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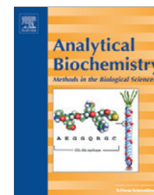

Three-dimensional hydrogel structures as optical sensor arrays, for the detection of specific DNA sequences

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ABSTRACT

The fabrication and characterization of surface-attached PEG–diacrylate hydrogel structures and their application as sensing platforms for the detection of specific target sequences are reported. Hydrogel structures were formed by a photopolymerization process, using substrate-bound Eosin Y molecules for the production of free radicals. We have demonstrated that this fabrication process allows for control over hydrogel growth down to the micrometer scale. Confocal imaging revealed relatively large pore structures for 25% (v/v) PEG–diacrylate hydrogels, which appear to lie in tightly packed layers. Our data suggest that these pore structures decrease in size for hydrogels with increasing levels of PEG–diacrylate. Surface coverage values calculated for hydrogels immobilized with 21-mer DNA probe sequences were significantly higher compared to those previously reported for 2- and 3-dimensional sensing platforms, on the order of 10^{16} molecules cm^{-2}. Used as sensing platforms in DNA hybridization assays, a detection limit of 3.9 nM was achieved for hybridization reactions between 21-mer probe and target sequences. The ability of these hydrogel sensing platforms to discriminate between wild-type and mutant allele sequences was also demonstrated, down to target concentrations of 1–2 nM. A reduction in the hybridization time down to a period of 15 min was also achieved, while still maintaining confident results, demonstrating the potential for future integration of these sensing platforms within Lab-on-Chip or diagnostic devices.

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DNA microarrays have gained much popularity in recent years as an effective tool for genetic analysis, and are used in clinical diagnostics [1], drug discovery [2], and pathogen detection [3]. DNA microarrays have the advantage of low cost, higher throughput, and reproducibility when compared with more traditional methods such as Southern blotting. These microarrays (also known as DNA chips) rely on the immobilization of DNA onto a variety of substrates, and have numerous applications in expression studies or sequencing by hybridization [4].

Single-stranded DNA (ssDNA)[1] probes are often synthesized directly onto the substrate surface [5,6]. Technologies such as photolithographic DNA synthesis allow for the manufacture of high-density DNA microarrays, but are costly and time consuming, which tends to limit their widespread implementation [7]. Another approach involves the immobilization of presynthesized ssDNA probes onto solid supports, which is more commonly used. Several methods have been reported in the literature for the covalent attachment of modified oligonucleotides to preactivated solid supports such as glass [8–10], silicon wafers [11], gold surfaces [12], and plastic polymers such as poly(methyl methacrylate) (PMMA) [7,13]. One major limitation of two-dimensional (2D) DNA microarrays is the lower signal observed when probe DNA is confined to the substrate surface, since the efficiency and kinetics of hybridization are influenced by the probe density [14,15]. An alternative is to immobilize ssDNA within a three-dimensional (3D) hydrogel matrix, which can offer beneficial applications for various optical sensor arrays [16]. 3D supports such as polyacrylamide gels have demonstrated 100 times greater a capacity for the immobilization of oligonucleotides compared to 2D glass supports [17] and various methods for the immobilization of such biomolecules within these types of gels have been reported [18–20]. However, acrylamide-based hydrogels suffer from a number of limitations. In general, it is difficult to obtain water contents greater than 95 wt%, causing them to become diffusion-limited due to a small pore size of the gel. The localization of higher concentrations of immobilized oligonucleotides at the surface of these gels prevents us from using such 3D supports to their full advantage.

Polymeric hydrogels based on monomeric sugar acrylates or methacrylates do not experience such drawbacks, as they tend to

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[1] *Abbreviations used:* PBS, phosphate buffer solution; PEG, poly(ethylene glycol); PEGDA, PEG–diacrylate; PMMA, poly(methyl methacrylate); sAP, shrimp alkaline phosphatase; SNPs, single nucleotide polymorphisms; ssDNA, single-stranded DNA.

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have greater water content due to their pendant sugar moieties. Hydrogels formed from these compounds have equilibrium water contents above 95% resulting in pore sizes larger than 500 nm [21,22]. This makes them an attractive candidate for DNA immobilization and hybridization as the high water content is likely to allow for rapid diffusional transport of target DNA within the 3D matrix. Soto et al. demonstrated the immobilization of ssDNA and subsequent DNA target hybridizations in a microarray format using thin sugar-based hydrogel films [23]. Confocal microscopy data confirmed that ssDNA diffused completely through the hydrogel and was covalently coupled mostly within the interior regions. The use of sugar-based hydrogels containing carboxyl and amine terminal groups for the development of a 3D immunoassay for detecting cholera toxin and staphylococcal entertoxin B has also been reported [24]. Poly(ethylene glycol) (PEG), is a nontoxic biocompatible polymeric material that has been approved by the US Food and Drug Administration (FDA) for both oral and topical applications [25]. PEG-based hydrogels have been employed in the development of electrochemical and optical biosensors. The photopolymerization of enzyme-containing redox hydrogel films on electrode surfaces for the amperometric detection of glucose has been reported [26]. Examples of optical sensors include the fabrication of PEG-based hydrogel structures by UV-initiated photopolymerization, for the fluorescent detection of SNAFL-1-labeled acetylcholine esterase [27]. The fabrication of PEG–dialdehyde-based hydrogel chambers immobilized onto modified glass surfaces for the screening of small molecule–protein/protein–protein/nucleic acid–nucleic acid interactions using fluorescent Cy5 labels [28] has also been reported, as has the fabrication of 3D PEG hydrogel microstructures for the encapsulation of mammalian cells, using a photoreaction molding technique [29].

In this paper, we describe the fabrication and characterization of surface-bound PEG–diacrylate (PEGDA) hydrogel structures such as that previously reported [30,31], and their application in DNA hybridization assays for mutation detection and genotyping studies. The fabrication process involves the modification of glass substrates with Eosin Y photoinitiator molecules. Eosin Y requires the irradiation of a visible light wavelength (~514 nm) in order for it to become activated and produce free radicals, for a polymerization process to occur [32]. Hydrogel structures were thus formed by the irradiation of prepolymer solutions deposited onto modified glass surfaces at this wavelength, resulting in their covalent attachment to the surface. Because the polymerization process is initiated using a visible light source rather than UV, it has the advantage of causing no degradation to DNA molecules on light exposure. These surface-attached PEGDA hydrogel structures immobilized with amino-modified probe oligo sequences were used as sensing platforms in DNA hybridization experiments. Detection of complementary target sequences down to nanomolar concentrations was observed, allowing for low detection limits of target sequences that contain mutations p.W1282X and p.F508del relative to the cystic fibrosis transmembrane conductance regulator gene (CFTR; OMIM ID: 602421). These sensing platforms were successfully used to test for specific binding and for different hybridization kinetics between all probe sequences. Detection of the correct genotype of hybridized targets, even at very low concentrations, demonstrates these sensing platforms to be a highly effective and relatively fast tool for detecting different types of mutations in DNA sequences.

Materials and methods

Reagents and instrumentation

All chemicals used were of commercial grade, and purchased from Sigma–Aldrich unless stated otherwise. Oligonucleotides described in Table 1 were purchased from Metabion International AG, Germany. Probe sequences used in the detection of p.W1282X and p.F508del mutations were provided by The Wellcome Trust Centre, University of Oxford, and are also described in Table 1. Glass microscope slides with dimensions of 75 × 25 mm (J. Melvin Freed Brand, USA) were employed as glass substrates, and 20 × 20 mm glass coverslips (0.13–0.16 mm thick, purchased from Menzel-Glaser, Germany) were used alternatively in the case of confocal microscopy work.

Contact angle measurements were carried out using a Data-Physics video-based contact angle OCA system and SCA20 software. Epifluorescence images of air-dried hydrogel samples were obtained using an Olympus BX51 microscope and Cell^F software. Confocal fluorescence images of wet and air-dried hydrogel samples were obtained using an Olympus IX81 inverted microscope and Fluoview FV1000 software. UV absorbance measurements were recorded using a Libra S12 UV/Vis spectrophotometer, for the determination of surface coverage values of probe molecules immobilized within the hydrogel spots. PCR were carried out using an Eppendorf Mastercycler gradient system, and PCR products analyzed using a microfluidic-based electrophoresis method, by an Agilent 2100 Bioanalyzer system and 2100 Expert software.

Preparation of hydrogel structures

Glass substrates were silanized and modified with Eosin Y photoinitiator in accordance with previous protocols reported [30]. A prepolymerization solution was prepared in phosphate buffer solution (PBS), consisting of 25% (v/v) poly(ethylene glycol) diacrylate, 225 mM triethanolamine, and 37 mM 1-vinylpyrollidinone. Solutions were stored in a refrigerator at ~4 °C. Hydrogel structures were formed on Eosin Y-modified glass substrates by depositing prepoly-

Table 1
Sequence of probe oligonucleotides.

Name	Oligonucleotide sequence (5′ to 3′)	Length
Probe 1-Cy5 + NH_2	Cy5-TACAGGCTTACCGTCATAGGT-C7-Aminolink[a]	21
Probe 1-NH_2	C6-Aminolink[b]-TACAGGCTTACCGTCATAGGT	21
Target 1-Cy5	Cy5 Ester-ACCTATGACGGTAAGCCTGTA	21
Probe 2-Cy5 + NH_2	Cy5-GCCTAAGCCCTCTTTCTCAGT-C7-Aminolink[a]	21
Probe 2-NH_2	C6-Aminolink[b]-GCCTAAGCCCTCTTTCTCAGT	21
Target 2-Cy5	Cy5 Ester-ACTGAGAAAGAGGGCTTAGGC	21
W1282X-wt	AAAGGCTTTCCTCCACTGTTGCGATCATGTCGAAGGA	22
W1282X-mut	AAGGCTTTCCTTCACTGTTGCGATCATGTCGAAGGA	23
DF508-wt	AAATATCATCTTTGGTGTTTCCTATGGATCATGTCGAAGGA	26
DF508-mut	AAAGAAAATATCATTGGTGTTTCCTATGGATCATGTCGAAGGA	28

[a] Primary amino group at the end of seven carbon spacer.
[b] Primary amino group at the end of a six carbon spacer.

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