IMMOBILIZATION OF BIOLOGICAL ACTIVE MOLECULES ON CHEMICALLY INERT POLYMER CHIPS FOR BIO-ANALYTICAL DETECTION

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ABSTRACT

One of the important commercial applications of microfluidic devices is in molecular diagnostics, where the analytical targets are specific nucleic acids. One technology to separate these analytes of interest from the sample is the use of complementary nucleic acid capture probes or hybridization arrays to which these analytes can bind. While well-established binding chemistries for such arrays on e.g. silicon surfaces exist, the commercial use of disposable microfluidic devices commands the use of polymer substrates [1]. In this paper we present an injection-molded microfluidic device with two sample tracks and a surface modification for binding suitable capture probes.

KEYWORDS: Polymer microfluidic chip, surface modification, molecular diagnostics, hybridization array

INTRODUCTION

Micro-Total-Analysis-Systems (μTAS) or Lab-on-a-Chip (LOC) have been attracted continuously growing interest, because these platforms allow for an integrated, miniaturized, largely user independent, robust, and rapid analysis of markers of relevance for all areas within life sciences. The whole chain of such a (bio)-analytical process can encompass sample preparation, analyte purification, – optional – amplification, and, finally, detection.

Depending on the objective of the analysis, the analyte can belong to the protein or nucleic acid family. Although analyte quality as well as amplification efficiency strongly influence the sensitivity of an assay performed on the microfluidic platform, design and composition of the detection unit is a really critical parameter during analyte monitoring [2]. Hereby, an important strategy to discriminate between signal and background is to immobilize the analyte at defined areas within the microfluidic cartridge by trapping molecules and, concomitantly, to flush away molecules, which could contribute to the background signal. In this paper we resent a process and a device which contains two sample tracks with integrated arrays of immobilized oligonucleotides.

METHODS

Some materials such as glass, silicon, or silicone derivatives, are attractive carrier for immobilized catching molecules but are less suited for commercial mass production [3]. For such devices which are typically used as disposables, thermoplastic polymers such as polystyrene (PS), polycarbonate (PC), or poly-methyl-methacrylate (PMMA) are better suited substrate materials, because these materials can be structured by replication techniques such as injection molding or hot embossing [4]. For devices which are utilized in protocols which includes fluorescence detection, thermoplastic polymers with excellent optical properties, such as cyclic olefin copolymer (COC) or cyclic olefin polymer (COP), are recommended, because auto-fluorescent background will significantly disturb the measurement of the actual fluorescent signal. In order to be used as a suitable surface for the anchoring of biomolecules used as capture probes in molecular diagnostics assays, the surface of COC or COP chips has to be activated in those regions where the capture molecules and thus the detection unit are intended to be placed. A treatment with oxygen plasma (typically 60-90 s at approx. 1 mbar partial pressure) introduces functional groups, such as OH-, C=O, COOH-, or nitrogen-containing moieties into a surface layer of the chip with a depth depending on the mode of activation. An interconnecting reagent such as silanoxides provides an additional thin layer on the surface of the polymer mediating the linkage between catching bio-molecule and activated chip surface.

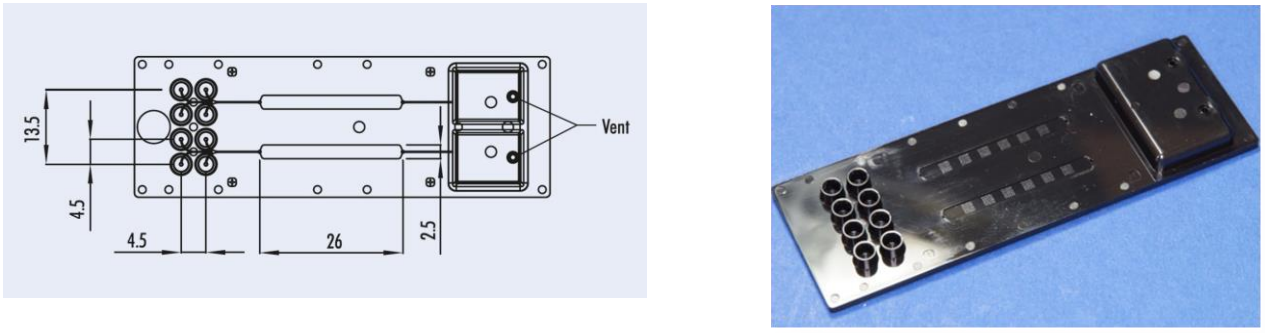

Figure 1: left CAD drawing of the arrays chip, right actual image of a molded and spotted chip. In each detection channel, six rectangular arrays of 8 × 8 spots with 160 pl probe volume can be seen.

MICROFLUIDIC DEVICE

The microfluidic device used in the experiments is shown in Fig. 1 (left CAD drawing, right actual molded device). The device has the dimensions of a microscope slide (25.5 mm × 75.5 mm). It contains two independent sample tracks. Each track has four input ports with female Mini-Luer ports for the introduction of different reagents. The capture probes can be spotted along a 2.5 mm wide, 200 µm deep and 26 mm long detection channel. All reagents used during the protocol can be stored on-chip in a 500 µl waste reservoir at the channel end which is equipped with an air vent sealed off with a hydrophobic membrane. The channel width was chosen in a way in order to allow on the one hand side the deposition of a sufficiently high number of spots while on the other hand allows for some free space between the outermost spots and the channel wall. This distance has to be kept due to the fact that the channel edge otherwise leads to a capillary flow of the deposited reagent and thus an undefined spot area and shape.

EXPERIMENTAL RESULTS

The volume of spots inside the chip based detection zone is typically in the range of 70 - 500 pl, corresponding to spot diameters between 80-800 µm. This can be realized using appropriate commercially available spot ting tool using piezoelectric actuation, see Fig. 2. Here we report on successful examples for the spotting of oligonucleotides (typically 25 bases) into the detection channel of the COP-chip. The spotting instrument has generated 6 arrays of 64 spots (8 × 8) with a volume of 160 pl each per detection channel. The immobilization of biological marker molecules on the chip surfaces were hybridized with the corresponding analyte in a PCR solution. Figure 3 shows a typical calibration curve of the fluorescent intensity as a function of the analyte concentration. Figure 4 shows the corresponding image of an agarose gel of the analytes in the respective concentration ranges giving additional support that a quantitative analysis of immobilized PCR/oligonucleotide hybrids is possible.

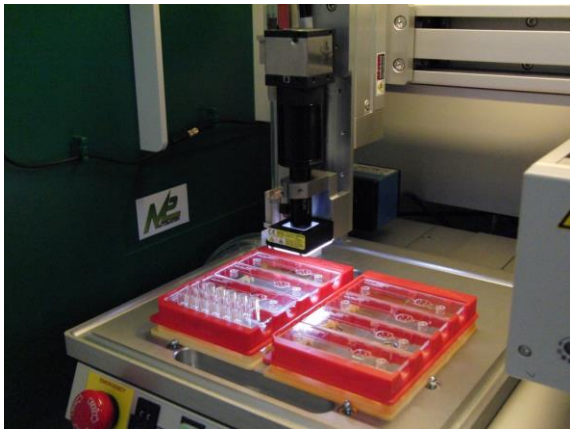

Figure 2: Spotting tool with piezoelectric dispenser depositing biomolecule onto polymer microfluidic chips.

ng/µl	Intensity	Standard diviation
3,83	19.460,436	1263,614
2,39	11.133,657	2781,279
2,08	7.431,538	616,717
1,82	7.087,205	818,297
0,44	2.130,772	94,930
0,53	1.990,384	86,197
0,15	1574,651	181,926
0,06	225,160	42,593

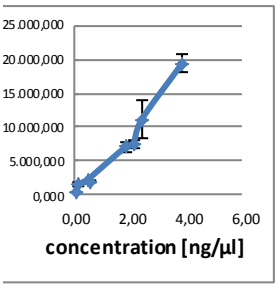

Figure 3: Relation between analyte concentration and the intensity of immobilized analytes represented by the fluorescent signal.

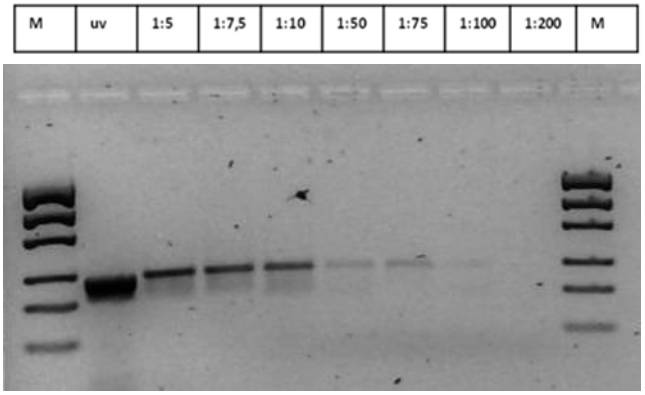

Figure 4: Agarose gel electrophoresis of the PCR products which have been analyzed in Fig. 3.

Figure 5 finally shows several other examples of immobilized biomolecule arrays on polymer surfaces. On the left hand side the image of an array with immobilized marker molecules which either interact strongly or intermediately or do not interact with the analyte tagged with a fluorophore is shown. The right hand side shows a second example of arrays after fluorescence detection with different concentrations of the analyte.

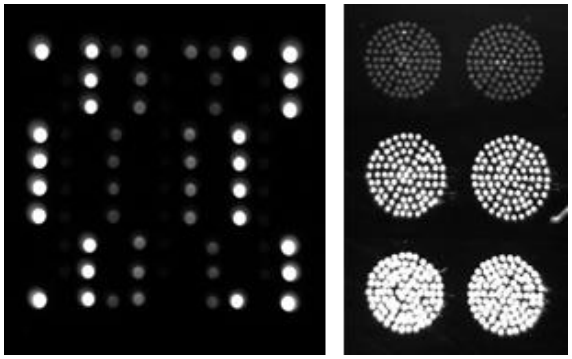

Figure 5: Further examples of arrays of immobilized capture probes after hybridization.

CONCLUSION

In the paper we have presented an injection-molded microfluidic device and surface modification method suitable for the generation of low and medium density arrays for the capture of biomolecules. These data show that it is possible to print biomolecule arrays on polymers of commercial relevance such as COC or COP in an automatic manner by applying a simple surface modification protocol. Comparison of Figs. 3 and 4 give rise to initial evidences indicating that the described technique is more sensitive than a conventional gel electrophoresis.

ACKNOWLEDGEMENTS

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