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Transfer of Surface Polymerase Reaction Products to a Secondary Platform with Conservation of Spatial Registration

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In this paper, we describe a method for directly transferring the product of a biological surface reaction from a primary reactant surface to a secondary product surface. Our approach, which is related to an array-replication method reported previously by us,[1,2] Stellacci,[3−5] and others,[6] is illustrated in Scheme 1. First, single-strand DNA (ssDNA), modified with a reactive amine group on the 5′ end, is spotted onto an epoxy-modified glass surface (complete experimental details are provided in the Supporting Information). This results in immobilization of the DNA template on the reactant surface. Second, biotinylated primer oligonucleotides are hybridized to the ssDNA template. Third, the primers are extended via a T4 polymerase reaction.[7,8] Fourth, a streptavidin-coated poly(dimethylsiloxane) (PDMS) monolith is brought into contact with the reactant surface.[1,2] This results in binding of the reaction product (the extended DNA complement) to the PDMS product surface via biotin/streptavidin interaction. Finally, the reactant and product surfaces are mechanically separated from one another, resulting in transfer of the product of the polymerase reaction to the PDMS surface. We show later that the product surface is able to selectively bind its complementary DNA and that a single reactant surface can be used multiple times to generate isolated product. Importantly, spatial registration is maintained between the reactant and product surfaces.

Scheme 1

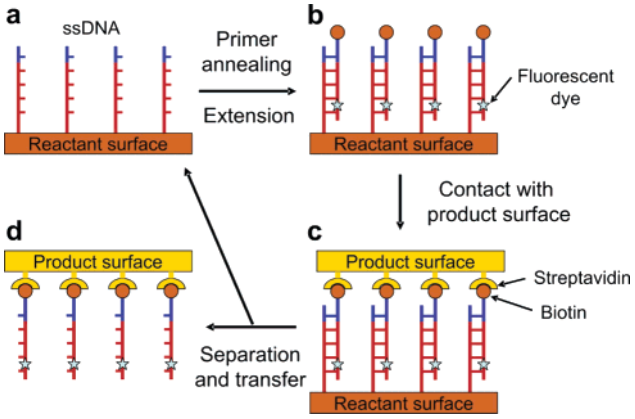

Figure 1 demonstrates ssDNA immobilization onto the reactant surface, primer annealing and extension, and product transfer. Specifically, Figure 1a is a fluorescence micrograph obtained after immobilizing the 38-base ssDNA template onto an epoxy-modified glass surface, annealing the primer to the template, and then extending the primer. In this case, the polymerase reaction mixture included dye-labeled deoxycytidine triphosphate (Cy3-dCTP), and therefore the extended primer is fluorescent (Scheme 1). Control experiments indicated that no fluorescence could be detected from the reactant surface after immobilization of the template and annealing of the primer, but before addition of Cy3-dCTP and primer extension (Supporting Information, Figure S1a). Likewise, no fluorescence was observed when the primer-annealed reactant surface was exposed to all reactants (including Cy3-dCTP) except for the T4 DNA polymerase and then rinsed with buffer (Figure S1b). This indicates no detectable level of nonspecific adsorption of the dye.

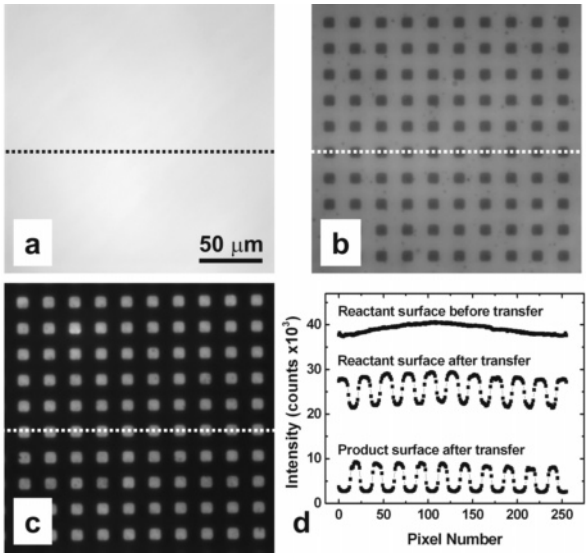

Figure 1. Fluorescence micrographs demonstrating extension of primers and transfer of the extended primers. (a) Fluorescence micrograph obtained from a reactant surface after a polymerase reaction incorporated Cy3-dCTP into the extended primers. (b) Fluorescence micrograph obtained from the reactant surface after transfer of the extended primers. (c) Fluorescence micrograph obtained from the product surface after transfer of the extended primers. (d) Fluorescence intensity profiles obtained along the dotted lines shown in (a)−(c). Integration time was 100 ms. Gray scales are 16 000−42 000 counts for (a) and (b), and 2500−15 000 counts for (c).

Parts b and c of Figure 1 are fluorescence micrographs of the reactant and product surface, respectively, after transfer of the extended primer. The dark regions on the reactant surface (Figure 1b) correspond to DNA incorporating Cy3-dCTP that was transferred to the product surface, and the light regions in Figure 1c correspond to the transferred DNA on the product surface. The checkerboard pattern results from drainage canals (20 μm on center, 10 μm wide, and 3 μm deep) on the product surface (Supporting Information, Figure S2). These canals are necessary for successful DNA transfer, because they provide a means for buffer solution trapped between the reactant and product surfaces to escape.[1,2] Figure 1d shows fluorescence intensity profiles obtained along the dotted lines in Figure 1a−c. The average intensity difference between the bright and dark regions on the reactant surface ((6.8 $\pm$ 0.2) $\times$ 10^3 counts, Figure 1b) is very close to that on the product

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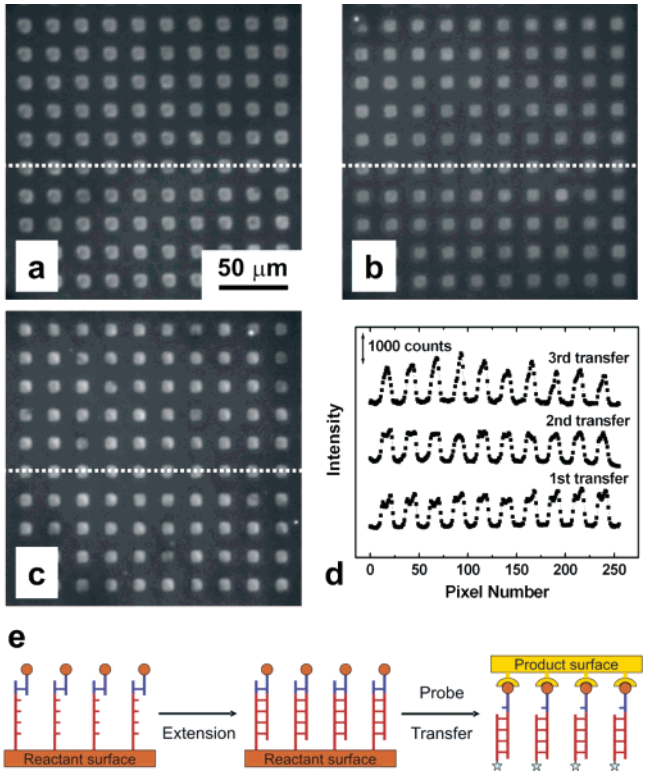

Figure 2. Fluorescence micrographs demonstrating multiple transfers of extended primers from a single reactant surface. (a) Fluorescence micrograph obtained from a product surface after primer extension, transfer of the extended primers, and hybridization of a fluorescent probe complementary to the extended primer (but not to the primer itself). (b) Same as (a), but after a second round of primer extension, transfer, and hybridization. (c) Same as (a), but after a third round of primer extension, transfer, and hybridization. (d) Fluorescence intensity profiles obtained along the dotted white lines shown in (a)−(c). (e) Scheme showing the experimental approach used to obtain the data in (a)−(d). The star symbols represent the fluorescent dye. The integration time was 1000 ms. The gray scale is 2500−5500 counts for (a)−(c). The fluorophore attached to the probe oligonucleotide was FAM (fluorescein).

surface ($(6.0 \pm 0.4) \times 10^3$ counts, Figure 1c), indicating little net loss of extended primers during transfer.

Figure 2 shows that multiple primer-extension reactions and transfers can be carried out using a single reactant surface. These experiments were executed using the approach shown in Figure 2e. After the primers were annealed to the immobilized template DNA, the polymerase reaction was performed using an unlabeled mixture of deoxyribonucleotide triphosphates (dNTP). This results in a surface that is not fluorescent. Next, the extended and nonfluorescent primers were transferred to a product surface. Finally, fluorescently labeled probe DNA, complementary to only the extended sequence (not to the primer), was exposed to the product surface. This process was carried out three times using the same reactant surface, and fluorescence micrographs of the three resulting product surfaces are shown in Figure 2a−c. Note that, in the absence of the T4 polymerase, no fluorescence was detected on the product surface (Supporting Information, Figure S3). There are three important conclusions that arise from this set of experiments. First, it demonstrates that multiple product transfers can be carried out using the same reactant surface. Figure 2d provides line scans corresponding to the three micrographs. These show that the average modulation in fluorescence is 1040 ± 110, 920 ± 50, and 1190 ± 190 for the first, second, and third transfers, respectively. A duplicate of this experiment was carried out using a different reactant surface, and in that case there was more variation among the first (1070 ± 90), second (1090 ± 110), and third (630 ± 50) replicates (Supporting Information, Figure S4). Second, when this experiment was carried out in the absence of internal spacers between the template oligonucleotide and the surface (18-atom hexa-ethyleneglycol spacers repeated five times: $iSp18_5$; Integrated DNA Technologies, Coralville, IA), no detectable hybridization of the fluorescently labeled complement was observed. This is likely a consequence of steric hindrance between the T4 polymerase and the glass surface, which results in incomplete primer extension. Third, Figure 2 clearly shows that the transferred reaction product is functional, because it hybridizes to its fluorescent complement.

There are two important conclusions resulting from this study. First, very small amounts of reaction products can be transferred from the reactant surface to the product surface. Here, we demonstrated transfer of $\sim 10^{-14}$ mol of DNA oligonucleotides,[9,10] but there is no technological barrier for reducing this to as few as $\sim 10^{-19}$ mol.[3] Second, the spatial relationship between reactant and product surfaces is preserved with micrometer-scale resolution after transfer, and it seems likely that this could be reduced still further.[3] This approach is demonstrated for a DNA polymerase reaction, but it should be useful for other chemical and biological reactions, too. Applications to high-throughput screening and separation of very small amounts of reaction products from a complex milieu are easily envisioned.

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Supporting Information Available: Information about the chemicals and materials, experimental procedures, and the control experiments described in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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