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Immobilization of acrylamide-modified oligonucleotides by co-polymerization

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ABSTRACT

A flexible chemistry for solid phase attachment of oligonucleotides is described. Oligonucleotides bearing 5′-terminal acrylamide modifications efficiently co-polymerize with acrylamide monomers to form thermally stable DNA-containing polyacrylamide co-polymers. Co-polymerization attachment is specific for the terminal acrylamide group. Stable probe-containing layers are easily fabricated on supports bearing exposed acrylic groups, including plastic microtiter plates and silanized glass. Attachment can be accomplished using standard polyacrylamide gel recipes and polymerization techniques. Supports having a high surface density of hybridizable oligonucleotide (~200 fmol/mm^2) can be produced.

INTRODUCTION

Solid phase nucleic acid hybridizations are widely used in the life sciences and diagnostics. Despite the general utility of this procedure, there is little consensus on the best chemical approach for attaching nucleic acid probes to supports. A great number of attachment methods have been published, which vary widely in chemical mechanism, ease of use, probe surface density and attachment stability (1–27).

Among the most promising solid phase attachment methods developed over the last decade are those that utilize polyacrylamide supports (5,7,15,25–27). The chief advantages of these supports are high probe capacity, low non-specific binding levels and relatively high thermal stability. Moreover, it is relatively easy to manipulate probe density for normalizing hybridization properties of a probe array (7). Despite these advantages, the DNA attachment methods cited above are inconvenient in that they require activation of the gels, probes or both with labile reactive chemicals.

During development of a solid phase PCR process (E.S.Abrams *et al.*, submitted for publication), we developed a primer attachment method based on the co-polymerization of acrylamide-modified oligonucleotides into a polyacrylamide co-polymer. The method has significant advantages in stability and convenience over previous methods. First, it can be performed with standard, widely used gel polymerization techniques. Second, it provides surfaces with very high probe density. Third, the attachment is extremely stable and can withstand PCR cycling conditions. These features, along with the low non-specific nucleic acid binding of polyacrylamide supports, make the method useful for many applications and accessible to a wide range of molecular biologists and chemists.

MATERIALS AND METHODS

Oligonucleotides

Oligonucleotides containing 5′-terminal acrylamide groups were obtained commercially from Research Genetics (Huntsville, AL) or Operon Technologies (Alameda, CA). The 5′-terminal acrylamide groups were added during automated synthesis using a commercially available acrylamide phosphoramidite (Acrydite™; Mosaic Technologies, Boston, MA). All other oligonucleotides, including 5′-fluorescein-labeled, 5′-amine-modified and unmodified oligonucleotides were obtained from Ransom Hill Bioscience (Ramona, CA). Lyophilized oligonucleotides were dissolved in TE buffer (10 mM Tris–HCl, pH 8.3, 1 mM EDTA) and stored frozen at –20°C. Concentrations were determined from absorbance at 260 nm (assuming 33 µg/ml oligonucleotide per 1 OD unit). All concentrations refer to oligonucleotide strands.

Electrophoresis assay of immobilization efficiency

Glass plates for a vertical polyacrylamide minigel (10 × 10 cm, 0.75 mm spacers) were assembled and the sandwich was filled approximately half way with 20% polyacrylamide (19:1; Bio-Rad), 1× TBE (90 mM Tris–borate buffer, pH 8.3, 2 mM EDTA). Polymerization was initiated by inclusion of 10% aqueous ammonium persulfate (APS) and TEMED at 1/100th and 1/1000th gel vol, respectively. After polymerization, three additional spacers were inserted vertically into the top of the plate sandwich so that they contacted the top of the gel layer. The spacers were spaced to create four laterally arranged compartments of approximately equal size on top of the gel layer. Four different 300 µl aliquots of gel solution (20% polyacrylamide, 1× TBE, 4 µl 10% APS and 4 µl 10% TEMED) were polymerized in these compartments, each aliquot containing a different oligonucleotide at a final concentration of 3 µM. Two different sequences were used for the four oligonucleotides [oligo 1, d(TTT TTT TTT ACG

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CAG CGA CGA GCA CGA GAG); oligo 2, d(TTT TTT TTT GAC TGC TGG CGG AAA ATG AGA AAA)]. Each sequence was synthesized in two versions: one with a 5′-terminal primary amine and one with a 5′-terminal acrylamide group. After polymerization of the four oligo-containing compartments, the three spacers separating the compartments were removed and the remaining space in the plate sandwich was filled with a 20% gel layer. This composite gel was then assembled in a minigel apparatus containing 1× TBE and subjected to electrophoresis at 100–150 V for ~45 min. After electrophoresis, the gel was stained with ethidium bromide and photographed using a CCD video camera with UV transillumination.

Preparation of optical fiber supports

The polished ends of silica optical fibers (1 mm diameter) were cleaned by soaking in 10% aqueous nitric acid for 2 h. The fibers were rinsed with water and acetone and then air dried. The fiber tips were then soaked in 10% 3-methacryloxypropyltrimethoxysilane (Bind Silane; Pharmacia Biotech) in acetone (v/v) for 1 h. The tips were washed in acetone and air dried.

Photochemical attachment of 5′-acrylamide oligonucleotides to optical fibers

To attach primers the silanized tips were immersed in solutions of 8% (w/v) acrylamide (17:1 w/w acrylamide:bis-acrylamide in 0.1 M phosphate buffer, pH 6.8) containing 1 µM capture probe 13B, d(TT TTT TTT TCG GGA TCC CAG GCC CGG GAA CGT ATT CAC), with a 5′-terminal acrylamide group (M samples) or 1 µM 13B probe with a 5′-terminal phosphate group (U samples). Riboflavin was added to a final concentration of 0.0006% w/v and light from a 100 W halogen lamp was passed through the distal end of the fiber for 5 min. The photopolymerized fiber tips were immersed in an agarose minigel box containing TE buffer and subjected to electrophoresis for 30 min at 100 V/cm to remove non-immobilized oligonucleotide.

Measurement of attachment stability under PCR conditions

Polyacrylamide beads containing 5′-acrylamide-modified and unmodified 13B capture probes (sequence above) were prepared by pipetting 25 µl aliquots of gel solution into microtiter plates filled with mineral oil. Gel solutions contained 20% acrylamide (19:1 w/w acrylamide:bis-acrylamide), 1× TBE, 0.5% w/v APS, 0.5% v/v TEMED and 10 µM oligonucleotide. Following polymerization, beads were washed in TE buffer and subjected to electrophoresis as described above to remove unpolymerized oligonucleotides. Beads were immersed in PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$) and thermocycled as indicated using a cycle of 94°C for 55 s, 55°C for 25 s and 72°C for 1 min. Following cycling, beads were subjected to electrophoresis again to remove any unbound oligonucleotides and hybridized with a complementary ^{32}P-labeled oligonucleotide target.

Measurement of probe density on optical fibers and beads

Probe density was determined by hybridizing complementary ^{32}P-labeled oligonucleotide, d(GTG AAT ACG TTC CCG GGC CT), to bead or fiber optic hybridization supports. The probe was labeled with terminal transferase (Amersham/Pharmacia Biotech) and [α-^{32}P]dCTP at 3000 Ci/mmol (NEN Life Sciences). Labeled oligonucleotides were separated from unincorporated nucleotides by silica adsorption (QiaAmp; Qiagen) followed by three cycles of centrifugal ultrafiltration on Microcon 3 (Amicon). Radioactivity was determined by Cerenkov counting in a liquid scintillation counter (Beckman LS-3801). Hybridizations were carried out for 30–60 min at room temperature using 200 µl reactions containing 0.1 µM ^{32}P-labeled target, TE buffer with 0.2 M NaCl and 0.5% SDS (HYB buffer). During hybridizations with bead supports, reactions were mixed gently on a rotating wheel. Following hybridization, supports were washed with three changes (~1 ml/change for fiber tips, ~0.5 ml/change for the beads) of HYB buffer and the amount of hybridized probe was determined by Cerenkov counting.

Microtiter plate hybridization

Gel solutions containing 0.5× TBE, 20% acrylamide (19:1 w/w acrylamide:bis-acrylamide), 0.07% w/v APS, 0.2% v/v TEMED, 10–50 µM oligonucleotide capture probe d(GAC TGC TGG CGG AAA ATG AGA AAA) modified with a 5′-acrylamide group were prepared. Gel solutions were pipetted into the wells of a dry untreated polystyrene microtiter plate (Nunc Polysorb, U-shaped well, 50 µl/well) and allowed to polymerize for 30 min at room temperature. Following polymerization, the wells were washed extensively with 0.5× TBE, soaked overnight at 4°C in 0.5× TBE and washed again immediately before hybridization. Hybridization was carried out at room temperature for 20 min using 1 µM complementary, d(TTC TCA TTT TCC GCC AGC AG), or non-complementary, d(TGA GGC TTG CTG TTA TGG TAC), 5′-fluorescein-modified target oligonucleotide in TE with 0.32 M NaCl. Following hybridization, wells were washed six times with 200 µl 0.5× TBE and fluorescence remaining in the wells was measured using a Molecular Dynamics Fluorimager.

Preparation of acrylamide arrays on slides

Gel solutions were prepared containing 75% glycerol, 10% total acrylamide (29:1 w/w acrylamide:bis-acrylamide), 5 µM 5′-acrylamide oligonucleotide, 0.125% w/v APS and 0.125% v/v TEMED. Aliquots of gel solution (0.2 µl) were manually pipetted onto silanized slides (acrylic silane-treated slides; CEL Associates, Houston, TX). Spotted slides were placed in a humid nitrogen atmosphere at room temperature for 5 min to allow polymerization. Polymerized slide arrays were subjected to electrophoresis in an agarose minigel box (50 mM Tris–acetate, pH 7.8, 2 mM EDTA, 20 V/cm, 20 min) to remove non-immobilized probe. Slides were rinsed in TE buffer or water and dried with a stream of nitrogen. In some cases, slide arrays were stored in TE buffer. Slides stored wet (immersed in TE) or dry (ambient temperature and humidity) showed similar performance (data not shown). The 5′-acrylamide capture probes used were: probe 1, d(CAG AAT CGT TAG TTG ATG GCG A); probe 2, d(AAT CCA AAA CGG CAG AAG); probe 3, d(GTT GCC CGT CTC GCT GGT GAA A). The capture probes were attached to the 5′-acrylamide group by an 18 atom polyethylene glycol spacer (Spacer 18 phosphoramidite, catalog no. 10-1918-90; Glen Research, Sterling, VA).

Generation of fluorescent asymmetric PCR product

A primary symmetric PCR amplification was carried out using ϕX174 virion target DNA using primers d(GAC TGC TGG CGG

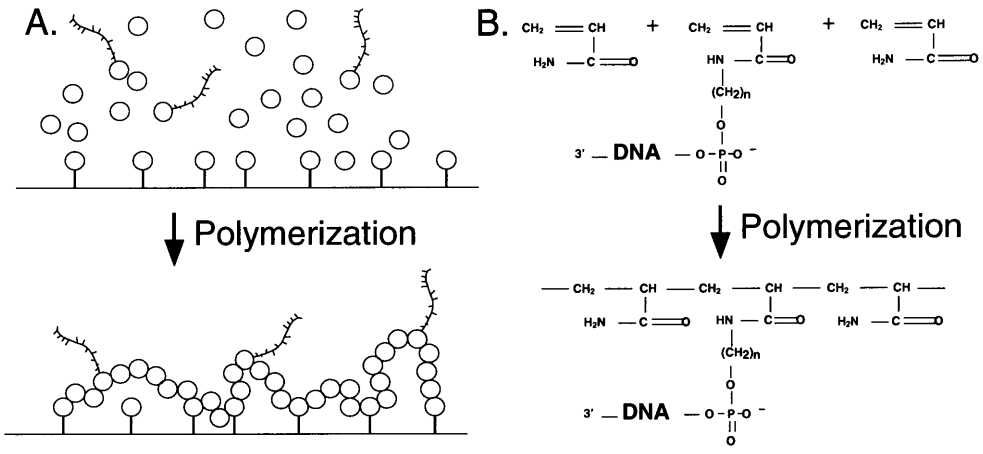

Figure 1. Co-polymerization attachment concept. (**A**) The circles represent polymerizable functional groups. In this paper, acrylamide and acrylamide derivatives were used. The horizontal line indicates a support that has exposed polymerizable surface groups. Oligonucleotides with 5′-terminal acrylamide groups are mixed with acrylamide monomer and bis-acrylamide and the mixture is placed in contact with the surface. After polymerization, the oligonucleotides are covalently incorporated into a polyacrylamide co-polymer which is attached to the support at multiple sites. (**B**) The structure of the polymer linkage between the DNA oligonucleotide and the polyacrylamide backbone is shown schematically. The acrylamide group on the 5′-end of the oligonucleotide is added during automated synthesis using an acrylamide phosphoramidite. Polymerization is catalyzed using standard chemical or photochemical methods.

AAA ATG AGA AAA) and d(ACG CAG CGA CGA GCA CGA GAG CGG TCA GTA G). The reaction contained target (10^7 copies), 1 µM each primer, 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 µM each dNTP and 2.5 U Taq polymerase (Promega) in a 50 µl volume. Thirty cycles of amplification were performed using steps of 94°C for 20 s, 60°C for 20 s and 72°C for 40 s. A 2.5 µl aliquot of the symmetric amplication product was used as target for a 50 µl asymmetric PCR reaction using the same reaction conditions and cycling conditions except that a single 5′-fluorescein primer was used [d(GAC TGC TGG CGG AAA ATG AG)]. The asymmetric PCR product was used in slide hybridization experiments without purification.

Slide array hybridization

Hybridization reactions were performed using a 100 µl plastic chamber attached to the slide by a rectangular adhesive spacer (Frame-Seal; MJ Research, Watertown, MA). Hybridization mixtures (100 µl) contained 50 µl asymmetric PCR product in slide hybridization buffer (50 mM Tris–HCl, pH 8.0, 50 mM sodium phosphate buffer, pH 6.0, 5 mM EDTA, 0.01% w/v SDS, 200 mM NaCl). Hybridization was carried out overnight at room temperature. After hybridization, the hybridization chamber was removed and the slide was washed in 200 ml slide hybridization buffer for 1 h at room temperature with vigorous stirring. Washed slides were covered with slide hybridization buffer and scanned to detect fluorescein-tagged target using a Molecular Dynamics Fluorimager.

RESULTS

The original motivation for the work described in this paper came from our efforts to develop a primer attachment chemistry of sufficient stability for use in solid phase PCR methods. In a survey of several published oligonucleotide attachment methods, we found that all provided adequate stability and primer loading for low temperature (<50°C) hybridization procedures, but that none would withstand standard PCR cycling protocols. Because of the good thermal stability of polyacrylamide-based supports demonstrated by Mirzabekov *et al.* (25), we conceived the co-polymerization attachment method shown schematically in Figure 1. Oligonucleotide capture probes, chemically modified with a polymerizable acrylamide group (circles in Fig. 1A), are mixed with acrylamide monomer (and/or other suitable monomers) and placed in contact with a support that is also chemically modified with polymerizable groups (Fig. 1A). During polymerization of the mixture, a polymer layer containing the co-polymerized DNA probes is formed on the surface. The probes are held within the co-polymer by stable carbon–carbon bonds (Fig. 1B) and the polymer layer is attached to the underlying support at many points (Fig. 1A), creating an extremely durable hybridization surface.

Oligonucleotides containing polymerizable acrylamide groups at their 5′-terminal positions were synthesized using standard automated β-cyanoethyl phosphoramidite chemistry with a commercially available acrylamide phosphoramidite (Acrydite™ phosphoramidite; Mosaic Technologies, Boston, MA). To illustrate the specificity of co-polymerization attachment, oligonucleotides with 5′-terminal acrylamide groups were co-polymerized into block-shaped regions within a 20% polyacrylamide (non-denaturing) minigel. As controls, identical oligonucleotides containing 5′-terminal amines instead of acrylamide groups were cast into adjacent regions within the gel. Following polymerization, the samples were subjected to electrophoresis for sufficient time to move non-immobilized oligonucleotides out of the blocks. An image of the ethidium bromide stained gel is shown in Figure 2. Quantification of the fluorescent signals demonstrates that ~83–84% of the oligonucleotides with 5′-acrylamide modification were firmly attached within the blocks. In contrast, only 6–9% of the 5′-amino oligonucleotides were immobilized within the blocks. These results demonstrate that immobilization of 5′-acrylamide oligonucleotides by co-polymerization is dependent on the presence of the acrylamide group and that non-specific attachment by other groups occurs at a 10-fold lower level.

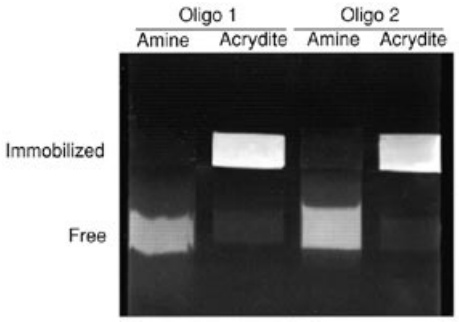

Figure 2. Specificity of co-polymerization attachment. Two pairs of oligonucleotides (lanes marked Oligo 1 and Oligo 2) were synthesized. Each pair of oligonucleotides had the same base sequence, but one had a 5′-terminal primary amine modification (Amine lanes) and the other had 5′-terminal acrylamide modification (Acrydite lanes). The four oligonucleotides were co-polymerized into gel blocks within a non-denaturing polyacrylamide electrophoresis gel as described in Materials and Methods. The gel was briefly subjected to electrophoresis, stained with ethidium bromide and imaged using a Molecular Dynamics Fluorimager.

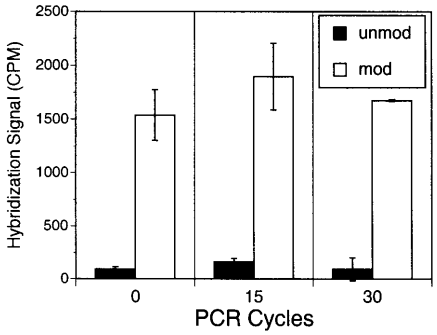

Figure 3. Stability of capture probes during PCR thermocycling. Oligonucleotides modified with 5′-acrylamide groups (mod, open bars) and unmodified oligonucleotides of identical sequence (unmod, solid bars) were co-polymerized separately into polyacrylamide beads (Materials and Methods). Beads of each type were thermocycled for the indicated number of cycles, electrophoretically washed to remove non-immobilized probe and hybridized to a complementary ^{32}P-labeled oligonucleotide target. The amount of hybridized target was determined by Cerenkov counting. The values represent the mean ± SD from duplicate measurements.

One of the original motivations for developing the co-polymerization method was the potential for achieving thermally stable attachment. This is expected since the connections between the polymer and oligonucleotide are stable carbon–carbon or carbon–nitrogen bonds. In addition, each oligonucleotide is attached to the support at many points through a crosslinked polymer network (Fig. 1). To test the thermal stability of the gel attachment, polyacrylamide beads (25 µl volume) containing 5′-acrylamide oligonucleotides or unmodified oligonucleotides of identical sequence were prepared (Materials and Methods) and subjected to a standard program of thermocycling suitable for PCR. Following thermocycling, the beads were subjected to electrophoresis to remove non-immobilized primers and then hybridized with a complementary ^{32}P-end-labeled oligonucleotide to assess primer loss during thermocycling. The results of the hybridization assay, shown in Figure 3, demonstrate that no systematic primer loss can be detected. The beads polymerized with unmodified oligonucleotide showed a low background signal, indicating that non-specific co-polymerization of unmodified oligonucleotides was very low. Using the specific activity of the probe and the calculated surface area of the beads, the apparent surface density of primer was estimated to be ~40 fmol/mm^2.

Co-polymerization attachment of oligonucleotide probes to plastic surfaces, in this case microtiter plates, is shown in Figure 4. Gels (50 µl/well) containing 5′-acrylamide oligonucleotides, 5′-amine oligonucleotides or no oligonucleotides were cast in duplicate wells of a standard untreated polystyrene microtiter plate. The plates were washed overnight to remove non-immobilized capture probe and hybridized with complementary and non-complementary fluorescein-labeled oligonucleotide. After washing away non-hybridized sample, hybridized target oligonucleotides were detected using a 2-dimensional scanning imager. As seen in Figure 4, complementary labeled oligonucleotides hybridized strongly to wells containing 5′-acrylamide probes. Hybridization of complementary probe to 5′-amine capture probes was not observed. Non-specific binding of non-complementary labeled oligonucleotides to any of the wells was not observed either. By comparison with a series of fluorescein standard samples (data not shown), the well containing 50 µM 5′-acrylamide probe captured ~10 pmol oligonucleotide. Using the simplifying assumption that all of the hybridization occurred on the surface of the acrylamide layer, the apparent capture probe density is estimated to be 260 fmol/mm^2. The polyacrylamide layers remained securely attached to the plate, even after a 1 h incubation in hybridization buffer at 70°C.

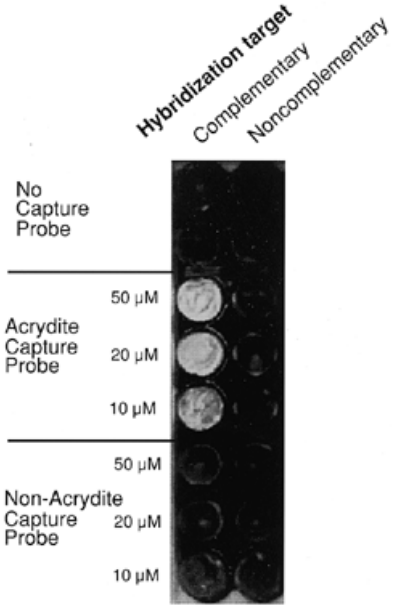

Figure 4. Immobilization of 5′-acrylamide oligonucleotides in microtiter plates. Acrylamide mixtures containing a 5′-acrylamide oligonucleotide (Acrydite Capture Probe), an unmodified oligonucleotide of identical sequence (Non-Acrydite Capture Probe) or no oligonucleotide (No Capture Probe) were polymerized in the wells of an untreated polystyrene microtiter plate. Non-immobilized probes were washed away by an overnight incubation in buffer. The wells were hybridized to complementary (left column) and non-complementary (right column) fluorescently labeled oligonucleotide targets. The fluorescent image of the plate was obtained using a Molecular Dynamics Fluorimager.

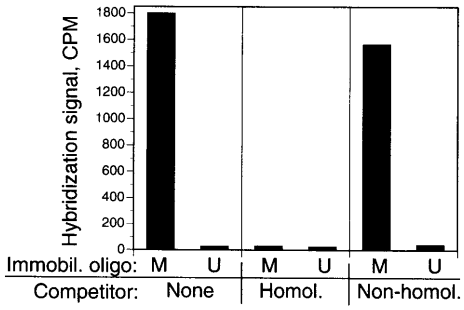

Figure 5. Hybridization to photopolymerized fiber optic supports. Polyacrylamide layers containing 5′-acrylamide capture probes were photopolymerized onto the ends of glass optical fibers as described in Materials and Methods. Fiber tips were hybridized to complementary ^{32}P-labeled oligonucleotide targets alone (left), complementary target in the presence of 100-fold excess of unlabeled complementary target (center) and complementary target in the presence of 100-fold excess of unlabeled non-complementary target (right). From the specific activity of the probe (~12 000 c.p.m./pmol), the probe density on the fibers is ~0.15 pmol, corresponding to 190 fmol probe/mm^2 area.

Numerous photochemical systems have been used to catalyze acrylamide gel polymerization. Light-directed polymerization has been developed by Walt and co-workers for fabrication of miniature multi-element chemical sensors and hybridization arrays (5,28). Photopolymerization is especially attractive for these purposes since highly focused light sources can be used to generate probe or primer arrays with small features. In addition, photopolymerization processes eliminate the need to dispense actively polymerizing monomer solutions.

To test the use of photochemical catalysts in our co-polymerization method, 5′-acrylamide oligonucleotides were photopolymerized onto the tips of glass optical fibers using riboflavin as a photoinitiator. Our procedure was adapted from Barnard and Walt (28). The fiber tips were functionalized with a polymerizable acrylic silane. To generate the photopolymerized layer, the silanized tip was immersed in acrylamide/probe solution and illuminated from the opposite end with a strong white light source. A small drop of polymerized polyacrylamide was formed on the fiber tip. Polymerized tips were washed electrophoretically and hybridized with a ^{32}P-labeled complementary target to assess capture probe density. Figure 5 shows that tips photopolymerized with 5′-acrylamide probes showed a strong hybridization signal. In contrast, tips polymerized with unmodified probes showed no hybridization signal above background. The specificity of the hybridization to the 5′-acrylamide probes is seen from the effect of unlabeled competitor hybridization targets: homologous competitor in 100-fold excess eliminated hybridization signal while non-homologous competitor reduced signal by only 15%. Estimating the surface area of the tip as a flat circle with the same diameter as the fiber, the estimated surface density of the capture probes is ~200 fmol/mm^2, in rough agreement with the value obtained from the microtiter plate experiment (Fig. 4).

The co-polymerization attachment technology is easily adapted for use in fabricating glass hybridization arrays as demonstrated in Figure 6. Acrylamide mixtures containing three different 5′-acrylamide probes were spotted in triplicate and polymerized onto the surface of a glass microscope slide. The slide had been pretreated with an acrylic silane to introduce surface co-polymerizable groups. The slide was hybridized to a fluorescein-labeled asymmetric PCR product complementary to two of the immobilized probes.

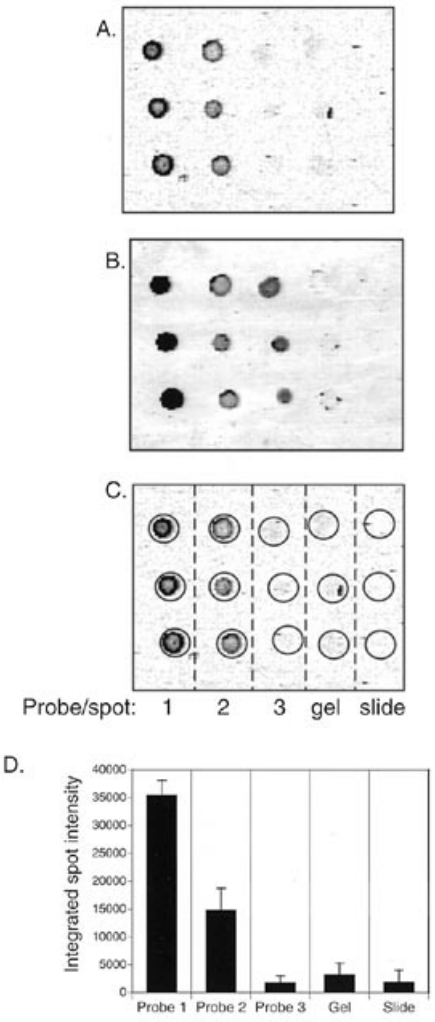

Figure 6. Hybridization of PCR products to co-polymerized 5′-acrylamide probes on glass slides. (**A**) Acrylamide mixtures containing three different 5′-acrylamide probes [spots 1–3 in (C)] or lacking probe [spots gel in (C)] were manually spotted onto the slide in triplicate and allowed to polymerize. The array was electrophoretically washed to remove non-immobilized probe. The slide was hybridized with a 435 base fluorescently labeled asymmetric PCR product. Probes 1 and 2 are complementary to the PCR target, but probe 3 is not. Following hybridization, the slide was washed to remove unhybridized target and scanned using a Molecular Dynamics Fluorimager. (**B**) To confirm the presence of the non-complementary probe 3, the hybridized array was stained with SYBR green I, which stains both single-stranded and double-stranded DNA, and rescanned. (**C**) The image of (A) is reproduced, showing the positions of each probe spot in the array. The regions circled were used for calculation of integrated fluorescence intensity as shown in (D). The positions marked gel contain three polyacrylamide spots without co-polymerized probe and positions marked slide are three equivalently sized areas of the bare slide. (**D**) The average integrated fluorescence intensity from each set of spots in the image of (A) is shown. Error bar length represents twice the SEM.

Following hybridization and washing, the slide was scanned on a fluorescence imager to reveal the distribution of hybridized target as shown in Figure 6A. That image shows high fluorescent signal on the spots containing probes 1 and 2, which are complementary to different positions within the asymmetric PCR product. Very little fluorescent signal is seen on the spots containing non-complementary probe (probe 3) or underivatized polyacrylamide gel (gel). To verify that the probe 3 spots did

contain immobilized oligonucleotide, the slide was stained with a fluorescent DNA stain (SYBR green I) and rescanned to produce the image shown in Figure 6B. Averaged hybridization and background signals from the image shown in Figure 6A are plotted in Figure 6D, using the spot definitions shown in Figure 6C. Non-specific target binding to non-complementary probe 3 is indistinguishable from the background signals of underivatized polyacrylamide gel (gel) or adjacent regions of the glass slide (slide, Fig. 6C and D). Hybridization signals from the spots containing complementary probes 1 and 2 exceeded background levels by 19- and 8-fold, respectively.

DISCUSSION

The co-polymerization attachment method described in this report has several important advantages over existing methods for DNA immobilization. It is easy to prepare co-polymerizable probes by automated DNA synthesis using an acrylamide phosphoramidite (Material and Methods). Probe immobilization can be accomplished with standard inexpensive gel polymerization techniques that are already widely used in molecular biology laboratories. Immobilization does not require highly reactive and unstable chemical crosslinking agents. The 5′-acrylamide probes co-polymerize efficiently with acrylamide and probe attachment is highly specific for the terminal acrylamide group (Fig. 2). Larger co-polymerizable probes can also be generated using PCR with 5′-acrylamide primers, since the thermocycling reaction has no effect on the terminal acrylamide groups (data not shown).

Attachment of co-polymerized probes is thermally stable. The immobilized probes are joined to the polyacrylamide layer by carbon–carbon bonds and the layer is attached to the support at multiple points (Figs 1 and 3). The results of Figure 3 have been confirmed in other experiments using 5′-acrylamide primers immobilized on glass for solid phase PCR applications (E.S.Abrams *et al*., in preparation).

High probe densities are achievable. Inclusion of 5′-acrylamide probes at 10 μM in the polymerization mixture yields gels with apparent surface densities of ~200 fmol hybridizable probe/mm^2 (Figs 4 and 5). This value compares favorably with literature values using other attachment methods, which range from 20 to 500 fmol/mm^2 (4,8,10,12,17,29). Interestingly, in two studies using different supports, aminated polypropylene (29) and glass with a phenylenediisothiocyanate linkage (4), the optimum probe densities for hybridization were similar, ~300 and 330 fmol/mm^2, respectively. These values are very close to the apparent probe density achieved by our co-polymerization method (Figs 4 and 5). Further experiments will be required to evaluate hybridization efficiency in our system at higher probe density.

Co-polymerization of oligonucleotide probes into acrylamide-based co-polymers has been independently proposed by two other groups (30,31). Ozaki *et al*. (30) used a method very similar to ours to coat the walls of silica capillaries with oligonucleotide-containing co-polymers for affinity capillary electrophoresis applications. Muscate *et al*. (31) generated linear, uncrosslinked oligonucleotide-containing co-polymers also for affinity capillary electrophoresis applications.

Our method also resembles that of Livache *et al*. (11,32) in some aspects. Their method for DNA attachment is based on co-polymerization of pyrrole-modified oligonucleotides into a polypyrrole co-polymer. Their method differs from ours in that co-polymerization is electrochemically catalyzed and the probe-containing co-polymer is deposited onto an electrode. The polypyrrole hybridization surface had good thermal and chemical stability.

Acrylamide-modified probes can also be immobilized in standard polyacrylamide slab gels for electrophoresis, as shown in Figure 2. In other work, we have demonstrated that electrophoresis of single-stranded samples through polyacrylamide gels containing immobilized probes is an efficient and highly specific method for performing hybridization reactions (33; P.W.Hammond and T.C.Boles, in preparation).

The co-polymerization process described here should be generally useful for fabricating a wide variety of DNA-containing polymers, including microparticles, dendrimers, linear soluble polymers and polymer coatings. It should be especially useful for synthesis of self-assembling polymers that utilize specific nucleic acid base pairing interactions as an organizing principle (1,13,15,34,35). The use of DNA-containing co-polymers could significantly enhance future efforts to develop polymeric self-assembling systems, which have been primarily based on polymer–polymer and polymer–solvent interactions (36). The co-polymerization method described here should accelerate development of these materials.

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