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ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 2012

Impact Factor: 5.64 · DOI: 10.1021/ac300025v · Source: PubMed

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Activated Paper Surfaces for the Rapid Hybridization of DNA through Capillary Transport

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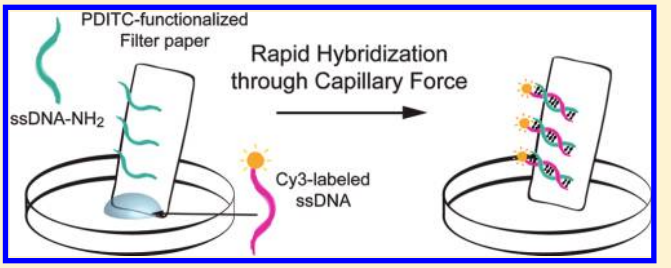
Supporting Information

ABSTRACT: The development of low-cost, accurate, and equipment-free diagnostic tests is crucial to many clinical, laboratory, and field applications, including forensics and medical diagnostics. Cellulose fiber-based paper is an inexpensive, biodegradable, and renewable resource, the use of which as a biomolecule detection matrix and support confers several advantages compared to traditional materials such as glass. In this context, a new, facile method for the preparation of surface functionalized papers bearing single-stranded probe DNA (ssDNA) for rapid target hybridization via capillary transport is presented. Optimized reaction conditions were developed that allowed the direct, one-step activation of standard laboratory filters by the inexpensive and readily available bifunctional linking reagent, 1,4-phenylenediisothiocyanate. Such papers were thus amenable to subsequent coupling of amine-labeled ssDNA under standard conditions widely used for glass-based supports. The intrinsic wicking ability of the paper matrix facilitated rapid sample elution through arrays of probe DNA, leading to significant, detectable hybridization in the time required for the sample liquid to transit the vertical length of the strip (less than 2 min). The broad applicability of these paper test strips as rapid and specific diagnostics in "real-life" situations was exemplified by the discrimination of amplicons generated from canine and human mitochondrial and genomic DNA in mock forensic samples.



Since the first production of paper sheets from cellulosic plant fibers centuries ago, paper-based materials continue to be harnessed in a growing number of applications, motivated, in part, by a low-cost, high strength-to-weight ratio, and inherent resource renewability. Beyond traditional uses as a medium for writing and the graphic arts, paper and paperboard are used widely in advanced technical areas such as food packaging, barrier applications (e.g., disposable protective clothing, masks, and sheeting), and filtration. With a vision toward value-added applications, increasing attention is now being focused on the development of "bioactive paper," that is, advanced, cellulose-based functional materials that have the capacity to bind, detect, and/or deactivate biological substances, ranging from small molecules, proteins, carbohydrates, and nucleic acids to whole organisms such as fungi, bacteria, and viruses.[1−3] Indeed, there is a diversity of potential uses for bioactive papers, including environmental remediation, security applications, "smart" (i.e., indicator) packaging, pathogen filters, and not least, medical diagnostics.[4]

A key advantage of cellulosic paper over competing substrates such as glass or petrochemical-derived plastics (beyond weight or environmental concerns) is the intrinsic porosity of the sheet structure, which facilitates chromatographic separations and inexpensive microfluidics devices based on capillary flow. The functionality of paper-based diagnostics can be further elaborated by printing, coating, or impregnation technologies, as well as covalent chemical modification.[5] As such, paper-based assay platforms have been available since the mid-20th century, perhaps the most well-known example of which is the pregnancy test stick, an immunochromatographic assay based upon a lateral-flow device. Recent times have however witnessed a resurgence in the development of paper-based assays, using paper either as a separation medium or as a sample capture and transport matrix in "dipstick" analyses.[6−14]

Received: January 3, 2012
Accepted: February 27, 2012

   dx.doi.org/10.1021/ac300025v | *Anal. Chem.* XXXX, XXX, XXX−XXX

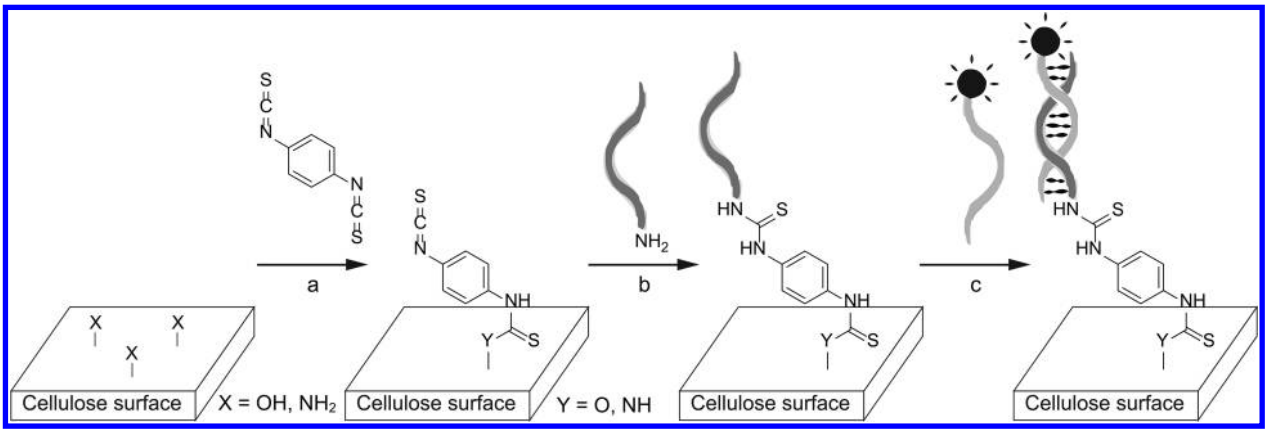


Figure 1. Schematic illustration of the methodology of activation of cellulose surfaces with 1,4-phenylenediisothiocyanate (PDITC) for DNA immobilization and subsequent rapid hybridization. (a) Surface activation with PDITC, (b) immobilization of aminated ssDNA probes, and (c) hybridization with complementary Cy3-labeled ssDNA.

Nonetheless, the development of paper-based diagnostic tests, which are at the same time simple, affordable, sensitive, specific, rapid, and robust, remains a major challenge in many application areas.[15−17]

In particular, the past decade has witnessed a significant increase in interest for new and improved deoxyribonucleic acid (DNA)-based diagnostic tests,[18] and there presently exists significant scope to further expand the use of cellulose-based paper substrates for the specific capture and detection of DNA originating from clinical or forensic samples. DNA-functionalized glass surfaces have traditionally been powerful tools for the hybridization-based isolation, detection, and analysis of specific DNA sequences. However, hour-long hybridization times and detection methods that require expensive and bulky equipment have imposed limitations on the broad commercial application of glass-supported DNA assays, especially in field diagnostics, including those in resource-poor regions.[16]

In this context, we present here a new, facile method for the preparation of surface functionalized papers that bear single-stranded probe DNA (ssDNA), for rapid target hybridization via capillary transport (Figure 1). The intrinsic wicking ability of the paper matrix, in harness with optimized surface chemistry for the covalent attachment of ssDNA sequences in an array format, facilitated rapid sample elution and sequence-specific hybridization. As a practical example, these paper-based arrays were able to accurately differentiate sequence-tagged amplicons of canine and human DNA in mock forensic samples.

MATERIALS AND METHODS

Chemicals and Substrates. *N*,*N*-Dimethylformamide (DMF) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich and dried over 4 Å molecular sieves for at least 24 h prior to use. Ultrapure water (18 MΩ·cm) was obtained from a Milli-Q purification system (Milipore) and was used in all experiments. 1,4-Phenylenediisothiocyanate (98%, PDITC) was obtained from Sigma-Aldrich. Whatman filter paper sheets (7.5 × 10 cm) grade 1 (catalog no. 1001-824) were used as a cellulose surface for ssDNA array production and Whatman filter paper disks (diameter ⌀ = 1 cm) grade 1 (catalog no. 1001-6508) were used in the binding isotherm determinations. Xyloglucan (XG, M_w 4345 Da, M_w/M_n 1.2) and amine-functionalized xyloglucan (XG-NH_2,[19] synthesized from the aforementioned XG by reductive amination) was obtained from SweTree Technologies (Stockholm, Sweden).

Preparation of Activated Surfaces. *Binding Isotherms of XG-NH_2 on Cellulose.* Whatman No. 1 filter paper disks (⌀ 1 cm, total mass 14 mg) were immersed in 700 μL of an aqueous solution containing 0.11, 0.22, 0.43, 0.87, 1.75, 3.5, and 7 mg of XG-NH_2 in glass vials and incubated at room temperature with orbital shaking for 24 h. The amount of XG-NH_2 adsorbed onto cellulose was measured by a subtractive ninhydrin assay:[20] The amount of XG-NH_2 in solution (after equilibrium) was calculated from a standard curve with XG-NH_2 solutions of increasing concentrations (Figure S-1, Supporting Information). The amount of bound XG-NH_2 was then calculated by reduction of the amount of XG-NH_2 in solution from the amount of XG-NH_2 added to the vial ($XG_{sorbed} = XG_{added} - XG_{solution}$, Figure S-2, Supporting Information).

Preparative Adsorption of XG and XG-NH_2 onto Filter Paper. Whatman No. 1 filter paper sheets (7.5 × 10 cm, total mass 630 mg) were each cut in half lengthwise immersed in 32 mL of an aqueous solution of XG or XG-NH_2 (32 mg) in a rectangular glass tray with a glass cover. The mixture was placed on an orbital shaker for 24 h at room temperature, after which time it was washed twice with water (2 × 60 mL) for 5 min and dried under a gentle stream of air. Under these conditions, 50% of the total amount of the XG-NH_2 was sorbed into cellulose at this concentration (cf. Figure S-2, Supporting Information).

Activation of Filter Paper with PDITC. Unmodified filter paper (FP), filter paper containing adsorbed XG-NH_2 (FP/XG-NH_2), and filter paper containing adsorbed nonfunctionalized XG (FP/XG) were immersed independently in a series of solutions of PDITC, either in dry DMSO (250−1 mM) or dry DMF (500−1 mM), in a glass container (30 mL per sheet) and placed under orbital shaking for 12 h at room temperature, after which time the sheets were washed twice with DMSO or DMF and water (each with twice the volume of the PDITC solution) for 5 min and then dried under a gentle stream of air.

Oligonucleotide Design. Oligonucleotides for immobilization and detection were designed using software developed in-house, and ordered from the manufacturer (Sigma-Aldrich, St. Louis, USA and MWG, Ebersberg, Germany). Unique sequences for surface immobilized oligonucleotides T_1 through T_4 (Table S-1, Supporting Information) were created through generating 30-mer randomized ID tag sequences and mapping against the human and dog genomes, and to each other. The top ranking sequences with maximum difference to each other and minimum cross-reactivity were selected.

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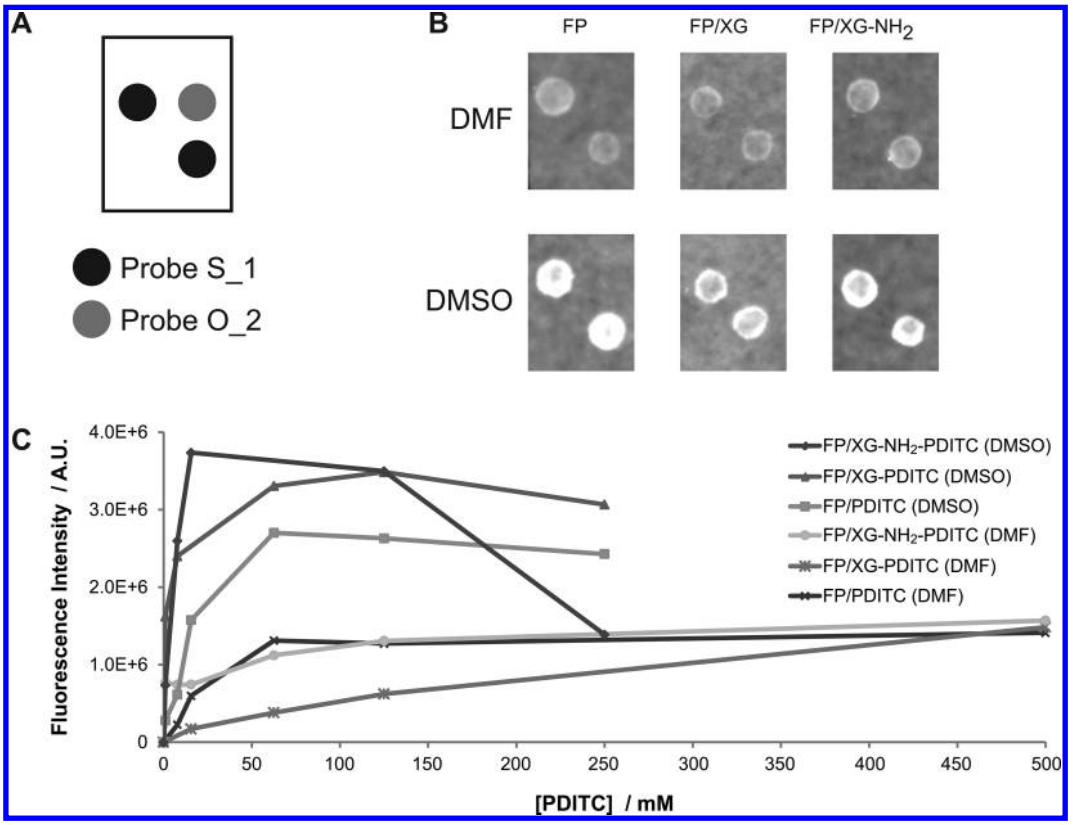

Figure 2. Cellulose surface chemistry optimization. (A) Schematic representation of the probe array used for optimization experiments (full probe sequences are given in Table S1, Supporting Information). (B) Fluorescent images of filter paper surfaces activated by different surface treatments following hybridization. In all cases, paper strips were reacted with 15.6 mM PDITC in either DMF or DMSO. FP is native Whatman No. 1 filter paper, FP/XG is Whatman No. 1 treated with xyloglucan (XG), and FP/XG-NH_2 is Whatman No. 1 treated with aminated xyloglucan. (C) Measured fluorescence intensity (A.U., arbitrary unit) as a function of PDITC concentration for all activated filter paper surfaces. In the absence of PDITC treatment, fluorescence was undetectable for all surfaces (FP, FP/XG, and FP/XG-NH_2) in both solvents (DMSO and DMF).

Sequences S_1, O_2, and PS_1 (Table S-1, Supporting Information) were generated with less stringency and were used as test probes for surface chemistry optimization.

Primers for polymerase chain reaction (PCR) amplification of unique target sequences in dog mitochondrial DNA, dog genomic DNA, human mitochondrial DNA, and human genomic DNA, respectively, were designed to incorporate ID tag sequences, which matched the surface probes in the resulting amplicons (Table S-2). Synthetic probe PS_1 was used as a positive control.

Array Preparation. DNA arrays were prepared on the surface of the activated filter paper chips through either manual or robotic printing.

Manual Printing. To analyze the optimization of surface chemistry for PDITC activation of filter papers (FP, FP/XG, and FP/XG-NH_2), paper "chips" (0.8 cm × 1.2 cm) were cut from larger sheets. Two synthetic oligonucleotide probes were used (S_1 and O_2, Table S1, Supporting Information) and an array pattern of three features per filter paper chip (Figure 2A) was printed manually by pipetting 0.1 μL solutions containing 20 μM oligonucleotide probe in 50 mM sodium phosphate buffer, pH 8.5, for each of the probes. After spotting, the paper chips were stored in a humidification chamber at room temperature overnight.

At this time, the chips were treated by submersion in a prewarmed (50 °C) "blocking" solution of 0.1 M TRIS and 50 mM ethanolamine, pH 9.0, to quench unreacted isothiocyanate groups. Chips were then washed sequentially with deionized water (3 × 2 mL, 2 min), a prewarmed (50 °C) solution of 4× saline-sodium citrate (SSC) buffer containing 0.1% sodium dodecyl sulfate (2 mL, 30 min), and deionized water (3 × 2 mL, 2 min), followed by drying in air.

Robotic Printing. Arrays of oligonucleotide probes were printed with an array printer (Nano-Plotter NP2.1, GeSiM, Germany) on PDITC-activated FP/XG-NH_2 surfaces. A pattern of eight arrays per filter paper chip (7.5 × 2.5 cm) was prepared by plotting a solution containing 20 μM oligonucleotide probe in 50 mM sodium phosphate buffer, pH 8.5, respectively for each probe. Six features were included in each array, consisting of four oligonucleotide ID_tag probes (T_1 through T_4), one positive control (S_1), and one negative control (buffer only) (Figure 3A). Following printing, chips were maintained in a humid atmosphere overnight, blocked with ethanolamine, washed, and dried, according to the protocol described above for manually printed chips.

Preparation of Sample DNA. DNA from canine blood samples and human blood samples had been previously extracted using a commercial DNA extraction kit (DNeasy, Qiagen, Carlsbad, CA, USA).

Amplification of Target DNA. Four pairs of primers were designed to target unique amplicons in dog mitochondrial DNA, dog genomic DNA, human mitochondrial DNA, and human genomic DNA, respectively. Primers were modified with biotin and Cy3 tags to allow processing into single-stranded DNA products by magnetic bead capture and subsequent detection by fluorescence, respectively.

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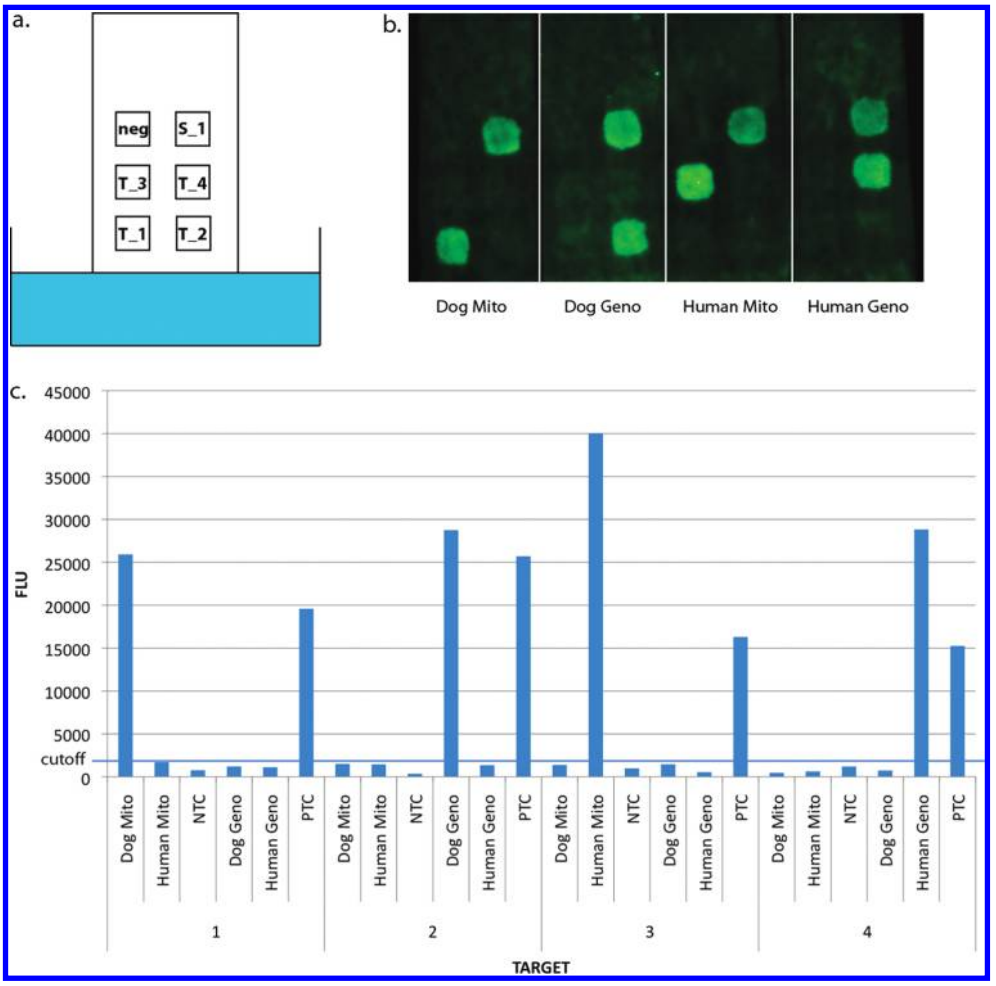

Figure 3. Mock forensic application of filter paper-based DNA arrays. (A) Array configuration. The immobilized oligonuleotides are complementary to (T_1) Dog Mitochondrial amplicon, (T_2) Dog Genomic amplicon, (T_3) Human Mitochondrial amplicon, and (T_4) HumanGenomic amplicon. (neg) is a negative control and (S_1) is complementary to the positive control probe PS_1 (see Table S-1 for full sequences). (B) Fluorescent detection of Cy3-labeled hybridized ssDNA sample. For visualization, a cutoff was set according to the mean signal of the maximum recorded background. (C) Signal intensities from the four detected samples show that intensities (FLU, arbitrary fluorescence units) are similar among the samples and the corresponding positive controls (PTC), and that both background signal measured at the negative control (NTC) spot and nonspecific hybridization are minimal.

Each 50 μL PCR mixture contained 1U Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA), 1X Platinum Taq PCR buffer (Invitrogen), 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 200 nM each of a Cy3-labeled forward primer and a biotin-labeled reverse primer, and 1 μL extracted sample DNA. The reaction mixture was processed at 94 °C for 5 min, and cycled 45 times through 94 °C for 30 s, 58 °C for 40 s, and 72 °C for 60 s, and after the cyclic amplification final elongation step of 72 °C for 10 min was performed.

Generation of Single-Stranded DNA. Ten microliters of 1 μm streptavidin-coated paramagnetic beads (MyOne C1, Dynal, Invitrogen) were pipetted into a 1.5 mL tube and put on a magnetic stand to remove the bead storage buffer. Three picomoles of PCR product and an equal volume of 2× Bind and Wash buffer (10 mM Tris-HCl, 1 mM ethylenediaminetetraacetic acid, 2 M NaCl, 1 mM β-mercaptoethanol, 0.1% Tween 20, pH 7.5) was added to the beads and the final mixture was incubated at 50 °C for 15 min with 10 s of mixing every 30 s at 300 rpm in a Thermomixer Comfort Exchangeable MTP thermoblock (Eppendorf, Hamburg, Germany). Double-stranded amplicons were denatured using 11 μL of 0.1 M NaOH to generate single-stranded DNA. The supernatant containing the eluted Cy3 labeled strand was kept and neutralized with 10.2 μL of 0.1 M HCl. 1× Klenow Fragment reaction buffer (Fermentas, St. Leon-Rot, Germany) was added to the collected sample to a total volume of 30 μL.

Hybridization by Capillary Transport. For surface chemistry optimization experiments, 25 μL of 0.5 pmol single-stranded PS_1 target (Table S-2, Supporting Information) in 1× Klenow Fragment reaction buffer (Fermentas, St. Leon-Rot, Germany) was placed as a drop in the bottom of a Petri dish prior to capillary force hybridization. For experiments with ssDNA obtained from dog and human DNA, 0.5 pmol single-stranded PS_1 probe was mixed with half of the sample collected after single-strand generation to yield a final volume of 15.5 μL placed on the Petri dish. The respective filter paper chips were then contacted with each sample solution, which resulted in the vertical wicking of the solution through the paper matrix and, consequently, the printed probe arrays. Immediately as wicked fluid reached the top of the paper chip, it was removed from contact with the Petri dish and washed with 2× SSC containing 0.1% SDS at 55 °C for 10 min in a Thermomixer Comfort Exchangeable MTP thermoblock (Eppendorf, Hamburg, Germany) with shaking at a speed of

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300 rpm, followed by 1 min of washing with 0.2× SSC buffer and 1 min washing with 0.1× SSC buffer at room temperature. The filter paper chips were then air-dried at room temperature in the dark.

The limit of detection of the method was determined using 0, 0.04, 0.2, 1, and 5 pmol of single-stranded DNA probe PS_1 following the hybridization protocol described in the preceding paragraph.

For experiments probing single-stranded DNA obtained from dog and human samples, the hybridization through capillary wicking and subsequent washing and drying steps were performed twice on the same chip. In the second hybridization cycle, the PS_1 oligonucleotide was not added.

Signal Analysis. Following hybridization, the filter paper chips from the surface chemistry optimization were scanned in a Fujifilm Luminescent Image Analyzer and the signal intensity was measured using the associated software (Fujifilm, Tokyo, Japan). Filter papers used in the dog/human sample detection assays were scanned in a Tecan LS Series Laser Scanner (Tecan, Switzerland) and the images were analyzed using the GenePix 5.1 software (Molecular Devices Corporation, USA).

RESULTS AND DISCUSSION

Optimization of Cellulose Surface Chemistry for Covalent Immobilization of Aminated ssDNA. As a prelude to the elaboration of paper-based DNA diagnostics, covalent attachment is essential to overcome the inherent low affinity between polynucleotides and cellulose.[21] Carbodiimide-mediated coupling between these biomolecules has been reported as early as the 1960s.[22] Since then, DNA sequences have been coupled to micro- and nanocrystalline cellulose, nonwoven cellulose fabric, and regenerated cellulose (cellulose II allomorph), using a diversity of methods, including epichlorohydrin- and bis(epoxide)-mediated coupling, reductive amination, photo-cross-linking, and cellulose-binding DNA aptamers (refs 21, 23, and 24 and references therein). In contrast, silicate glass surfaces, for example, microscope slides, are typically activated toward coupling with aminated DNA via initial reaction with an aminoalkyltrialkoxysilane, such as 3-aminopropyltriethoxysilane (APTES), to install surface amino groups, followed by treatment with the amine-reactive, homobifunctional linker PDITC.[25,26]

Earlier work in our laboratory established that reducing-end derivatives of the water-soluble, plant cell wall polysaccharide xyloglucan (XG) could be utilized to deliver reactive amino groups to paper surfaces for subsequent functionalization by harnessing the unique, essentially irreversible, sorption of XG on cellulose.[19,27,28] A key advantage of this approach is that cellulose surface amination occurs under gentle conditions (shaking in aqueous solution at room temperature), which maintain fiber and sheet structures. Thus, we envisioned that PDITC-based cross-linking chemistry, widely employed for glass supports, could be readily transferred to porous filter paper as a matrix for aminated ssDNA probe immobilization, following initial activation with aminated xyloglucan (XG-NH_2).

Whatman No.1 filter paper was selected as a matrix because of its low nonspecific biomolecule adsorption, good aqueous flow characteristics, cost-effectiveness, and ready availability. Filter papers bearing XG-NH_2, as well as control sheets bearing unmodified XG of the same molar mass, were initially prepared by adsorption over 24 h at room temperature under orbital agitation. An adsorption isotherm using a ninhydrin assay for primary amino groups indicated that saturation of the paper surface with XG-NH_2 was achieved at addition levels of 250 mg XG-NH_2 per gram of filter paper (24 h adsorption, Figures S-1 and S-2, Supporting Information). The high loading achieved at saturation (ca. 100 mg of XG-NH_2/g filter paper) is remarkable; this value is ca. 10-fold higher than that for native XG on the same substrate.[19] We attribute this difference to the dual effects of the lower molecular mass of XG-NH_2, which may allow further penetration of the polysaccharide into the porous fibers, and the chemical effect of the amino group, which may facilitate binding through ionic interactions with anionic groups on the processed fiber surface. For subsequent modification, an addition level of 50 mg of XG-NH_2 per gram of filter paper (5% w/w) was selected to economize the use of the polysaccharide reagent.

The reaction of PDITC with aminoalkylsilane-modified glass surfaces is typically performed in organic solvents such as DMF or dichloromethane. Because of the highly polar, hydrophilic nature of the filter paper surfaces, we were interested in exploring polar solvents for PDITC activation of cellulose and XG-modified cellulose. PDITC is, however, practically insoluble in water; thus, filter papers bearing XG-NH_2 (FP/XG-NH_2), unfunctionalized XG (FP/XG), and control filter papers (FP) were immersed and orbitally shaken in solutions of the bis(isothiocyanate) of increasing concentration up to 500 mM in *N*,*N*-dimethylformamide (DMF) or 250 mM in dimethylsulfoxide (DMSO). Subsequent array spotting of aminated ssDNA by hand pipetting, capping of unreacted surface thiocyanate groups with ethanolamine, and hybridization with a fluorescently (Cy3) labeled complementary strand under capillary flow conditions allowed the relative immobilization capacity of these papers to be quantified under functional assay conditions. Optimized washing steps were derived from those used for glass microarrays to minimize background signal. Figure 2B shows a representative set of paper strips produced by varying the surface preparation and solvent conditions. As this figure highlights, signal is clearly visible above the background in all cases, and the cross-reaction between the target and a second sequence probe immobilized on the surface is undetectable.

Figure 2C summarizes the quantitative data similarly obtained for the range of PDITC concentrations. The presence of surface-bound amino groups from XG-NH_2 did not appear to dramatically enhance PDITC activation of the papers, except at the lowest concentrations of PDITC in DMF and DMSO. Rather, the solvent appeared to be the biggest factor affecting the relative capacity of the papers, with the more polar DMSO proving to be significantly more effective than DMF. Unfortunately, solubility limitations of PDITC in DMSO restricted the working range, and apparently limited the activation of the papers, at the highest concentration (or more correctly, mixture compostion) tested, 250 mM. Preliminary experiments using fluorescein isothiocyanate (FITC) as a model compound indicated that addition of the bases triethylamine or pyridine (1% v/v) to either solvent did not improve the surface coupling efficiency (data not shown). On the basis of these results, the activation of FP/XG-NH_2 with 5 mM PDITC in DMSO was selected for all subsequent analyses. Under these conditions, the limit of detection was established to be ca. 0.2 pmol ssDNA in a 25 μL sample volume, whereas signal saturation occurred at >1 pmol ssDNA (Figure S-3, Supporting Information).

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Importantly, although the preaddition of aminated xyloglucan (XG-NH_2) conferred a slight improvement in coupling efficiency/detection sensitivity and allowed reduced PDITC consumption, the bis(isothiocyanate) was clearly reactive not only with the surface-bound amino groups but also with hydroxyl groups present on cellulose. It may, therefore, be adventitious to activate paper directly with higher concentrations of PDITC, thus avoiding the XG-NH_2 activation step when scaling up the method for eventual application (Figure 2).

Additionally, a preliminary study with Whatman RC55 regenerated cellulose membranes (catalog no. 10410212) was performed to evaluate the performance of these readily available surfaces for DNA hybridization and detection. The results showed that regenerated cellulose membranes are deformed by repeated wetting/drying cycles occurring during chemical treatment and washing, and, further, are not capable of effective capillary wicking because of the lack of a porous structure.

Application of ssDNA-Functionalized Paper for Sample Discrimination. With an optimized surface chemistry at hand, a diagnostic application of the DNA-bearing filter papers was developed. Using robotic nanoplotter printing, originally developed for printing of microarrays on microscopic glass slides, we created an array of six distinct features, comprised of five different oligonucleotide probes and one negative control, in which only buffer was printed. One of the oligonucleotides was identical to the positive control used in the optimization of surface chemistry (PS_1), whereas the other four (T_1, T_2, T_3, and T_4) each included a unique ID_tag sequence generated using software developed in-house. The use of generic ID_tags allows the generation of a versatile diagnostic array, the specificity of which is determined by the design of PCR primers used for target amplification from essentially any sample type. Full probe sequences are provided in (Table S-1, Supporting Information), and their positions on the array are given in Figure 3A.

PCR primers against four unique targets in dog mitochondrial DNA, dog genomic DNA, human mitochondrial DNA, and human genomic DNA, respectively, were generated with unique ID_tag sequences from the surface-attached oligonucleotide probes included in each of the reverse primers (Table S-2, Supporting Information). Each primer pair was designed with a biotin-labeled reverse primer and a Cy3-labeled forward primer. Following PCR amplification and biotin-mediated capture on streptavidin-coated magnetic beads, strand separation at high pH allowed the facile isolation of the Cy3-labeled single-stranded product for hybridization to the paper-based array (Figure 4).

After sample transport by capillary action, a signal from the positive control and one of the unique ID_tag oligos is expected (Figure 3A). Figure 3B shows the results after detection with each of the four amplicon samples on independent paper chips. Integrated signal intensities were comparable for similar amounts of sample, as shown in Figure 3C. Together, these data illustrate the use of activated filter papers for the rapid detection of DNA samples in a variety of applications, which is driven by the intrinsic capillary transport properties of the cellulosic fiber matrix. The hybridization step, in particular, is exceptionally rapid because of the high rate of flow of sample DNA across the surface-bound oligonucleotide probes. Thus, a traditionally rate-limiting step in DNA-based diagnostics was removed.

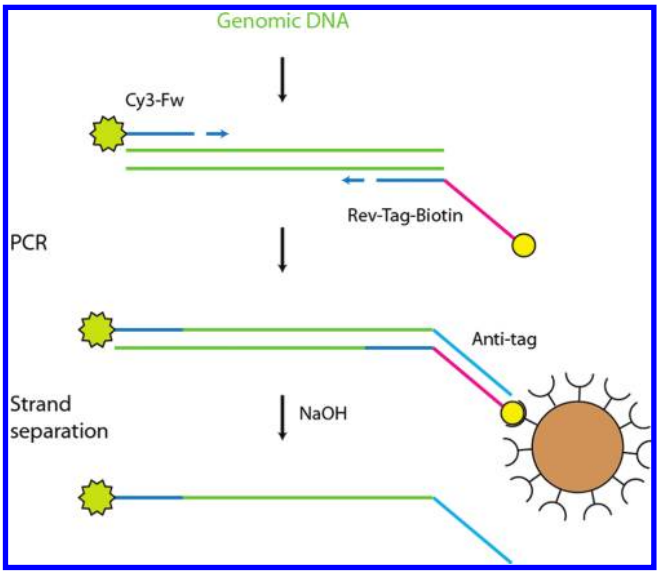

Figure 4. Overview of target DNA sample preparation through PCR, bead capture, and strand separation. The forward primer is coupled to a Cy3 label, while the reverse primer contains a sequence ID_tag (T_1 through T_4, respectively) and a terminal biotin moiety. During strand separation the biotinylated PCR product is bound to streptavidin-coated paramagnetic beads. The Cy3-labeled forward strand is eluted with NaOH and used for downstream detection.

CONCLUSION

In the development of alternatives to silicate glass for the production of DNA biosensors, a variety of materials, including latex beads, polystyrene, carbon, gold, and oxidized silicon, have been explored.[29] Nonetheless, traditional paper—macroporous sheets produced by the rapid filtration of individual, native cellulose fibers from herbaceous plants or wood—continues to evoke interest as a substrate for DNA diagnostics because of its unique material properties. In the present study, we were able to adapt convenient chemistry that has traditionally been used for glass surfaces to activate cellulosic fibers in paper for coupling with aminated DNA. The devised gentle activation conditions maintained the porous sheet structure (with no visible morphology changes whatsoever), which was essential for subsequent capillary action-driven sample hybridization. Equally vital, sufficient reactivity to yield high signal-to-background ratios was simultaneously obtained.

The concerted design of an array of probes with unique ID_tag sequences makes the printed arrays broadly applicable to any DNA sample by the straightforward incorporation of ID_tag sequences into the PCR primers used in sample amplification. Here, the concept was demonstrated with a tetraplex differentiation of human and dog mitochondrial and genomic DNA; higher order multiplexing can be readily achieved. As such, diverse diagnostic and detection applications can be readily envisaged in which the paper substrate confers certain advantages in terms of ease-of-transport and disposability. To be truly versatile, however, the detection method must be simplified to provide an immediate visual readout.[30,31] Efforts toward this goal are currently underway in our laboratories.

dx.doi.org/10.1021/ac300025v | Anal. Chem. XXXX, XXX, XXX–XXX

■ ASSOCIATED CONTENT

Supporting Information

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Pelin Akan for generation of unique ID_tag sequences and Afshin Ahmadian for fruitful discussions. Mårten Sundberg, Peter Nilsson, and Peter Gyarmati are thanked for their help with robotic printing and image analysis. The authors also thank Rikard Slättegård and Marcus Ruda (SweTree Technologies) for supplying XG-NH_2 and advice on synthetic methods, respectively. Work in the Division of Glycoscience, KTH Biotechnology, was funded by The Swedish Research Council Formas via CarboMat − The KTH Advanced Carbohydrate Materials Consortium (A Formas Strong Research Environment); work at the Science for Life Laboratory was funded by The Swedish Science Council (*Vetenskapsrådet*). H.B. thanks *Vetenskapsrådet* and the Knut & Alice Wallenberg Foundation (via the WWSC) for additional funding. A.C.A. and Y.S. contributed equally to this work.

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