate binding specificities and characterizing binding inhibitors.

2. Materials and methods

2.1. Materials

A list of materials used in this study and the sources of each can be found in the supplementary information. All buffers were made with ultrapure DI water (Barnstead Nanopure; ThermoFisher Scientific) and brought to the correct pH using 1 M HCl or 1 M NaOH. Phosphate buffered saline (PBS, pH 7.4) contained 10 mM phosphate (1.9 mM KH_2PO_4 and 8.1 mM Na_2HPO_4) with 150 mM NaCl. HEPES buffer (pH 7.3) with divalent cations was composed of 20 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$. PBS with 0.1% Tween-20 (w/v) (PBST) was mixed for washing the chips. Thiolated sugars with oligoethylene glycol spacers (HS-OEG_3-sugar) and thiolated OEG (HS-OEG_3) were synthesized in the Ratner laboratory as previously described (Ratner et al., 2004).

2.2. BSA conjugate synthesis

BSA conjugates were synthesized to provide a facile method to immobilize and display small ligands (biotin and sugars) on PPy-coated microelectrodes. Thiolated biotin, thiolated sugars, and thiolated OEG were attached to the free amines of the BSA via the heterobifunctional cross-linker sulfo-SMCC (see supplementary information for full description). BSA conjugates were stored at a concentration of 4 mg/ml at −20 °C and diluted in buffer to 0.5 mg/ml for functionalization of the microelectrodes.

2.3. Bacterial growth and preparation

All strains of *E. coli* and *S. enterica*, were graciously donated by the Sokurenko lab at the University of Washington (Seattle, WA). All bacteria were genetically modified to either (a) express fimbria containing a terminal FimH mannose-binding adhesin or (b) lack FimH binding adhesin and act as control strains. Details of each strain, including the growth conditions, can be found in the supplementary information. For brevity, the mannose-binding *E. coli* will be referred to as "*E. coli* FimH+" and the non-binding strain will be referred to as "*E. coli* FimH−." The mannose-binding *Salmonella* will be referred to as "*S. enterica* FimH+" and the non-binding *Salmonella* will be "*S. enterica* FimH−."

Following growth, the bacterial suspensions were transferred to 15 ml conical tubes and centrifuged for 5 min at 4000 rcf. The broth supernatant was discarded, and the pellet was washed with 10 ml PBS. The bacteria were then resuspended in PBS and diluted to an OD_{600} of 0.8 (±0.05).

Binding of *E. coli* was detected using labeled antibodies, so no further modification was necessary prior to binding studies. *S. enterica* were labeled with Syto 62, a cell-permeable fluorescent nucleic acid stain. *S. enterica* in PBS (OD_{600} = 0.8) were pelleted by centrifugation at 4000 rcf for 5 min, the PBS was removed, and the bacteria were resuspended in PBS containing 5 μM SYTO 62. After a 15 min incubation, the *Salmonella* were pelleted again at 4000 rcf for 5 min, washed twice with PBS, and then resuspended in PBS containing 0.2% (w/v) BSA at an OD_{600} = 0.8.

2.4. Platform: microelectrode array and instrumentation

The microelectrode array (Fig. 1) and supporting instrumentation were developed by CombiMatrix (Mukilteo, WA; now CustomArray, Bothell, WA), as described in detail previously (Cooper et al., 2010; Ghindilis et al., 2007; Roth et al., 2006). The array used in these studies contains 12,544 platinum microelectrodes, each 44 μm across, fabricated using standard CMOS processing. Each microelectrode is separated from the counter electrode with an insulating layer of silicon oxynitride. The array is

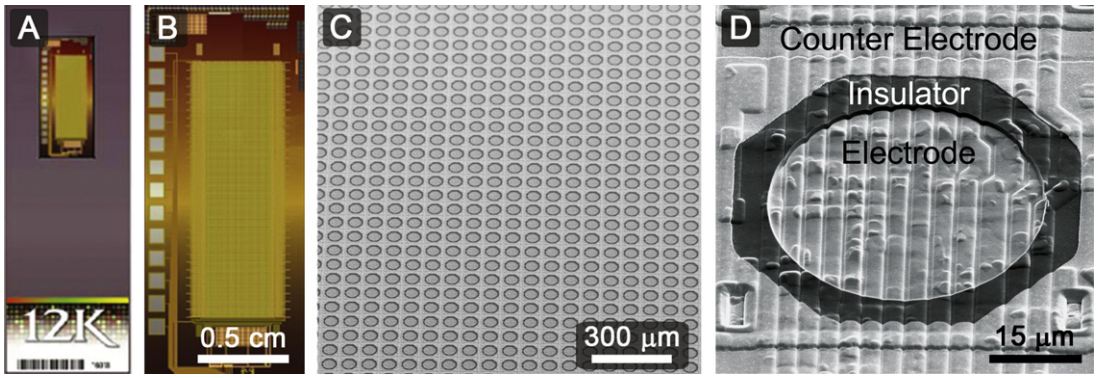

Fig. 1. The CustomArray microarray contains 12,544 individually-addressable microelectrodes. Each electrode is 44 µm across and is separated from the counter electrode by an insulating layer of silicon oxynitride. The microelectrode array at increasing levels of magnification: (A) The array is embedded on a ceramic 1 in. × 3 in. holder; (B) just the microelectrode array; (C) an SEM of a field of microelectrodes; (D) an SEM of a single microelectrode.

embedded in a ceramic support the size of a standard 1 in. × 3 in. microscope slide to facilitate handling and ensure compatibility with fluorescent microarray scanners.

A commercial hybridization cap (hyb cap) is used to isolate the microelectrode array from the electrical contact pads and prevent assay solutions from shorting the electronics. Two hyb cap configurations are available: a single chamber cap allows exposure of all microelectrodes to the same solution, while the four-chamber flow cell divides the microelectrodes into four regions of ~2000 electrodes each. Hyb caps are removable and can readily be switched from one configuration to another (e.g. single-chamber to four-chamber) without disrupting or distorting binding on the microarray. Through on-chip integrated microelectronic circuitry and supporting instrumentation and software (Potentiosense microarray reader, CustomArray), each electrode or designated patterns of electrodes can be specifically controlled and interrogated, facilitating high-throughput and specific deposition of PPy.

The microelectrode array supports both fluorescent detection and enzyme-enhanced ECD (Ghindilis et al., 2007; Roth et al., 2006). Fluorescent labeling of the analyte or of a secondary probe enables detection on a standard microarray scanner, such as the GenePix 4000B (Molecular Devices; Sunnyvale, CA) used in these studies. ECD utilizes the CustomArray ElectraSense®, a palm-sized instrument that can quantitate redox reactions, such as those generated by the reaction of horseradish peroxidase (HRP) with tetramethylbenzidine (TMB) and hydrogen peroxide. The ElectraSense® connects to a personal computer through a USB cable that also provides power to the unit.

2.5. Microelectrode functionalization with BSA-conjugates via polypyrrole

2.5.1. PPy deposition

A functionalization map for the ElectraSense instrument was created using CustomArray software for PPy deposition. The map designates which electrodes to activate, as well as the current to maintain and the amount of time to keep the electrodes activated. PPy was deposited from a solution of 0.1 M pyrrole in 0.1 M sodium sulfate buffer using a current of 90 nA for 1 to 4 s. The optimal deposition time was found to be 2 s, and all quantitative measurements were, therefore, performed on electrodes functionalized using these optimized conditions. The microelectrode array was divided into four regions, each corresponding to a single chamber of the four-chamber flow cell, to control for intrachip heterogeneity and allow screening of up to four analytes per chip. Each one of these regions was further divided into four rows of five 5 × 5 blocks of electrodes, with each of these four rows containing a different biomolecule (Fig. 2). Each row was individually functionalized via a stepwise addition of PPy, biomolecule, BSA block, and rinse (see Array Biofunctionalization section, below).

2.5.2. Array biofunctionalization

Blocking solutions used during the functionalization process were either a saturated casein solution (see Materials in supporting information) or 2% (w/v) BSA in PBS. Bare CustomArray microelectrode microarrays were placed in a single-chamber hyb cap and blocked for 5 min with blocking solution to prevent non-specific binding. The chip was rinsed with PBST (3 times), PBS (3 times), and 0.1 M sodium sulfate (3 times) – the standard wash sequence – prior to the addition of pyrrole solution (0.1 M pyrrole in sodium sulfate) and PPy electrodeposition. Immediately following deposition of the first row of PPy, the array was washed twice with PBS and then blocked again with 2% BSA for 5 min. This row served as a negative control for binding. Depending on the experiment, BSA conjugates were deposited on the next three rows by iterative application of the aforementioned wash sequence, PPy deposition, and bioconjugate functionalization. Each bioconjugate, at a concentration of 0.5 mg/ml, was incubated on the chip for 15 min at room temperature to facilitate adsorption to the most recently deposited row of PPy. To prevent cross-functionalization, the chip was immediately blocked for 3 min with 2% BSA. This process was repeated for each remaining row using the desired biomolecules. A final incubation in 2% BSA for 1 h at room temperature was employed to prevent non-specific binding during subsequent assays. Prior to binding with fluorescently-labeled proteins, HRP-conjugated probe molecules, or bacteria, chips were washed twice with PBS. In most cases, binding assays were performed immediately after functionalization. However, to verify the stability of functionalized arrays, several chips were rinsed with water, dried, and stored over desiccant at room temperature for up to four months. Unless otherwise indicated, all chips contained a control for the protein carrier (BSA), a negative control sugar-BSA conjugate for which the target analyte has no known specificity, and a sugar-BSA conjugate to which the analyte is known to bind.

2.6. Protein and bacterial binding and inhibition

2.6.1. Proteins

For imaging studies, fluorescently-labeled proteins were diluted in buffer (PBS pH 7.4 for all proteins except for ConA, which was diluted in 20 mM HEPES with 150 mM NaCl, 1 mM $CaCl_2$, and 1 mM $MnCl_2$, pH 7.3) to the following working concentrations: streptavidin, 37 nM; ConA, 400 nM; and RCA_{120}, 4 µM. The functionalized chips were incubated with the probe for 30 min and then washed with PBST (twice) and PBS (twice) before the hybridization chamber was removed. The microarray was immediately covered with

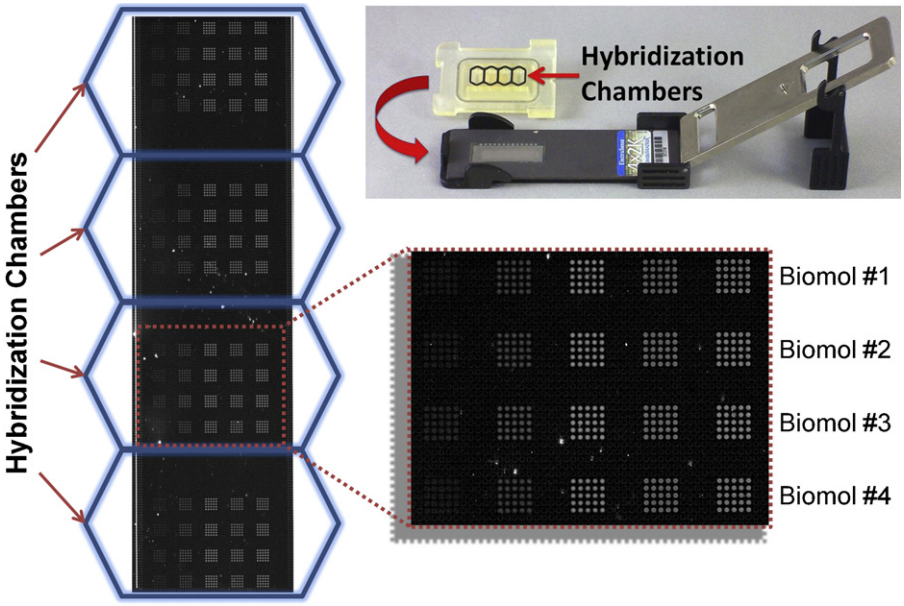

Fig. 2. During the binding assays, the array can be divided into four fluidically-isolated hybridization chambers using the 4 × 2k hyb cap that is secured on the microelectrode array using a custom clamp. The fluorescent image on the left and the magnified inset on the right show the PPy deposition layout that was used for all experiments reported herein. Each sector corresponding to the 4 × 2k hyb cap contains four different biomolecules (Biomol). Image was created using a GenePix 4000B observing PPy autofluorescence at 532 nm. The PPy deposition time was varied for the array in this figure, which can be observed in the difference in fluorescent intensity between columns of 5 × 5 regions of electrodes.

PBS and a cover slip to prevent drying, and imaged on a GenePix 4000B. Arrays were scanned using instrument settings appropriate for DyLight 649 dye (ex: 635 nm, em: 670 nm) with a photomultiplier gain of 400.

For ECD, RCA_{120}-HRP, diluted to a final concentration of 50 μg/ml in 0.1% BSA (w/v) in PBS, was incubated on the chip for 30 min at room temperature. Unbound RCA_{120}-HRP was removed by washing the chip with 2% BSA (twice), PBS (twice), and with pH 4 Conductivity Buffer Substrate (BioFX, Owings Mills, MD) (twice). TMB Conductivity 1 Component HRP Microwell Substrate (BioFX) was added to the array, and it was scanned immediately with the ElectraSense® microarray reader.

The CustomArray software creates a pseudo image of the array where the intensity of individual electrodes, depicted as square pixels, is proportional to the current produced from the HRP-mediated reaction with TMB. The average intensities of each row were plotted for quantitative comparisons.

2.6.2. Bacterial adhesion

In all bacterial adhesion assays, bacterial suspensions were incubated on the chip for 1 h at room temperature and unbound bacteria were washed with PBST (4 times) and PBS (4 times). Bacteria bound to the microelectrodes were detected through either fluorescently-labeled antibodies (for *E. coli*) or a fluorescent nucleic acid label (*S. enterica*). Antibodies against fimbrial protein A (fimA), expressed on *E. coli*, were labeled with DyLight 649 using the same methods as reported for the lectins.

For *E. coli*, the DyLight-labeled antibody probe was incubated on the chip for 30 min at room temperature, and washed twice with PBST and PBS. Since the *S. enterica* were pre-labeled with the nucleic acid stain, these arrays could be imaged immediately after the 1 h binding step. After removing the flow cells, the arrays were covered with PBS and a cover slip to prevent drying, and then imaged at 635 nm with a photomultiplier gain of 400.

2.6.3. Inhibition of bacterial adhesion

Methyl-α-D-mannopyranoside (αMM) inhibition of *S. enterica* binding to mannose was investigated at concentrations of 0.01 mM, 0.05 mM, 0.1 mM, and 1 mM αMM in PBS. Each region of the chip had BSA-mannose and two different control biomolecules (BSA-lactose and BSA only). For inhibition studies, bacteria were labeled with Syto 62 and resuspended in PBS with 0.2% BSA (w/v) and the correct concentration of αMM. Bacterial suspensions were pre-incubated for 30 min at room temperature with the binding inhibitor. After the incubation, bacterial suspensions containing the inhibitor were added to the functionalized microelectrode array using the four-chamber hyb cap to test multiple inhibitor concentrations on the same chip. On each array, one chamber contained *S. enterica* without inhibitor and the three remaining chambers contained *S. enterica* with different concentrations of inhibitor. We also included two controls: (1) a *S. enterica* fimbrial knockout strain (*S. enterica* FimH−) to verify that the bacteria were not binding through other interactions, and (2) *S. enterica* FimH+ incubated with 5 mM galactose in place of αMM to ensure that the inhibition was specific. Attachment was allowed to occur for 1 h before the suspensions were removed and the chip was washed. For washing, each chamber of the four-chamber flow cell was rinsed with 200 μl PBST (3 times), at which point the four-chamber hyb cap was replaced with the single hyb cap and the chip was washed again with PBST (4 times) and PBS (4 times). For imaging, the hyb cap was removed, the array was covered with PBS, a cover slip was added to prevent drying, and imaged at 635 nm with a photomultiplier gain of 400.

3. Results and discussion

3.1. Verification of functionalization approach

Carbohydrate-mediated bacterial binding represents a diverse class of biomolecular interactions that play a significant role in pathogenesis and are of great interest to both basic and applied biomedical research. Our objective is to develop a fundamental research platform that is simultaneously capable of interrogating these host-microbe interactions and screening potential inhibitors against microbial adhesion. To achieve this goal, we required a functionalization strategy that could satisfy the following criteria: (1)

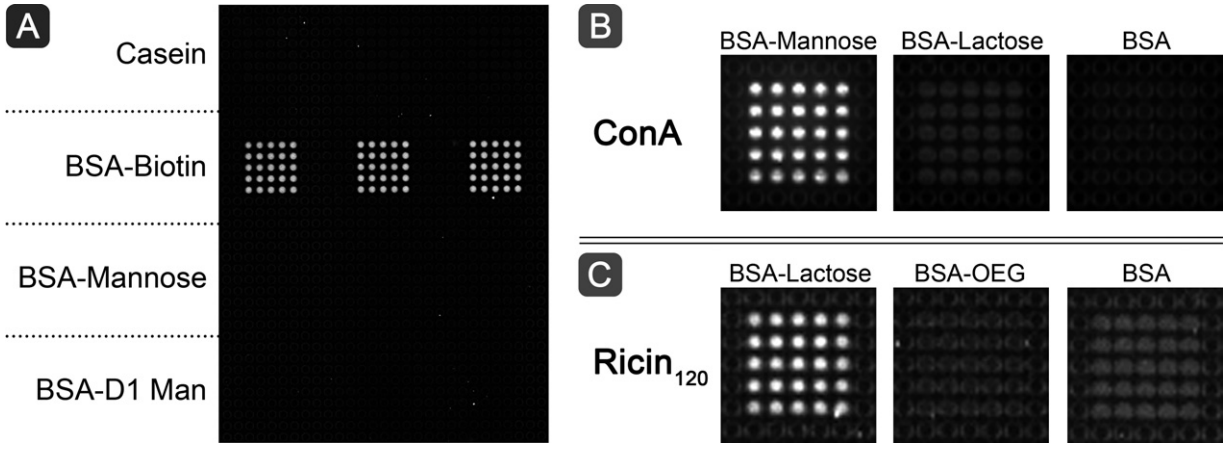

Fig. 3. The PPy–BSA conjugate microelectrode array was tested using several ligands and fluorescently-labeled complementary proteins. (A) A representative sector of the microelectrode array functionalized with three different BSA conjugates and blocked with casein was probed with fluorescently-labeled streptavidin. Binding is specific to biotin, as expected. (B and C) Fluorescently-labeled lectins (ConA and $Ricin_{120}$) specifically bind to their complementary glycoconjugate.

the ability to screen analyte binding to multiple immobilized ligands including controls, (2) a proper display of ligands to allow binding of interrogating proteins and bacteria, (3) reproducible binding between assays, and (4) stable ligand immobilization chemistries. The PPy method meets all of these criteria and is a versatile strategy for microelectrode functionalization (Ahuja et al., 2007; Ramanaviciene and Ramanavicius, 2002). Further, this approach has previously been characterized and optimized for stable and reproducible functionalization using the CustomArray microelectrode array (Cooper et al., 2010).

We confirmed the bioactivity of immobilized small molecule ligand BSA-conjugates using three different binding pairs: biotin-streptavidin, mannose-ConA, and lactose/galactose-RCA_{120}. Thiolated ligands were conjugated to the free amines of BSA using sulfo-SMCC and deposited on specified microelectrodes. Utilizing BSA as a scaffold to display ligands is advantageous because: (1) it reduces non-specific interactions and is frequently used as a carrier and blocking agent, (2) it adheres strongly to PPy, and (3) the BSA neoglycoprotein conjugate mimics the presentation of carbohydrates found at the cell surface. In addition to the specific ligand-BSA conjugate, each array included negative control electrodes with unmodified BSA and orthogonal sugar-BSA conjugates to which the labeled protein should not bind.

Biotin-streptavidin, a model binding pair with strong binding affinity, was employed to validate our microelectrode array platform. Biotin is a useful model for carbohydrates, as it is similar in size to the mono- and disaccharides used for subsequent carbohydrate functionalization. As illustrated in Fig. 3A, fluorescently-labeled streptavidin bound strongly to electrodes functionalized with BSA-biotin and not to either of the controls (BSA-Man and BSA-D1Man). After confirming the functionalization approach with biotin-streptavidin, we tested carbohydrate-lectin binding pairs to demonstrate that the approach would be compatible for interactions with lower binding affinities. Many carbohydrate-mediated interactions are low affinity (in the μM to mM range); a more stable binding state is usually achieved with multivalency, when the lectin binds multiple residues at once (the glycoside cluster effect) (Bertozzi and Kiessling, 2001; Lee and Lee, 1995). The BSA conjugate strategy employed for this study readily facilitates multivalent display through the attachment of multiple pendant ligands to the core BSA scaffold. We demonstrated that fluorescently-labeled ConA and RCA_{120} lectins bound to electrodes functionalized with their complementary BSA-carbohydrate conjugates with low background and low nonspecific binding (Fig. 3B and C).

3.2. Electrochemical detection of ricin

To demonstrate the versatility of the platform, we used ECD to detect RCA_{120} binding to galactose. One of the traditional challenges to ECD with electrode arrays is the technical difficulty of extracting an output signal from each electrode because of the wiring complexity and the need for a multi-channel detector. The integrated microelectronics of CustomArray's chips allows high-throughput electrical readings of the entire array in 15 sec using the ElectraSense®. These signals can be readily analyzed on the computer to which the reader is attached. It has been shown that ECD has equivalent sensitivities as fluorescent detection for genotyping and gene expression assays (Ghindilis et al., 2007); ECD is more attractive for POC applications because the measurement equipment is compact, less expensive, and more robust. In this work, the ElectraSense® can be used for both PPy deposition and for acquiring ECD data, thus eliminating the need for additional equipment (e.g. fluorescent microarray scanner). An HRP-mediated redox reaction generated a current that was measured by each electrode (Fig. 4A). Measuring these outputs, the ElectraSense® produced a data file that contains the total current detected on the microelectrode array. This data file was analyzed and converted into a pseudo image where the intensity of each pixel corresponded to measured electrical activity. ECD was successfully applied to detect ricin binding to BSA-galactose, as shown in Fig. 4B and C.

3.3. Mannose-mediated capture of E. coli

Following the validation of the functionalization approach, we investigated the bioactive microelectrode platform for studying whole-cell carbohydrate-mediated bacterial interactions. To test bacterial binding, we used the well-known interaction of type-1 fimbriated K12 *E. coli* and mannose (Ofek and Doyle, 1994; Ofek et al., 1977; Sokurenko et al., 1995). Type-1 fimbria bind mannose through the FimH lectin domain which is expressed at the fimbrial tip (Choudhury et al., 1999; Krogfelt et al., 1990). The array was functionalized with BSA-mannose and BSA-lactose (orthogonal sugar control), BSA-OEG (linker control), and BSA (blocking control). As an additional control, arrays containing BSA-mannose and BSA-blocked electrodes were also probed with *E. coli* FimH−, which lacks the FimH binding lectin. Binding of *E. coli* FimH+ with mannose was verified: *E. coli* FimH+ bound specifically to mannose-coated electrodes (Fig. 5A–C) while *E. coli* FimH− did not show an observable binding response (Fig. 5D). These binding results were further confirmed using SEM (Fig. 5B and C), demonstrating

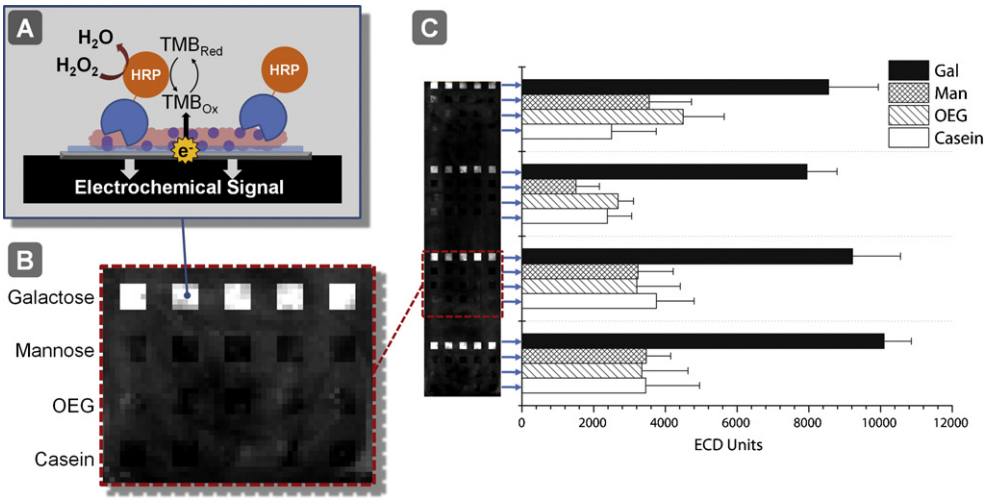

Fig. 4. Electrochemical detection of ricin binding to galactose-functionalized electrodes. (A) The reduction of TMB solution by the HRP label on the ricin produces a flow of electrons away from the electrodes. CustomArray instrumentation and software (the ElectraSense®) detects the localized current on each electrode. (B) A pseudo image is generated where the intensity of each pixel corresponds to the electrical activity detected on the microelectrode array. (C) The average signal from each row of electrodes, corresponding to a single glycoconjugate, is shown graphically. (Gal = Galactose; Man = Mannose; OEG = oligoethylene glycol).

the ability of the CustomArray microelectrode biosensor to detect specific binding interactions between bacteria and their complementary carbohydrate ligands immobilized on the microelectrodes.

A common problem with microarrays and pre-functionalized biosensors is their declining bioactivity over time. In order to test the long-term stability of the modified microelectrodes, we stored a functionalized array for four months at room temperature over desiccant. This array is shown in Fig. 5D, which also demonstrates that the *E. coli* binding was FimH-mediated. As can be seen, the BSA-mannose conjugate retained its activity, as indicated by binding of *E. coli* FimH+. Further, non-specific binding remained low and *E. coli* FimH− did not bind to the aged surfaces.

3.4. Inhibition of Salmonella binding to mannose-functionalized electrodes

Bacterial binding inhibition studies were performed to demonstrate a medically relevant application of the microelectrode biosensor. As previously discussed, anti-adhesive therapies are gaining interest in the medical community to prevent and treat bacterial infections. High-throughput tools are needed for screening

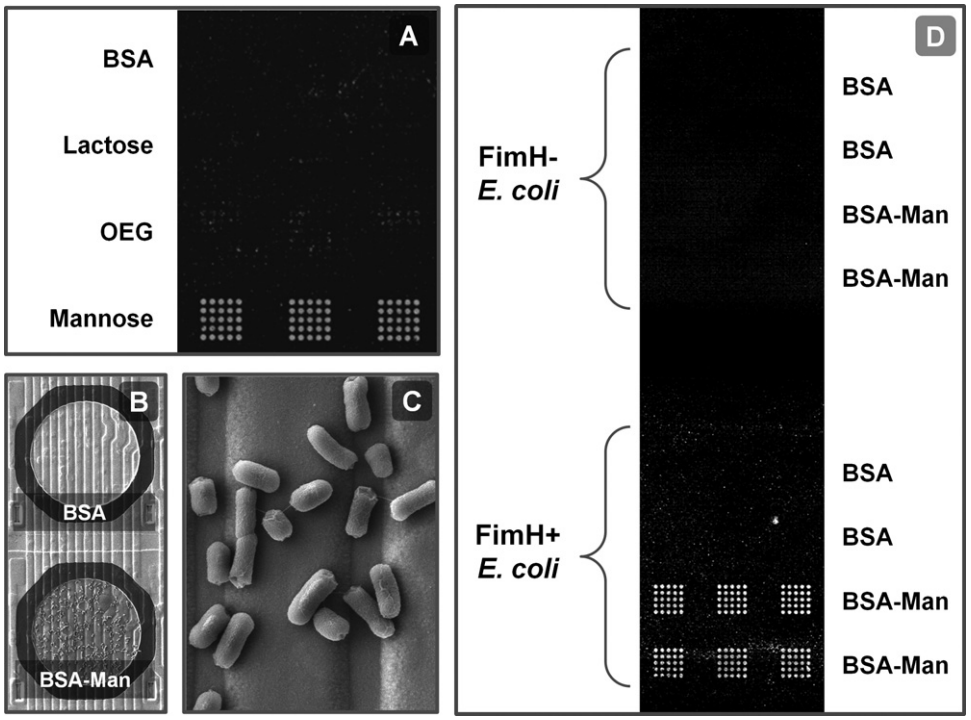

Fig. 5. Verification of the platform for detecting carbohydrate-mediated bacterial adhesion using type 1 fimbriated *E. coli* binding to BSA-mannose conjugates. (A) *E. coli* binding visualized using fluorescently-labeled antibodies reveals binding to BSA-mannose with low non-specific binding. (B and C) SEM confirms the binding specificity seen using fluorescence. (D) A FimH knockout strain of *E. coli* (FimH−) does not bind to BSA-mannose, confirming the FimH mediated interaction. FimH- and FimH+ *E. coli* were incubated on the same array using the 4 × 2k Hyb cap. This chip was stored at room temperature for four months and thus demonstrates an extended shelf-life for pre-functionalized arrays.

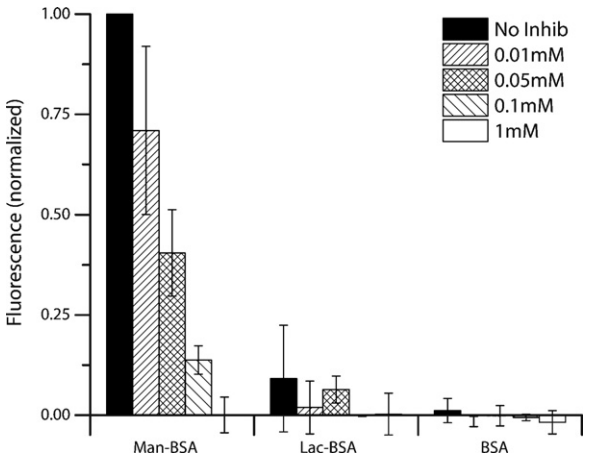

Fig. 6. *S. enterica* binding to mannose-BSA is inhibited in a dose-dependent manner. Methyl-α-D-mannopyranoside (aMM) was pre-incubated with the bacteria at concentrations of 0.01, 0.05, 0.1, and 1 mM aMM. Error bars represent one standard deviation from the mean of the fluorescent intensities from at least four different microarrays. Values were normalized to the fluorescent intensity of uninhibited *S. enterica* on each microarray.

different binding inhibitors for their inhibitory strength prior to their use in *in vivo* experiments. We investigated the use of our bioactive CustomArray platform for studying the inhibition of mannose-binding strain of *Salmonella* (*S. enterica* FimH+) by αMM as a model for screening bacterial anti-adhesives.

As shown in Fig. 6, dose-dependent inhibition of *S. enterica* FimH+ was observed using concentrations of αMM ranging from 0.01–1 mM. An IC_{50} of 40 μM was determined from these results. The inhibition results were obtained as an average of all electrodes across at least four different arrays (i.e. four different experiments), demonstrating the ability of this functionalization method to obtain reproducible experimental results, a requisite feature for the implementation of this platform for studying bacterial binding inhibition.

4. Conclusions

This study demonstrated the successful application of a functionalized CMOS microelectrode microarray biosensor for use in interrogating carbohydrate-mediated protein and bacterial interactions. Employing a versatile PPy-based strategy capable of selectively modifying individual electrodes within the array, this platform was used to determine the IC_{50} for a specific carbohydrate bacterial binding inhibitor for FimH expressing *S. enterica*. In addition to fluorescent detection, ECD was used to highlight the multimodal detection capabilities of the platform and its potential to be used outside of a traditional laboratory setting. The microelectrode biosensor and the microarray naturally combine to create a single high-throughput platform for identifying bacterial binding specificities and for screening of binding inhibitors.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2012.02.017.

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