DNA Double-Write Photolithography: A Synergy for Top-Down and Bottom-Up Nanofabrication (Abstract # 611)

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ABSTRACT

Intrinsic programmability and other properties of DNA allow its use for bottom-up self-assembly and as a top-down photolithographic material. These capabilities provide considerable potential for many DNA technology applications. However, one limitation of UV patterning of DNA is that hybridization of complementary DNA sequences can only be carried out in areas not exposed to UV. Such UV *single-write* methods restrict the full potential for programmed DNA self-assembly. We now demonstrate a DNA *double-write* process that uses UV to pattern a uniquely designed DNA write material, which produces two distinct binding identities for hybridizing two different complementary DNA sequences.

Keywords: DNA, photolithography, self-assembly, top-down, bottom-up, nanofabrication

1. INTRODUCTION

Functional biomaterials based on DNA allow for patterning by a variety of methods [1-6], as well as self-assembly nanofabrication of DNA derivatized with fluorophores, nanoparticles, and other entities[7-11]. A key advantage for using DNA self-assembly is that oligonucleotide sequences with as few as 16 bases can provide a huge number ($>10^9$) of sequence programmed nanoscale structures (1.25 nm x 6 nm). Each of the DNA sequences can be designed with a unique and highly specific binding identity that can form a stable hybrid with its complementary DNA sequence. Self-assembly by hybridization of complementary DNA sequences derivatized with fluorophores, nanoparticles, and other entities is a rapid, simple, and selective process compared to methods that utilize chemical binding agents**.** Patterning on DNA substrates that produce a single binding identity has been carried out using pre-patterned polymer-blocking materials such as polymethyl methacrylate (PMMA) and electron beam lithography[12], and also by destructive micropatterning and rolling-circle amplification methods [13,14]. Other approaches for patterning with DNA and DNA nanoparticles that attempt to merge aspects of top-down and bottom-up techniques include micro-contact printing[15], silk screen patterning[16], patterning on nanosize pre-patterned etched template substrates[17,18], and patterning using AFM tips[19,20]. A DNA patterning photolithography process has been developed where UV exposure produces thymine base dimerization preventing hybridization in the exposed areas[21]. This process is single write in that it only allows subsequent self-assembly by hybridization to occur in the masked areas. Other work has been carried out where UV sensitive agents such as psoralen have been used to produce cross-linking between two DNA strands[21,22,23]. More recently, DNA sequences modified with the UV crosslinker cinnamate have been used to produce inter-strand cross-linking[24]. Further efforts have also been carried out on developing wavelength dependence for the photoreversal of a psoralen-DNA cross-linking[25,26].

2 RESULTS AND DISCUSSION

Here, we demonstrate a novel DNA *double-write* material, based solely on the design of DNA sequences. The DNA write sequence is designed with discrete placement of thymine bases which are sensitive to UV irradiation [21, 27], while one of the two complementary DNA sequences is designed to produce a displacer probe effect[28, 29]. Upon UV patterning of the DNA write sequence, which is immobilized on a support (glass, silicon, etc.), two distinct binding identities are produced to which two different complementary DNA sequences can be hybridized. This allows DNA-based self-assembly as well as further UV patterning to be carried out in both the UV exposed and non-exposed areas. Together these unique sequence design parameters enable the *DNA double write* process. The mechanism for blocking hybridization in the DNA write sequence is thymine dimerization induced by deep UV irradiation. Exposure of DNA sequences containing thymine

bases to short wavelength UV light (~254 nm) causes formation of thymine dimer cyclobutane 6-4 photoproducts[21, 27]. The dimerization of the thymine bases prevents hydrogen bonding to the adenine bases in the complementary DNA sequence. The unique DNA write sequence used in the *DNA double write* process is designed to allow two complementary DNA probe sequences with distinguishable identities to bind (hybridize) to the DNA material after UV patterning. The scheme for the DNA double write process and the DNA sequences are shown in Figure 1a and 1b.

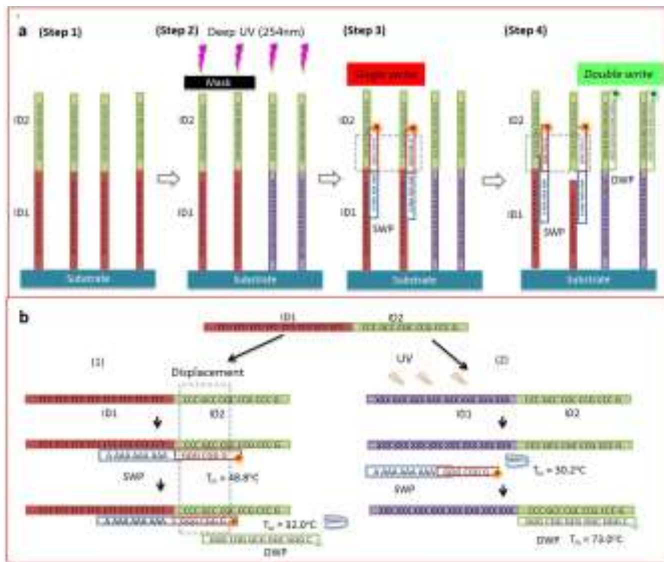

Figure 1 - Scheme for the DNA double write process. a, *Step 1 Immobilization of the DNA double write sequence (FWS:ID1&ID2) onto the glass substrate. Step 2 photomasking and UV exposure. Steps 3 and 4 hybridization of the red fluorescent SWP probe sequence 5'-Alex546-GGG CGG GAA AAA AAA AA-3' and the green fluorescent DWP probe sequence 5'-Alex488-GGG CGG GCG GGC GGG C-3'.* ***b,*** *Effect of displacer and thymine dimer formation on probe hybridization and melting temperatures (Tm).*

The first step in the overall process is the immobilization of the specially designed DNA double write sequence (FWS:ID1&ID2) onto the glass substrate surface (Fig. 1a, Step 1). The second step in the process is the photomasking and UV exposure, which is carried out for 5 minutes (Fig. 2a, Step 2). The third and fourth steps are hybridization of the red fluorescent SWP probe sequence 5'-Alex546-GGG CGG GAA AAA AAA AA-3' and the green fluorescent DWP probe sequence 5'-Alex488-GGG CGG GCG GGC GGG C-3' (Fig. 1a, Steps 3 and 4). Generally the hybridization steps take about 30 minutes, including the washings. The first DNA write sequence (FWS:ID1&ID2) 5'-TTT TTT TTT TTT TTT TTT TTT TTT (ID1)-CCC GCC CGC CCG CCC G (ID2)-3' is designed with two distinct sections, ID1 (red, Fig. 2a and 2b) and ID2 (green, Fig. 2a and 2b). This design allows two different complementary DNA probes to be specifically hybridized to the unexposed regions (red, Fig. 1a and 1b (1)) and the UV exposed regions (purple, Fig. 1a and 1b (2)), respectively. The first complementary DNA probe sequence is a red fluorescently labeled single write probe (SWP) 3'-AAA AAA AAA AGG GCG GG-5'-Alex546, and the second complementary DNA probe sequence is a green fluorescent double write probe (DWP) 3'-GGG CGG GCG GGC GGG C-5'-Alex488. The design of the UV sensitive thymine base sequence in the ID1 (red) area of the DNA write sequence FWS:ID1&ID2 is of key importance for the double write process. However, the design of the ID2 (green) sequence, and the design of the SWP and DWP complementary DNA probe sequences are equally important. Special consideration is given to melting temperature (Tm) of the sequences, as well as incorporation of a *displacer* property into the SWP complementary DNA probe sequence. The displacer property is enabled by designing the SWP probe so it is not only complementary to the ID-1 (red) sequence of the FWS:ID1&ID2, but also has a short segment of sequence (GGGCGGG) that is complementary to a section of the ID-2 (green) sequence (boxed areas in Fig. 2a and 2b). The SWP and DWP hybridization and Tm data in Table 1 show for Case 1 with no UV exposure, the hybridization efficiency of SWP probe to the FWS-ID1&ID2 sequences is high (Tm=48.8 ^{o}C). For Case 2 with UV exposed, the hybridization efficiency of SWP probe to the FWS-ID1&ID2 sequences is much lower (Tm=30.2 ^{o}C). For Case 3 with no UV exposure, the hybridization efficiency of the DWP probe to the FWS-ID1&ID2 sequence with the SWP probe hybridized is now much lower (Tm=21-32 ^{o}C). This reduction in Tm for DWP is due to the displacer effect of SWP. For Case 4 with UV exposure, the hybridization efficiency of DWP probe to the FWS-ID1&ID2 sequences is high (Tm=73 ^{o}C). In this final case, the hybridization of the SWP probe is significantly reduced due to thymine dimerization in write sequence ID1 region, and the DWP probe can now hybridize with very high efficiency. Figure 2 shows five sets of images for the patterned feature, where line widths for the letters are 10 µm (Fig. 2a), 5µm (Fig. 2b) and 2 µm (Fig. 2c). The line edges of

the letters still appear to be very sharp. Figure 2d and 2e provide further results for patterned line images, showing line widths from 10 µm down to 500 nm, with 20 µm spacing gaps (Fig. 2d), and patterned spacing gaps from 100 µm to 5 µm with 10 µm line width (Fig. 2e). These results show three times better feature size resolution for the DNA double write process compared with previous results reported for photo-crosslinking chemistry-based DNA patterning[24].

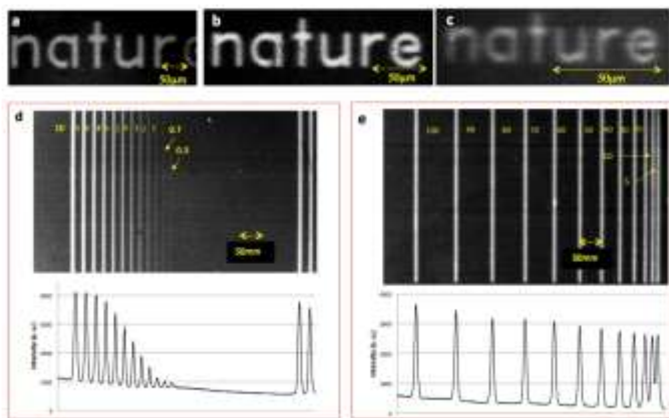

Figure 2 - DNA double write line widths with first level write. a, b, c, *The images of letters at 10 µm, 5 µm and 2 µm linewidths.* ***d,*** *The linewidth resolution from 10 µm to 500 nm.* ***e,*** *the gap resolution from 100 µm to 5 µm.*

3. CONCLUSIONS

In this study we have shown a *DNA double write* material and process could be used to create patterns with 500 nm linewidth resolution. Ultimately, much higher resolutions and shorter exposure times may be possible by using a sophisticated stepper and a high energy deep UV (192 nm, 254 nm) source. It should also be possible to produce more homogeneous DNA write layers immobilized on smoother, more defect free substrates. A variety of multiple layered DNA hybridization patterns and biotin streptavidin binding reactions were also demonstrated. We have introduced a novel method for UV photolithographic patterning of a unique DNA write material and subsequent self-assembly nanofabrication via hybridization. This approach creates two distinguishable identities after UV exposure, allowing two different complementary probes to be hybridized to the UV exposed and un-exposed areas. The key to this new process is in the DNA sequence design, which includes the presence of UV sensitive thymidine bases in the write sequences, and the specially designed complementary probe sequences which include a displacer effect for specificity to the UV exposed and un-exposed areas. Most importantly, the write sequences and complementary probe designs shown in this study do not preclude encoding ~~a~~ much larger numbers of unique binding identities into DNA as well as RNA and PNA sequences. The DNA double write process we have described represents a true synergy of top-down (photolithography) and bottom-up (self-assembly) approaches that will have an impact on DNA nanotechnology[32]. For example, it may be possible to combine our technique with DNA origami[33] and use UV patterning to produce additional conformational changes and interactions within the DNA origami structures. Since our work actually shows selective DNA information changing by UV photolithography, the new materials and process may be used with techniques employed with DNA chemical computing system[34-36]; for example, using the displacer effect of ss-DNA with higher melting temperatures[37]with surface connected DNA computing[38] to build an enzyme-free process that allows a translation system for higher sequence specificity. These represent just a few of the potential DNA nanofabrication applications that may be possible using the new DNA double write materials and process.

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