FOIL-BASED DNA MELTING CURVE ANALYSIS PLATFORM FOR LOW-COST POINT-OF-CARE MOLECULAR DIAGNOSTICS

A. Ohlander[1*], S. Bauer[1], H. Ramachandraiah[2], A. Russom[2], K. Bock[1,3].

[1]Fraunhofer Research Institution for Modular Solid State Technologies EMFT, Munich, Germany

[2] Division of Proteomics and Nanobiotechnology, KTH Royal Institute of Technology, Stockholm, Sweden.

[3]TU Berlin, Center for Technologies of Microperipherics, Berlin, Germany

ABSTRACT

We report on genotyping of single nucleotide polymorphisms (SNP) by melting curve analysis (MCA) on DNA microarrays in a plastic microfluidic system with integrated heaters using lamination foils. Thin-film micro-heaters were processed on polyethylene napthalate (PEN) foil in only one metallization step. DNA microarrays were prepared directly on the PEN surface without the need of surface pre-treatment via UV-mediated immobilization of the spotted DNA microarrays. MCA in microfluidic channels is demonstrated at a ramping rate of 60°C/min, enabling ultra-rapid (50 seconds) SNP analysis.

KEYWORDS: micro-heater, microarray, single nucleotide polymorphism, lab-on-chip

INTRODUCTION

Lab-on-chips are predicted to play an important role in global health applications. However, they are depending on low material and manufacturing cost without the expense of reduced device functionality to enable a real breakthrough as diagnostic tools. Roll-to-roll processing can promote this through high functionality integration at high manufacturing throughput [1]. We recently demonstrated MCA on DNA microarrays in a lab-on-foil system with integrated heaters [2], [3]. The system uses Parylene C for heater-encapsulation and subsequent multi-step co-polymer modification for covalent oligonucleotide immobilization of DNA microarrays. The multi-step coating and surface modification process has been identified as a clear limitation in further implementation of the method for successful mass fabrication. To address this, we present a dramatically simplified system where poly(T)-poly(C) tagged oligonucleotides are directly immobilized via UV treatment. UV-mediated DNA immobilization on plastic substrates was recently proven to be a simple and robust method, with the added benefit of about 70% cost reduction of the probe material alone [4], [5]. Here, we have applied the immobilization protocol to develop a microfluidic MCA platform using an adhesive foil for heater encapsulation as a roll-to-roll compatible cost-efficient processing solution.

EXPERIMENTAL

Cu mesh heaters with a foot print of 3x1.5 mm^2 and 5/50 µm line/space ratio were fabricated using lift-off on 125 µm thick PEN as described in [3]. The heaters were encapsulated by laminating a 150 µm thick single-sided pressure sensitive adhesive foil (PSA) over them. The thermal homogeneity in the heater area was evaluated by spin coating a 15 µm thick encapsulated thermocromic liquid crystal (TLC) layer (LCR Hallcrest) onto the surface and observing the color changes in the TLC layer upon heating. Microarrays of poly(T)-poly(C) tagged match, mismatch and heterozygous oligonucleotides (Biomers.net, Germany) were diluted in 1xPBS (pH 8.5, 0.04% TritonX-100) to a concentration of 20 µM and spotted on the back side of the PEN foil using a Nano-Plotter NP2.1 (GeSiM, Germany). The spotted microarrays were let to dry and immobilized by exposure to UV light (254 nm) using a Spectrolinker™ XL-1000 (Spectronics Corporation, USA), and washed in 0.1 x SSC + 0.1 % SDS for 10 minutes. The contour of channels was cut by laser in a 50 µm thick, double-sided PSA foil which was laminated over the heater on the backside of the PEN foil. A second, 125 µm thick PEN foil, was used to close the system. An image of the copper mesh heater and the microfluidic assembly can be seen in figure 1 a and b.

a) *b)*

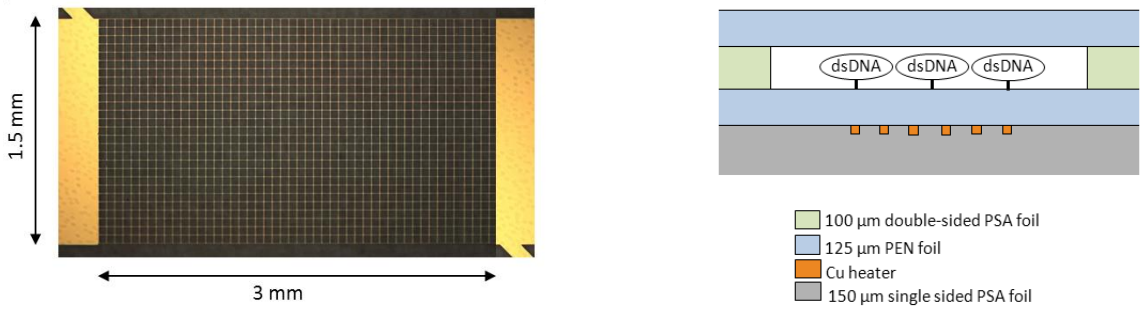

Fig 1a: Copper mesh heater with 5/50 µm line/space ratio on transparent PEN foil. The foil is placed on dark background for clarity. The heater uses a four-point connection for power and sensing. Fig 1b: Cross-section of lab-on-foil system with integrated heater for SNP analysis. The DNA is immobilized on the backside of the PEN/heater foil.

Complementary Cy3 labeled target (Biomers.net, Germany) were diluted in Perfect Hyb (Sigma Aldrich) to a concentration of 1 µM. The target solution was injected in the channel using a syringe and let to hybridize for 1 minute at room temperature. The channel was then washed with 2x100 µL 0.1 x SSC + 0.1% SDS buffer. Before melting curve analysis, the channel was filled with 5xSSC buffer, the heater was connected to a Source Measure Unit (SMU) and assembled under a Zeiss Axioplan2 microscope. The heater was ramped from room temperature to 100°C at a rate of 8°C/min using an in house developed PID controller software. Microscope images were taken each 10 seconds while heating. In a second experiment a heater was ramped at a rate of 60°C / minute. The intensities in the microscope images were evaluated using ImageJ and Origin 8.

RESULTS AND DISCUSSION

As thermal homogeneity on the heater surface is crucial to be able to distinguish point mutations by surface bound MCA, thermal imaging on the back side of the PEN substrate used for DNA immobilization was performed by coating a 15 µm thick encapsulated-TLC layer on to it (figure 2.)

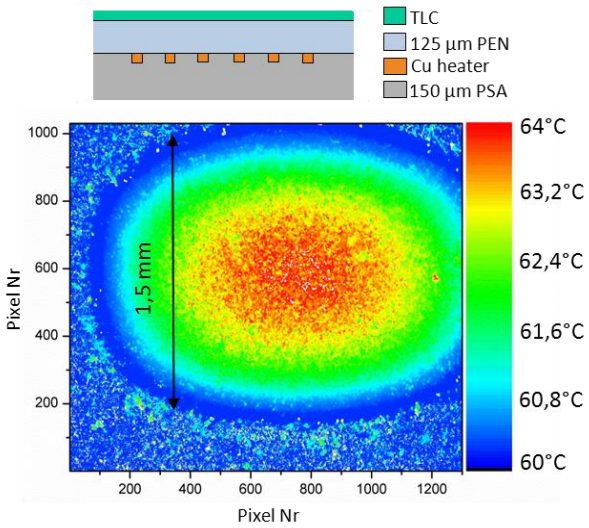

Fig 2: The colour changes in a 15 µm thick TLC layer after image processing, coated on the backside of the PEN carrier foil of a heater passivated by PSA tape.

It can be seen that a 700x1000 µm area in the center (red colour) exhibit the thermal homogeneity required for MCA. Hence, it was defined as the active area in the heater. In this area, microarrays of matching, mismatching and heterozygous oligonucleotides were immobilized using poly(T)-poly(C) oligonucleotide modification and UV-crosslinking on the backside of untreated heater-PEN foils. After integration into microfluidics as in Fig 1b, the microarrays were allowed to hybridize with Cy3 labeled complementary strands of 1 µM concentration and MCA was performed. As the UV-induced immobilization method requires no surface modification of the substrate, no blocking step is required before hybridization as the naturally DNA repellant surface characteristics of the polymer substrate is preserved. This is indicated by the clear visibility of the Cy3 labeled, 50 µm diameter DNA spots in figure 3 a (upper right).

Increasing the temperature in the heater surface where the DNA duplexes are immobilized causes duplex dissociation and hence a decrease in fluorescence intensity of the DNA spots. Figure 3 a and b shows melting curves of match, mismatch and heterozygous microarray spots using two different heating rates, 8°C/min and 60°C/min. Three spots per sample type were evaluated generating highly reproducible melting curves with a clear distinction between the three SNP variants. Mismatched duplexes dissociate at a lower temperature than matching duplexes, producing the intensity drop at a lower temperature. The heterozygous sample being a 50-50 mix of match and mismatch generates a curve resulting from superposition of the two DNA variants. Using a heating rate of 60°C/minute slightly alternates the shape of the melting curves as compared to the common slightly s-shaped curves produced with a heating rate of 8°C/min. This could be due to differences in thermal diffusion characteristics from the thin film copper lines (high thermal conductivity material) through the PEN material (low thermal conductivity material) upon different heating rates. Nevertheless, the three curves from each sample type are highly reproducible and clear discrimination of SNPs can be made using a heating rate of 60°C/min enabling ultra-rapid MCA in only 50 seconds time.

a) *b)*

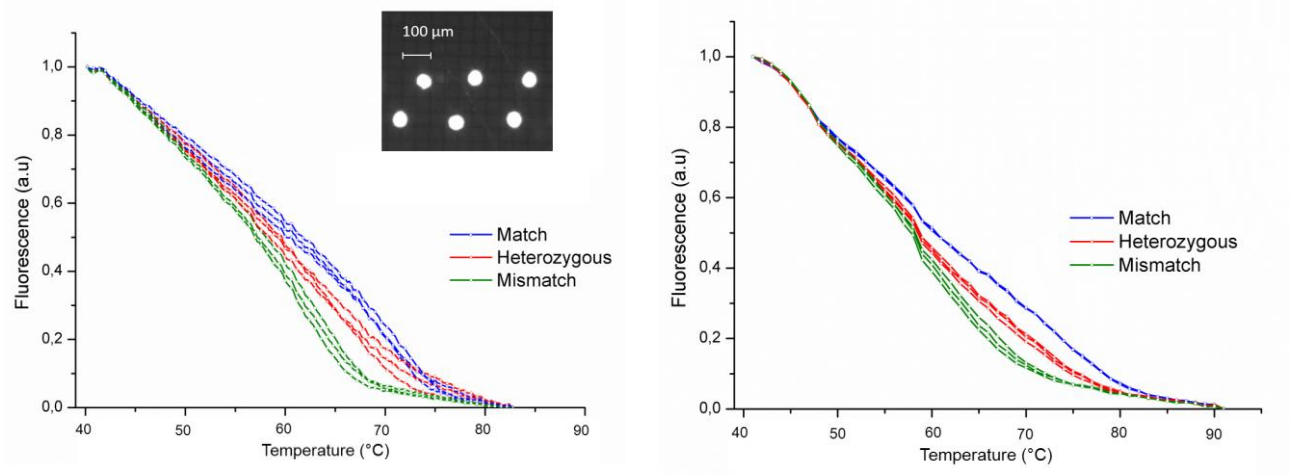

Fig 3a: Melting curves of 3 match, 3 mismatch and 3 heterozygous DNA spots immobilized by poly(T)poly(C)-tail and UV light on a Cu mesh heater (upper right) using a ramping rate of 8°C/min. Fig 3b: Rapid MCA using a heating rate of 60°C/min.

A covalent attachment of the immobilized probe to the substrate is crucial for surface bound MCA. The highly reproducible curves with clear discrimination between match, mismatch and heterozygous DNA samples, consolidates the robustness of the poly(T) poly(C) immobilization method as it withstands the elevated temperatures involved in the assay. The immobilization method has major advantages to conventional ones in terms of cost, as it requires no pre-surface treatment and no blocking to prevent unspecific binding. This promotes ease of processing in roll-to-roll manner and hence it reduces the manufacturing time and material cost immensely. Alone the simple 10T and 10C nucleotide modification on the immobilization probe, reduces the price by 70% of the probe material as compared to conventional amino-modified ones [4].

Proper encapsulation of the mesh heater is imperative to prevent degradation of the copper upon heating which would alternate the resistance in the wires and hence result in inaccurate feedback to the PID controller. Previously, we used Parylene C which is a common MEMS passivation material [2], [3]. For a low-cost approach, we now have replaced the CVD (Chemical Vapour Deposition) deposited Parylene C with a simple single sided PSA lamination foil who's assembly easily can be implemented in a roll-to-roll manufacturing process. Using the PSA foil as passivation material, the resistance in the circuit continued to have a linear behavior, indicating that the lamination foil provides the encapsulation needed for steady control of the heater.

CONCLUSION

A novel, foil-based, rapid MCA platform for SNP genotyping is presented. Highly reproducible melting curves was achieved on microarrays of immobilized oligonucleotides being able to discriminate between matching, mismatching and heterozygous samples using a rapid heating rate of 60°C/sec resulting in MCA in only 50 seconds. The microfluidic foil system uses roll-to-roll compatible and highly cost efficient manufacturing and DNA spotting technologies promoting a market-viable point-of-care molecular diagnostic device.

REFERENCES

[1] M. Focke, D. Kosse and C. Müller, *"Lab-on-a-Foil: microfluidics on thin and flexible films"*, Lab Chip, 2010, 10, 1365–1386.

[2] A. Ohlander, T. Hammerle, G. Klink, C. Zilio, F. Damin, M. Chiari, A. Russom, K. Bock. *„DNA melting curve analysis on semi-transparent thin film microheater on flexible lab-on-foil substrate"* µTAS 2012, M.8.180.

[3] A. Ohlander, C. Zilio, T. Hammerle, S. Zelenin, G. Klink, M. Chiari, K. Bock, A. Russom, *"Genotyping of single nucleotide polymorphisms by melting curve analysis using thin film semi-transparent heaters integrated in a lab-on-foil system"*: Lab Chip. 2013 Jun 7;13(11):2075-82.

[4] Y. Sun, I. Perch-Nielsen, M. Dufva, D. Sabourin, D. Duong Bang, J. Høgberg , A. Wolff, *"Direct immobilization of DNA probes on non-modified plastics by UV irradiation and integration in microfluidic devices for rapid bioassay"*, Anal Bioanal. Chem. 2012 Jan;402(2):741-8.

[5] D. Sabourin, J. Petersen, D. Snakenborg, M. Brivio, H. Gudnadson, A. Wolff, M. Dufva *"Microfluidic DNA micro arrays in PMMA chips: streamlined fabrication via simultaneous DNA immobilization and bonding activation by brief UV exposure"*. Biomed Microdevices (2010) 12:673–681.

CONTACT

*A. Ohlander, tel: +49(0)89 547 59 233; anna.ohlander@emft.fraunhofer.de