ENABLING DNA-MICROARRAYS IN POLYMERIC LAB-ON-A-CHIP SUBSTRATES FOR MULTIPLEXED TARGET ANALYSIS *VIA* SOLID-PHASE PCR

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ABSTRACT

A novel universal protocol is demonstrated for covalent grafting of solid-phase PCR primers oriented onto glass and various polymers (COP, PP, COC, PDMS) relevant for Lab-on-a-chip (LoaC) applications. Primers immobilized in a DNA-microarray format feature spots with high homogeneity and integrity. PCR compatibility of the novel immobilization protocol was confirmed by applying such arrays to solid-phase PCR (SP-PCR), improving previously reported "enhanced SP-PCR" [1] in terms of factorial signal increase from 9.9 to 86.8 and specificity from 11.7 to 45.9. Our method also enables to directly integrate DNA-microarrays amenable for SP-PCR into microfluidic LoaC cartridges of various materials circumventing the need of hybrid assemblies.

KEYWORDS: DNA immobilization, solid-phase PCR, polymers, microarray

INTRODUCTION

Hybrid systems consisting of glass- or silicon DNA microarrays integrated into plastic microfluidic LoaC systems are of advantage when established surface chemistries for manufacturing DNA-microarrays are combined with established prototyping techniques for LoaC cartridges [2]. Nevertheless, such hybrid systems pose technical challenges as well as economic disadvantages. These could be overcome by integrating DNA-microarrays monolithically into LoaC systems. We provide the currently missing link for such systems by an immobilization protocol for bonding primers onto typical LoaC polymers in a PCR-compatible way, enabling multi-target analysis via SP-PCR [3,4].

PRIMER IMMOBILIZATION

First, hydroxyl groups are generated on the surface by activation with an oxygen plasma. Second, 3-aminopropyltriethoxysilane (APTES) [5] is condensed to the hydroxyl groups. A covalently interconnected silane network is obtained by a subsequent curing step at 70°C. Next, PDITC is bound to the primary amino-groups of the APTES-silanized surface [6]. Finally, 5'-NH_2 modified solid-phase primers for later solid-phase reactions are spotted in 1 nL droplets to the now thiocyanate activated surfaces in dilution series with concentrations of 0.05, 0.10, 0.20, 0.40, 0.80, and 1.60 µM. A 5'-NH_2 and 3'-Cy5 modified primer is used to visualize primer immobilization and as spotting control. The DNA-microarrays are generated using a 6 x 4 nozzle Topspot® printhead, dispensing 24 spots per print (Figure 1). Three printing-blocks are deposited per substrate, each printing-block composed of 4 x 5 single prints, resulting in 480 spots per printing-block and 1440 spots per substrate in total. Prior to immobilization, the different polymer substrates where cut into the format of standard microscope slides. For each polymer substrate the DNA-microarrays are printed onto two separately processed and PDITC-activated slides.

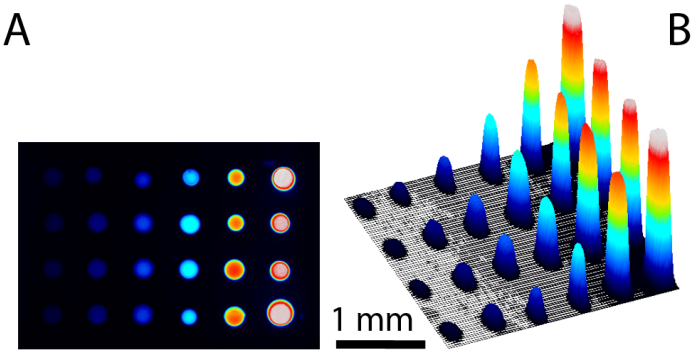

Figure 1: Cy5 scan of dilution series printed by a TopSpot® system onto PDMS showing flawless microarray spots with high integrity and homogeneity (A). Six droplets à 1 nL in quantities from 50 to 1600 amol are deposited in four replicas. 3-D plot of the dilution series bonded onto PDMS via PDITC (B).

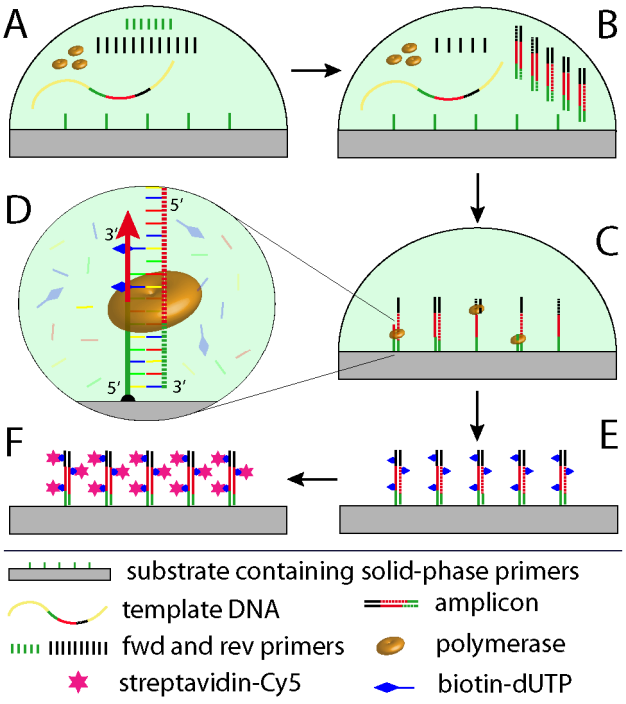

Figure 2: Schematics of solid-phase PCR used in our characterization experiments. Initially, a reaction compartment contains next to solid-phase primers forward (fwd) and reverse (rev) primers in an asymmetric ratio (A); in the beginning, PCR proceeds preferably in the liquid phase, until the forward (fwd) primer is depleted (B); then, solid-phase PCR dominates, where the 3' end of the immobilized primer is extended by polymerase activity, incorporating biotin-dUTPs (C, D); for visualizing the PCR product fixed to the solid-phase, streptavidin-Cy5 is bound to the incorporated biotin-dUTPs (E, F).

EXPERIMENTAL RESULTS

We demonstrate SP-PCR experiments (Figure 2) on microarrays with highly homogeneous and integer spots (Figure 1) containing *Cy5 primer* as spotting control, *extendable primer* as control for oriented grafting and thermal stability, and *not extendable primer* as negative control. After SP-PCR, surface-bound PCR products are stained and then scanned with a microarray reader (Figure 3). The intensities from 2 × 8 spots from two individually processed substrates were evaluated by dividing individual spot intensities before and after SP-PCR, giving a dimensionless number (Figure 4). Factorial signal increase of the *extendable primer* after SP-PCR is measured to 43.9 ± 4.8 (COC), 45.7 ± 6.9 (COP), 53.6 ± 5.4 (PP), 72.5 ± 6.7 (glass), and 86.8 ± 10.2 (PDMS) compared to 9.89 in literature [1]. Specificity, defined as the ratio between the signal increase of the *extendable-* and *not extendable primer* after SP-PCR yielding 21.7 ± 2.8 (COC), 45.9 ± 20.9 (COP), 21.6 ± 3.3 (PP), 31.7 ± 4.3 (glass), and 34.2 ± 6.7 (PDMS) compared to a specificity of 7.6 – 11.7 in [1].

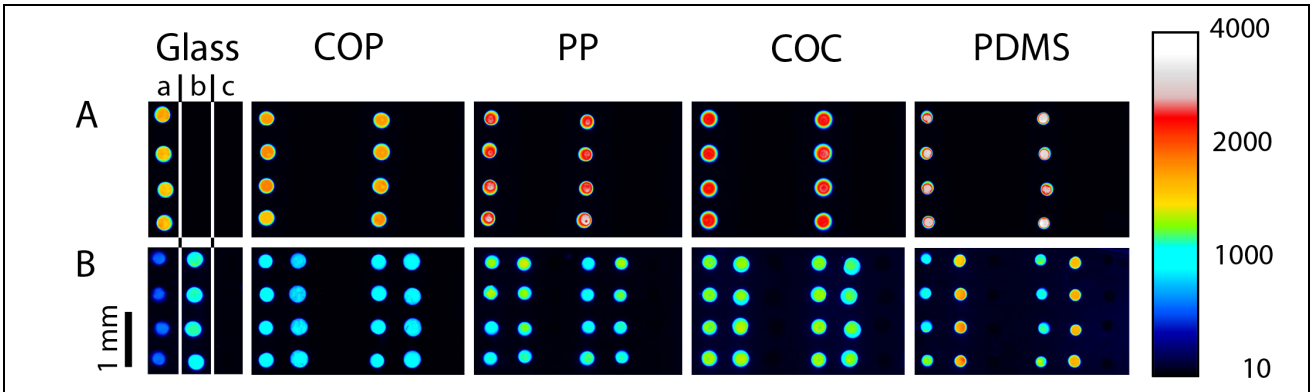

Figure 3: Scans containing the Cy5 control primer (lane a), extendable primer (lane b), and the not extendable primer (lane c) in rows of four spots per substrate. Scanning is done before (A) and after solid-phase PCR and staining (B). Highly specific extension of the solid-phase primer is observed on all polymers and also glass despite a thermally induced loss or degradation of primers (signal decrease of Cy5 primer before and after SP-PCR).

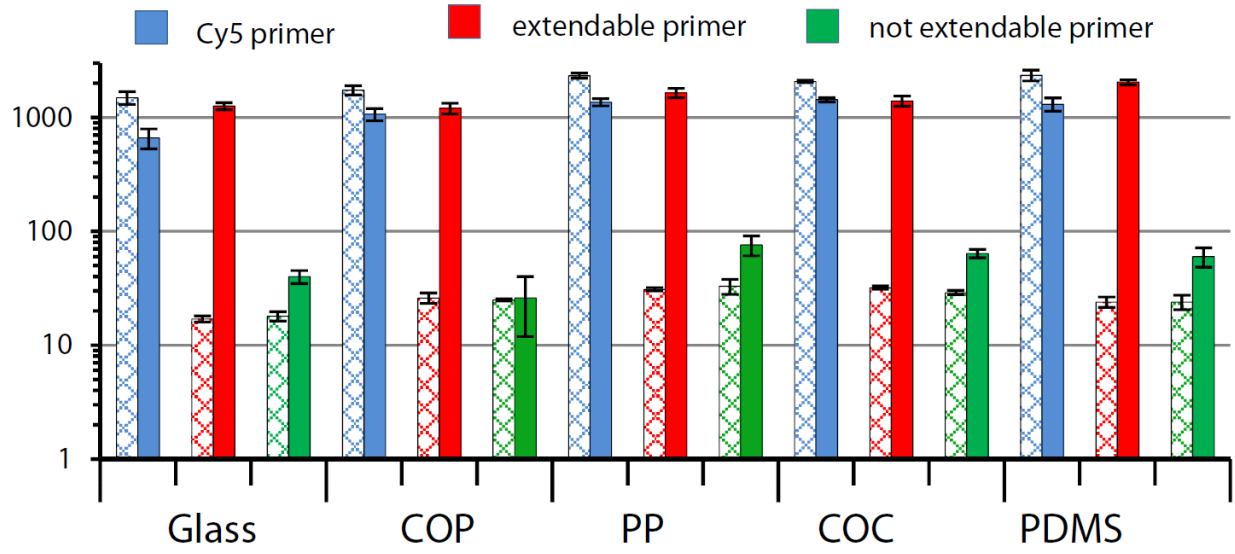

Figure 4. Measured fluorescence intensities for each substrate before (crossed bars) and after (bold coloured bars) solid-phase PCR. On all substrates, signals from the extendable primer significantly increase after SP-PCR, whereas signals remain close to the background for the not extendable primer, indicating an excellent SP-PCR system. All solid-phase primers are spotted in end-concentrations of 2.00 µM, for which reason intensities of the Cy5 primer before PCR are higher than intensities from Figure 2 (end-concentration of Cy5 primer: 1.60 µM). Gaussian standard deviations include slide to slide variations, n = 16.

CONCLUSION AND OUTLOOK

Reported achievements comprise a universal protocol for oriented covalently immobilization of DNA on COP, PP, COC, PDMS, and glass being compatible to PCR. Arrayed solid-phase primers on all substrates are extendable by polymerase, triggering highly intense and specific signals from SP-PCR with ~ 10 fold yield compared to state-of-the-art [1,3]. Our work paves the way to combine comprehensive LoaC functionalities with DNA-microarrays for highly multiplexed target analysis via SP-PCR [4], enabling manufacturing of "DNA-microarrays" monolithically integrated into "Lab-on-a-Chip" systems.

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