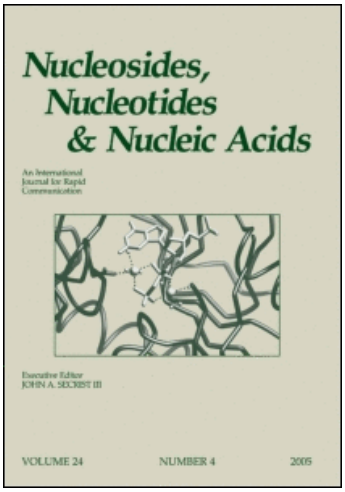

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THE USE AND EVALUATION OF 2 2 PHOTOADDITION IN IMMOBILIZATION OF OLIGONUCLEOTIDES ON A THREE DIMENSIONAL HYDROGEL MATRIX

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THE USE AND EVALUATION OF 2+2 PHOTOADDITION IN IMMOBILIZATION OF OLIGONUCLEOTIDES ON A THREE DIMENSIONAL HYDROGEL MATRIX

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ABSTRACT

Photochemical attachment of synthetic oligonucleotides on the three dimensional surface of a polyacrylamide based hydrogel was used in the specific detection of target oligonucleotides. Covalent attachment of the oligonucleotide to the hydrogel was mediated by the incorporation of a 2+2 photo-attachable functional group in both the hydrogel and the oligonucleotide probe. Expression and SNP assays were used to evaluate this platform.

Use of oligonucleotides in hydrogels has attracted a great deal of attention in the past six years. The three dimensional matrix of hydrogels are known to have higher loading capacity for incorporation of biomolecules than the two dimensional planar surface available on glass (1). The high loading capacity of these bio-compatible materials in turn provides for the enhancement of hybridization kinetics, which is an attractive feature for development of rapid hybridization based assays. Recent advances in the immobilization of oligonucleotides in hydrogel matrices have focused on the use of a variety of chemistries, including the generation of dialdehydes and activation of the hydrogel by introduction of hydrazide or amine functional groups (1), persulfate or photoinduced copolymerization of

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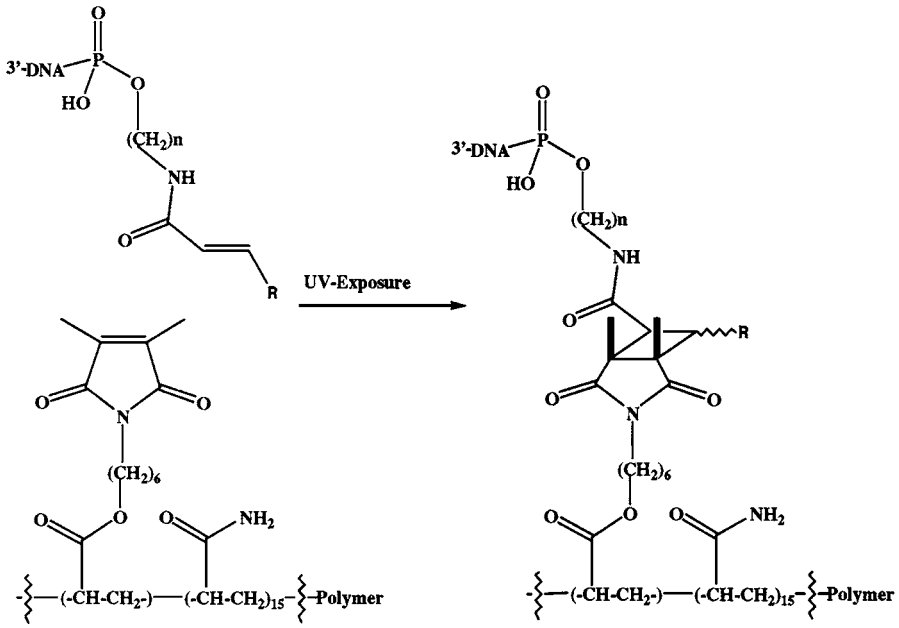

Figure 1.

allyl-oligonucleotides (2), and incorporation of biotinylated oligonucleotides on microelectrodes (3). The objective of our study was to develop a simple method that uses a minimum number of steps to manufacture an oligonucleotide bioarray starting with pure oligonucleotides of defined sequences. Hence, we report on the 2+2 photoinduced coupling of oligonucleotides mediated by the incorporation of a 2+2 residue (cinnamide or methacrylamide) (4) at the 5′ end of the oligonucleotide and a hydrogel bearing a dimethylmaleimide residue. The polymer (5) (Fig. 1) used in the study was pre-coated to a thickness of 600 microns by photochemical activation. The oligonucleotides bearing the 2+2 functional group were printed on the surface of the film by robotic deposition. A second dose of ultraviolet irradiation attached the oligonucleotide to the matrix via cyclobutane rings; a distilled water rinse removed the excess un-reacted reagents. The hydrogel was then used to carry out hybridization of target oligonucleotides in expression and single oligonucleotide polymorphism (SNP) assays.

Expression microarrays. Target is defined as the labeled nucleic acid sample applied to the DNA microarray. Probe refers to the DNA oligonucleotide photochemically attached to the hydrogel. Three probes on the microarray represent each gene. For photochemical attachment experiments, probes directed against eight different yeast mRNAs were included on the chip to determine sensitivity. One μg of human placental poly(A)$^+$ RNA was spiked with 17 pg of yeast poly(A)$^+$ RNA. Figure 2 shows a plot of the fluorescence intensity of different yeast transcripts detected when each was spiked into human placental poly (A)+ RNA at a mass

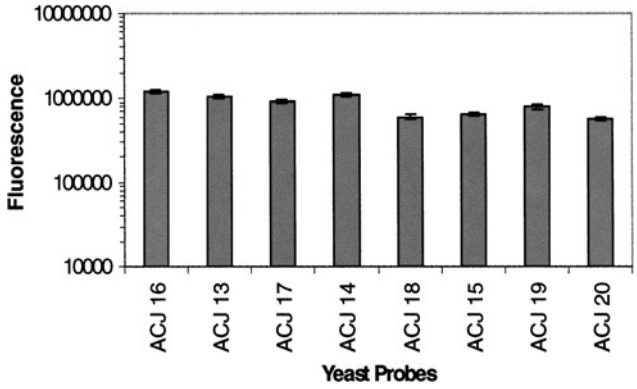

Figure 2. Human brain target sample (with yeast mRNA spikes diluted to 17 pg) was hybridized to 8 separate chips. The mean and standard deviation is shown for each yeast probe hybridization signal. The cutoff was determined to be the average background plus three standard deviations.

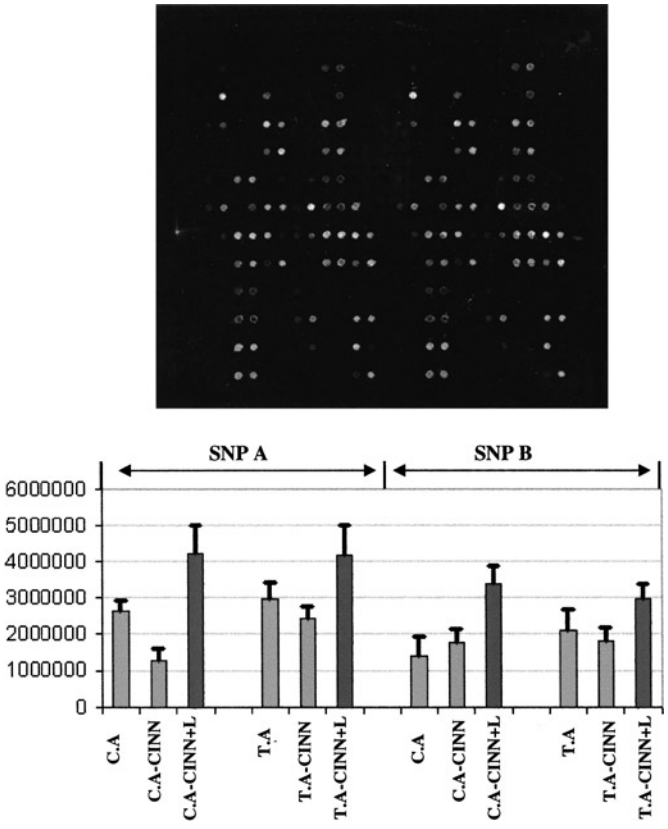

Figure 3. A comparison of signaling performance across the two attachment chemistries methacrylamide, cinnamide and cinnamide with a linker was made for two different allele pairs over 24 arrays (see figure). Consistently target dependent signal generated for probes attached with the three chemistries followed the same intensity pattern. Probes attached with cinnamide with a linker yielded higher signal intensities in comparison to either cinnamide alone or methacrylamide attached probes.

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ratio of 1 in 300,000 (equivalent to one copy per cell). Seven out of eight probes (each probe corresponding to a different yeast transcript) were significantly over the background cutoff fluorescence signal. Cutoff is defined as the mean signal of the blank pads plus three standard deviations which gives a 99.7% likelihood of having a real signal. The sensitivity of the assay on this platform has thus been shown to be down to 17 pg of starting poly (A)+RNA.

SNP micoarrays. In these experiments a comparison of signaling performance was also done using two different chemistries and length of linker. Specific probes for each allele of a bi-allelic SNP were robotically printed at different locations on the hydrogel. Both probes are required for an individual SNP assay. A fluorescent terminator is incorporated in the event that the correct target is present. The overall performance of the assays conducted on the hydrogel over 12 arrays yielded 15 out of the 16 SNPs calls made at $> 99\%$ accuracy. Accuracy is defined as the percent correct calls made for every SNP call made as confirmed by sequencing data. Probes attached with cinnamide with a linker yielded higher signal intensities in comparison to either cinnamide alone or methacrylamide attached probes as shown in Figure 3.

In summary, we have demonstrated the use of 2+2 photoattachment in the immobilization of oligonucleotides in hydrogels as an alternative approach to bioarray fabrication. This approach may provide a significant advantage because of it's simplicity, speed of reaction, and lack of post attachment processing. This data also demonstrates that the assay and oligonucleotide array formats are compatible with and perform well on this matrix.

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4. We found very little difference between the performance of the cinnamide or methacrylamide oligonucleotides in the photoattachment chemistry. However, due to the inherent hydrophobicity of the cinnamide group purification of these oligos are more convenient.

Further details of the photochemistry of the hydrogel, synthesis of the cinnamide oligos and the details of the assays used will be provided in follow up publications to this work.

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