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Parallel DNA Synthesis On Poly(ethylene terephthalate)

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Abstract

The fabrication of DNA arrays directly upon aminolyzed sheets of poly(ethylene terephthalate) (PET) is described. Array surfaces typically employ bifunctional linkers or layers of covalently attached polymers to provide substrate hydroxyl groups as synthesis attachment points. An amine treatment is used here to expose hydroxyl groups on films of PET. These hydroxyls can then be used to couple phosphoramidites and initiate the array synthesis without further steps of functionalization. Arrays fabricated on these substrates with a maskless array synthesizer are tolerant of the high number of chemical exposure steps required to synthesize relatively long oligonucleotides. The results may be of the greatest use to the synthetic biology community, where a flexible and robust substrate could enable new strategies to enhance the throughput of oligonucleotide synthesis.

Graphical abstract

Basically works: Poly(ethylene terephthalate) can be used as a DNA array substrate without having to install linker molecules between the surface and the DNA. Base treatment exposes hydroxyl groups which can be used to initiate DNA synthesis directly from the substrate. This finding is explored here using a maskless array synthesizer.

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Keywords

Maskless array synthesis; Photolithography; Oligonucleotide; DNA array; Synthetic biology

Addressed arrays of surface-bound DNA oligonucleotides have proven to be powerful analytical tools for the genomic sciences. Early applications of such arrays exploited their capacity to capture complementary DNA or RNA from solution to detect and quantitate the sequences present in a sample. The cost of DNA sequencing has since decreased far more rapidly than the cost of DNA synthesis which has encouraged the use of arrays as synthetic rather than analytical tools.^[1] While it is often now more affordable to sequence than to perform an array experiment, the parallel DNA synthesizers that fabricate arrays remain uniquely efficient means of generating complex oligonucleotide pools for gene synthesis, mutagenesis experiments, or selective enrichment for sequencing.^[2] This shift in array usage changes the considerations for the surface chemistry of the underlying array substrate. For analytical studies, it is crucial to minimize non-specific interactions between the surface and the analyte of interest. However, substrate restrictions for synthetic applications are more relaxed and typically only require that the material is robust enough to withstand the array fabrication chemistry.

Recent work on array substrates has shown that flexible sheets of poly(ethylene terephthalate) (PET) coated with multilayer polymer films can be used for the maskless array synthesis of DNA and RNA oligonucleotides.^[3,4] Such surfaces allowed the arrays to be cut and subdivided into smaller sections more readily than arrays made on rigid substrates.^[5] The oligonucleotide attachment chemistry also proved more stable to elevated temperatures than the silyl-ether bond found in arrays synthesized on silanized glass.^[6] However, a limitation of this work was that the polymer bilayers used to install hydroxyl groups on the PET were prone to delamination during the array fabrication. This limited the length of the oligonucleotides which could be synthesized as well as the complexity of the arrays. Here it is shown that phosphoramidite chemistry can be initiated from the hydroxyl groups in aminolyzed PET. The light-directed array fabrication process produces

significantly less damage on the base-treated substrates than on the previously reported thin-film modified substrates.

PET is readily functionalized using aminolysis.^[7] The amine cleaves the polymer at the ester linkage to generate a free hydroxyl (Scheme 1).^[8] To test this reaction for producing array substrates, a thin sheet of PET was treated with 6-amino-1-hexanol in methanol. A custom-built maskless array synthesizer was then used to synthesize a poly-(dT)₁₅ oligonucleotide.^[9] Figure 1A shows the results of staining this surface with Sybr Gold, indicating that DNA was synthesized only at the desired locations on the polymer.

The effect of the aminolysis conditions was then examined in more detail. First, the 6-amino-1-hexanol functionalization was compared to treatment with ammonia in methanol. PET surfaces were treated with either ammonia or 6-amino-1-hexanol of equal molarity and the resultant arrays were hybridized with fluorescently-labelled oligonucleotides. An example array is shown in Figure 1B and the intensity of the fluorescence signals is depicted in Figure 1C relative to an untreated PET surface. The similar signal intensities of the treated substrates establish that it is not crucial to use a hydroxyl-bearing amine to functionalize the surface. Whether the synthesis occurs preferentially from the hydroxyl of the 6-amino-1-hexanol or the PET backbone was not studied further, but it is likely that the benefit of using a hydroxyl-containing amine is offset by a lower reactivity relative to ammonia (data not shown). It may also be difficult in practice for a hybridization assay to detect both the expected hydroxyls if they remain in close enough proximity to hinder the DNA synthesis or duplex formation. Notably, it was also possible to synthesize DNA on untreated PET, albeit at a lower density. This likely results from residual hydroxyl end groups remaining from the polymerization process or those that accrue from various modes of degradation. A functional estimate of the new synthesis sites exposed by the aminolysis was then made using hybridization experiments. Arrays containing a single sequence were fabricated upon PET treated with various ammonia concentrations and hybridized with fluorophore-labelled oligonucleotides. Figure 2 depicts the relationship between the treatment conditions and the resultant signal intensity. This ability to control surface oligonucleotide density allows the surfaces to be optimized for either the synthesis of long DNA sequences or for yield, similar to the optimization of the controlled pore glass supports employed for standard solid-phase DNA synthesis. Wash-off experiments suggest that the oligonucleotide density of untreated PET is similar to that of silanized glass (~6 pmol/cm², a typical site density for array fabrication, see SI).^[10]

Covalently attaching the array oligonucleotides to the substrate material minimizes the points at which chemical degradation can occur. The functional stability of the PET substrate was assessed by synthesizing arrays with oligonucleotides of greater length than had been made previously upon flexible substrates. Figure 3 shows the results of hybridization of a fluorescently-labelled oligonucleotide complementary to the 18 nt termini of 88 nt features (sequence in Supplemental Table 3). The upper half of the image depicts a 100-feature sub-array where fluorescence is expected, while the lower half depicts the region where a control sequence was synthesized and no fluorescence should be observed. Each array was fabricated under a different set of conditions. Those depicted in [A] and [B] are synthesized upon PET coated with multiple polymer bilayers as previously described and

differ only in the oxidizer used during the phosphoramidite cycle; iodine, pyridine, and water in tetrahydrofuran (THF) was used in [A] and 0.5 M (10-camphorsulfonyl)oxaziridine (CSO) in acetonitrile was used in [B].^[4]

Both [A] and [B] exhibit surface defects and high non-specific binding that complicate potential analytical applications. The oligonucleotides on the surface may be usable for synthetic applications, but the poor feature definition suggests a loss of precision during the photodeprotection step of the synthesis. This would reduce the sequence fidelity and the ability to miniaturize the arrays.^[11] By contrast, the array in [C] shows no evidence of substrate degradation and the spatial separation between features is preserved. [C] was synthesized upon ammonia-treated PET using iodine/pyridine/water in THF as an oxidizer and included a step of acetylation to cap failed sequences during synthesis. Acetylation is commonly used in solid-phase phosphoramidite chemistry to truncate failed couplings so that sequences which would contain deletions can be removed from full-length products.^[12] In the light-directed format used here, acetylation also acts to reduce insertion errors. It had not been possible to use this step on previous PET-based substrates as the cumulative THF exposure the acetylation requires delaminated the bilayers from the surface.

This work establishes that poly(ethylene terephthalate) can be used as a substrate for DNA synthesis. The primary anchor for the first phosphoramidite coupling is the terminal hydroxyl of the polymer chains comprising the substrate. While amine treatments were explored here, any base treatment strong enough to cleave the PET ester would likely achieve a similar result. Synthesizing oligonucleotides directly from the substrate material improves resistance to the chemical processing needed for high-density array synthesis while preserving the desirable aspects of a flexible substrate.^[13] These findings show that base-treated PET is a simple yet attractive substrate choice for parallel DNA synthesis and suggest promising routes of further development. Various amines could be used to tailor the physical properties of the surface or introduce new functionalities to the arrays. A variety of cleavable linkers or sites for attaching other useful biomolecules, such as short RNA primers, could potentially be installed with such reactions as well.^[14] Similar base treatments may also be applicable to other polyesters, many of which are used as 3D printing materials. 3D printed array substrates could include wells for gene assembly or other enzymatic reactions, integrate with existing fluidics platforms, be rendered electrically conductive, or be divided into small sub-arrays which could be manipulated with magnets.^[15]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Synthesis of DNA on PET substrates. (A) Patterned poly-(dT)₁₅ stained with Sybr Gold on PET treated with 6-amino-1-hexanol. (B) Fluorescence micrograph of an array on ammonia-treated PET hybridized with Texas Red (yellow) and Cy5 (red) labelled oligonucleotides. (C). Fluorescence intensity after hybridization of features produced on different PET surfaces. Yellow bars denote signal intensity of features hybridized with Texas Red-labelled oligonucleotides and red bars denote the signal intensity of features hybridized with Cy5-labelled oligonucleotides. Error bars are $\pm$ one standard deviation of signals from 24 different features. The different background levels evident in Panels A and B reflect the differences in non-specific binding of Sybr Gold (Panel A) and fluorescently tagged oligonucleotides (Panel B).

Figure 2.

Fluorescence signal intensity as a function of aminolysis conditions. Signal intensities represent an average of 8 sub-features on the array and error bars are one standard deviation. Concentrations refer to the ammonia in methanol solution used to treat the surfaces. Signal intensities above the 16-bit limit were calculated by adjusting values acquired at a lower scanner gain during imaging (see SI). Inset depicts the feature pattern used for this set of arrays.

Figure 3. Hybridization to arrays produced with different synthesis chemistries. CSO denotes 10-camphorsulfonyl)oxaziridine.

Scheme 1.

Aminolysis of poly(ethylene terephthalate) for array synthesis.