PATTERNING AND FUNCTIONALIZATION OF THERMOPLASTIC MICROCHIP FOR AUTOMATED HIGH-THROUGHPUT MICROARRAY GENE SYNTHESIS

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ABSTRACT

The development of DNA microarray technology for high-throughput gene synthesis is hampered by poor sequence quality and inefficient gene assembly. We developed a programmable microchip using thermoplastic cyclic olefin copolymer (COC) functionalized with hydrophilic SiO_2 thin film arrays, creating physically isolated picolitter reactors that constrain liquid via differential wettability. These functionalized SiO_2-COC microchips exhibit enhanced droplet confinement and reduced edge-effect during *in situ* DNA synthesis, producing high-quality oligonucleotide arrays. Further, we demonstrated accurate and effective on-chip gene assembly from complex and heterogeneous microarray oligonucleotides by integrated amplification and assembly of sub-arrays in physically-isolated reaction chambers.

KEYWORDS: High-throughput Gene Synthesis, DNA Microarray, Thermoplastic Microchip

INTRODUCTION

Gene and genome syntheses play an increasingly important role in synthetic biology and biotechnology[1-5]. The high cost and limited throughput of column-based oligonucleotide synthesis have nevertheless become a bottleneck for large-scale gene synthesis and genome assembly projects in the new synthetic biology era. Emerging technologies on highly parallelized and miniaturized synthesis hold the potential to significantly increase the throughput and decrease the cost of gene synthesis. Recent advances in DNA microarrays[6-10] that can produce pools of up to a million oligonucleotides for gene assembly, albeit in minute quantities (~105–106 molecules per sequence), has driven the rapid development of gene synthesis technology.

THEORY

A number of challenges exist in adapting microarray technology for gene synthesis. In practice, sequence integrity can be significantly compromised by "edge effects" caused by droplets misalignment, light beam drifts or improper reagent sequestration. To achieve high-quality DNA synthesis, we sought to design microchip with reduced edge effect by utilizing differential wettability between hydrophilic features and their hydrophobic background. Glass and silicon have traditionally been used for microarray fabrication due to prominent physical properties and diverse surface functionalization chemistries[11].Recently, thermoplastic materials such as cyclic olefin copolymers (COC) have been tested as low-cost alternative array substrates, especially for devices integrating microarrays with microfluidics[12-14]. COC presents a chemically inert and highly hydrophobic surface with low auto fluorescence and moisture absorption.. Its compatibility with inexpensive and easily replicable fabrication techniques like injection modeling and hot embossing allows rapid prototyping and potential integration of microfluidics system. Silica deposition can be used to functionalize COC surface to create hydrophilic reactors by constraining liquid via differential wettability. The well-established surface chemistry of SiO_2 thin film allows a variety of functional groups and linkers to be readily conjugated to the surface. We describe here the fabrication and characterization of SiO_2-COC hybrid material with reduced edge effect, and demonstrate *in situ* synthesis of high-quality oligonucleotide arrays using a custom-built inkjet DNA synthesizer.

Oligo pools produced from microarray provide a cheap source of oligos but do not simplify or reduce the cost of down-stream gene assembly process. In many cases, large complex oligo pools make gene assembly more challenging and prone to error. A number of strategies have been developed to circumvent the problem, including more efficient assembly strategies[7] and selective amplification of oligos[8]. To address this issue, we divide the entire microarray into subarrays, each containing only the oligos needed to assemble a longer DNA molecule of about 0.5–1kb in total length. Subarrays were physically isolated from the rest of the chip by being located in individual wells, eliminating the need for post-synthesis partitioning of the oligo pool. We then integrate array oligo synthesis; amplification and gene assembly steps in physically isolated wells on the same chip. The entire process is performed on-chip to increase throughput and minimize turn-around time and spurious hybridization. Using this platform, we are able to produce full-length gene constructs from the patterned and functionalized COC microchip in a high-throughput and low cost manner..

EXPERIMENTAL

An array of microwells with bare COC bottom was created with a layer of positive S1813 photoresist following standard photolithography procedures. Silicon dioxide films were deposited by using a Kurt Lesker PVD 75 (Kurt J. Lesker Company, Pittsburgh, PA) RF magnetron sputtering system. Patterned samples were then immersed in silane solutions before removal of photoresist. The SEM images were taken on a FEI XL30 SEM machine. Chemical binding energy analyses were performed using a Kratos Analytical axis ultra-x-ray photoelectron spectrometer (Kratos Analytical Inc., New York) with a monochromatic Al KR x-ray source radiation at 1486.6eV. The deposited film thickness was measured with

an optical interferometer, Nanometrics 210 (Nanometrics Incorporated, CA). Surface roughness was measured with a Bruker Dektak150 profilometer (Bruker, Billerica, MA). *In situ* DNA microarray synthesis was carried out on a custom-built piezoelectric inkjet platform based on standard phosphoramidite chemistry. Oligos were cleaved from patterned samples under ~80 psi of pressure using a pneumatic clamp assembly (Biolytic Lab Performance Inc., Newark, CA) and separated by polyacrylamide gel electrophoresis. The gels were then stained with SYBR Gold and virtualized by UV illumination in a Fluorchem gel documentation and analysis system (Alpha Innotech, San Leandro, CA, USA). On-chip oligo amplification and gene assembly using combined nicking strand displacement and polymerase cycle assembly (nSDA–PCA) reaction was performed as described in our previous publications [15].

RESULTS AND DISCUSSION

Hydrophilic SiO_2 thin film arrays were pre-patterned on the inert and hydrophobic COC surface using RF sputtering technique as illustrated in Figure 1. An array of microwells with bare COC bottom was created with positive photoresist using standard photolithography procedure. The slides were then deposited a thin film of SiO_2 with radio frequency sputter, and optionally coated with hydroxyl-silane. The resist was then removed to reveal the silanized SiO_2 features on COC surface. XPS characterization and SEM imaging (Fig.2) confirmed proper formation of silica dioxide arrays. AFM analysis indicated that the sputtered SiO_2 surface (approximately 300nm thick) had flatness comparable to that of a COC or standard glass slides. The resultant microchips were able to constrain liquid via differential surface wettability between the COC and the deposited silicon dioxide (Table 1), effectively forming individual pico-liter reactors. This effect was even enhanced with conjugation of hydroxyl silanes, which reduced the consumption of synthesis chemicals by 50%. The coupling efficiency of the first nucleotide was drastically improved due to the presence of higher population of readily accessible hydroxyl groups on the surface.

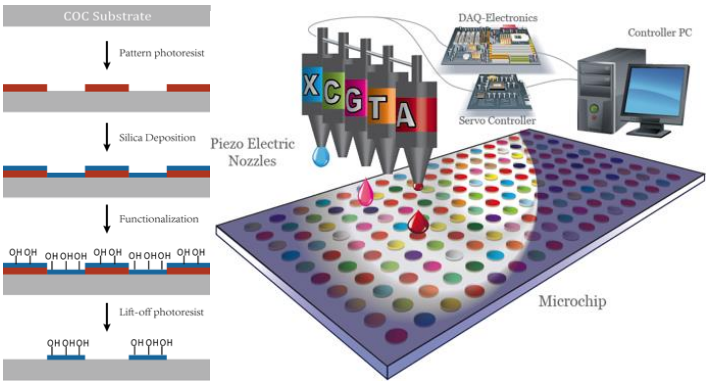


Figure 1. *Schematic illustration of in situ synthesis of DNA oligonucleotide microarray on patterned SiO2−COC slides using piezoelectric inkjet microarray synthesis technology*

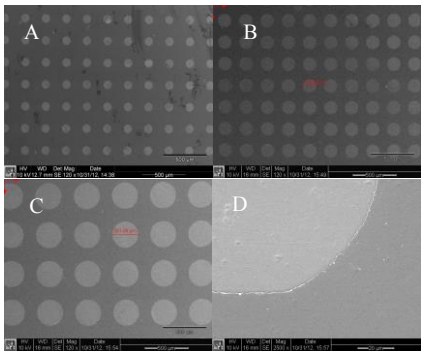
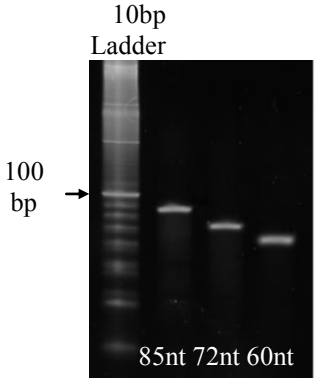


Figure 2. *SEM images illustrate the proper formation of silica dioxide arrays with different feature sizes: A) 100µm, B) 150µm, C) 300µm. D) 2500x magnification of one 300µm diameter feature*

Table 1. *Surface roughness (Ra, Rq) and contact angle measurements of COC samples with and without SiO_2 film deposition*

	Mean Surface Roughness (Ra)	Root-mean Surface Roughness (Rq)	Contact Angle
COC	3.44±0.84	4.32±0.74	100.05°
COC/SiO2	3.75±0.53	4.79±0.61	17°

These functionalized SiO2-COC microchips exhibited enhanced droplet confinement and reduced edge-effect during subsequent *in situ* DNA synthesis using a custom-built inkjet DNA synthesizer (Fig.1), and were able to produce high-quality oligonucleotide arrays of various lengths (Fig.3). We then divided the entire microarray as sub-arrays into physically isolated reaction chambers, where an integrated amplification and assembly reaction took place to construct full-length gene products at the local environment (Fig. 4). The entire process was performed on-chip to increase throughput and minimize turn-around time and spurious hybridization. Using this platform, we were able to produce full-length gene constructs with different sequences and lengths from the patterned and functionalized COC microchip in a high-throughput and low cost manner (Fig. 5)**.**



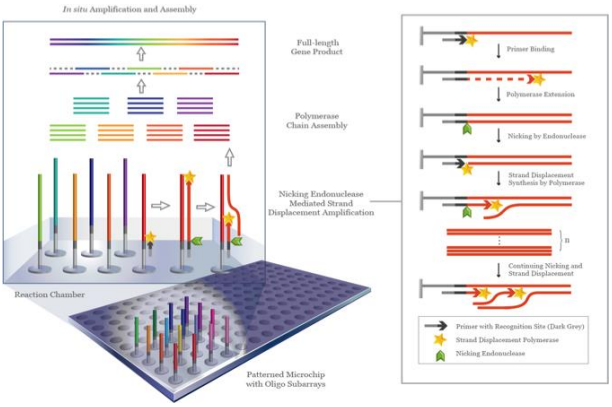


Figure 3. *Different length oligonucleotide synthesized and cleaved from functionalized microchip*

Figure 4. *Schematic illustration of the mechanism of integrated in situ oligo amplification and gene assembly*

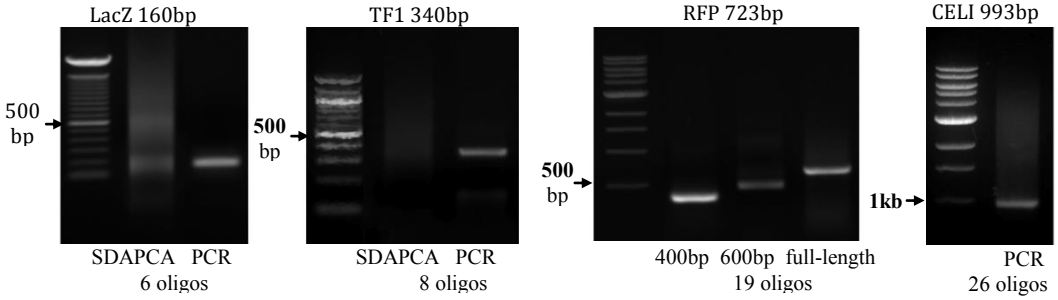


Figure 5. *Combined on-chip SDA-PCA and PCR amplification results of different length gene constructs from in situ synthesized oligonucleotide arrays.*

CONCLUSION

This study provides basis for further developments to integrate microarray with microfluidics for a broad array of bio-analytical, bio-fabrication, and diagnostic applications as various microstructures can be easily and directly molded on thermoplastic substrates. More importantly, we believe this technology could lead to breakthroughs in the development of accurate, low-cost and high-throughput gene synthesis technology, enabling quick design, construction and validation of DNA sequence in various biomedical applications.

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