BIOSTICKERS: PATTERNED MICROFLUIDIC STICKERS FOR RAPID INTEGRATION WITH MICROARRAYS

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ABSTRACT

We present a one-step, reversible, and biocompatible bonding method of a stiff patterned microfluidic "Biosticker", based on off-stoichiometry thiol-ene (OSTE) polymers [1], to state-of-the-art spotted microarray surfaces. The method aims at improving and simplifying the batch back-end processing of microarrays. We illustrate its ease of use in two applications: a high sensitivity flow-through protein assay; and a DNA-hybridization test. Read-out was performed in a standard high-volume array scanner, and showed excellent spot homogeneity and intensity. The Biosticker is aimed to be a plug-in for existing microarray platforms to enable faster protein assays and DNA hybridizations through mass transport optimization.

KEYWORDS: microarrays, OSTE, off-stoichiometry thiol-ene, bonding, microfluidic packaging

INTRODUCTION

Integration of microfluidics with microarrays provides advantages such as multiplexing, improved mass transport, faster reaction times and elimination of cross talk [2]. Previous integration methods utilize either i) a clamped or plasma bonded PDMS channel layer on a spotted substrate of bare silicon/glass/PDMS [3] or ii) a tape or plastic foil covering a thermoplastic substrate containing spots in custom-machined channels [4]. The former approach suffers from adsorption of small molecules into the PDMS and from PDMS channel deformation, whereas the latter approach is limited to patternable microarray surfaces and requires a stringent substrate alignment during spotting.

NOVEL POLYMER

The Biosticker chip was developed based on the recently introduced off-stoichiometry thiol-ene (OSTE) polymer platform, specifically designed for lab-on-chip applications [1,5,6]. By using the narrow glass transition temperature, the active surface and the excellent micromolding capability of the OSTE polymer, our advantages over previous work are threefold: a) The Biostickers have free thiol groups on their surface that can either directly covalently react with many standard microarray surfaces, or that can be designed to react via secondary functionalization (e.g. epoxyallyl monomer) with virtually any microarray surface. b) The bonds are still reversible: the Biostickers can be peeled off when heated above their glass transition temperature T_g=37 °C, i.e. when they soften and the covalent links are easily broken (Table 1, Fig 3). c) The material shows low diffusivity even for small molecules, preventing up-concentration and absorption [5,6].

Table 1. OSTE-Thiol (70) composition

Monomers	tetrathiol:tri-allyl-triazine, 1.7:1
Thiol excess	70%
Glass transition	37 °C
E-modulus @ 25 °C	1200 MPa
E-modulus @ 45 °C	30 MPa

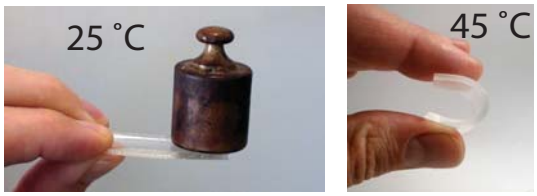

Figure 1: The Biosticker material is hard and stiff at room-temperature but when heated to 45 ˚C it becomes rubbery.

EXPERIMENTAL

For demonstration we prepared Biostickers containing a 9x9 mm^2 detection chamber of 30 µm height (aspect ratio > 1:300 !) by casting the OSTE-prepolymer (Table 1) on a 4" silicon/SU-8 wafer mold, followed by a planarization to 500 µm thickness using a polycarbonate carrier. The prepolymer was cured using a table-top UV-lamp (365 nm, 4 mW/cm^2, EFOS Lite, EXFOS) for 30 seconds (Fig 2A:1-2). The cured polymer sheet was released from the mold and carrier by heating the stack to 45 °C on a hotplate, where after access holes were drilled and the sheet was cut into Biostickers of 15x15 mm^2 (Fig 2A:3-5). Microarrays were prepared from silicon chips (15x15 mm^2) dip-coated with the polymer copoly(DMA-NAS-MAPS) as the receptor linker layer, recently demonstrated to improve sensitivity and limit-of-detection (LOD) [7].

The Biosticker was separately heated to 45 °C on a hotplate and thereafter lightly pressed against the microarray before it cooled down (Fig 2B:1-2). The heated biosticker conformed perfectly to the surface of the microarray and the thiol-groups could react with the activated esters (NHS) in the receptor linker layer to form a covalent bond.

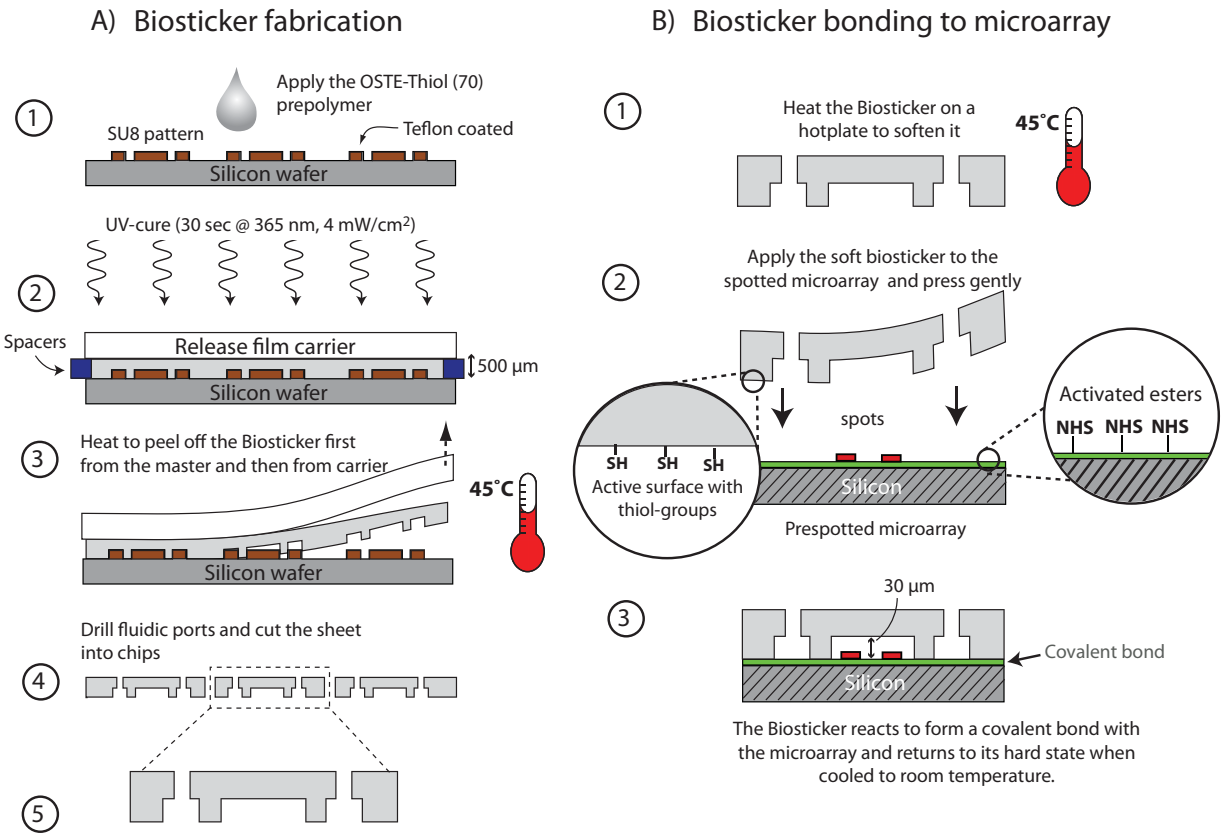

Figure 2: A) *The OSTE prepolymer is casted on a silicon/SU-8 master to 500 um thickness using spacers and a PC release liner and cured using a table top UV-lamp. By heating the polymer to 45 ˚C, it softens enough to be peeled off, first from the master and then from the carrier. When it cools down it reverts to its stiff state (Table 1) and fluidic ports are drilled and the sheet is cut into chips.* B) *To bond the Biosticker it is first heated to 45 ˚C to make it rubbery (Fig 1) and then applied to the already spotted microarray surface. In this case the thiol groups can directly react with the activated esters on the surface. Some other microarray surfaces present expoxy or isocyanate groups which can also be made to react with the Biosticker surface (experimentally verified but not shown here).*

When the Biosticker cooled down to room temperature it formed a covalently bonded, hard plastic cover with an integrated microfluidic network on the microarray (Fig 2B:3 and Fig 3). The fluidic ports were connected to a pump and deactivation was performed inside the chip using ethanolamine solution. We ran two bioassays: 1) a fluorescent protein experiment with spotted ß-lactoglobulin detecting 1 ng/ml of anti ß-lactoglobulin antibody, and 2) a DNA hybridization test using spotted 23 mer 5'- amine modified oligonucleotides and target complemetary oligonucleotide (1 µM). After completing the assay, the Biostickers were peeled off by heating the microarrays to 45 °C.

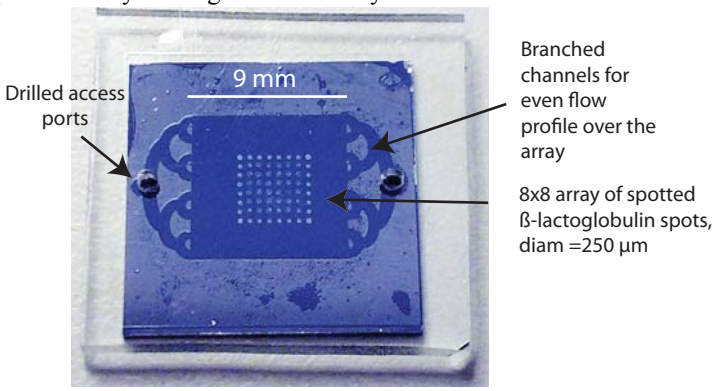

Figure 3: A Biosticker flow cell attached to the protein microarray. The spots are visible though the polymer. To guarantee an even flow profile over the spots, branched inlet and outlet channels are used.

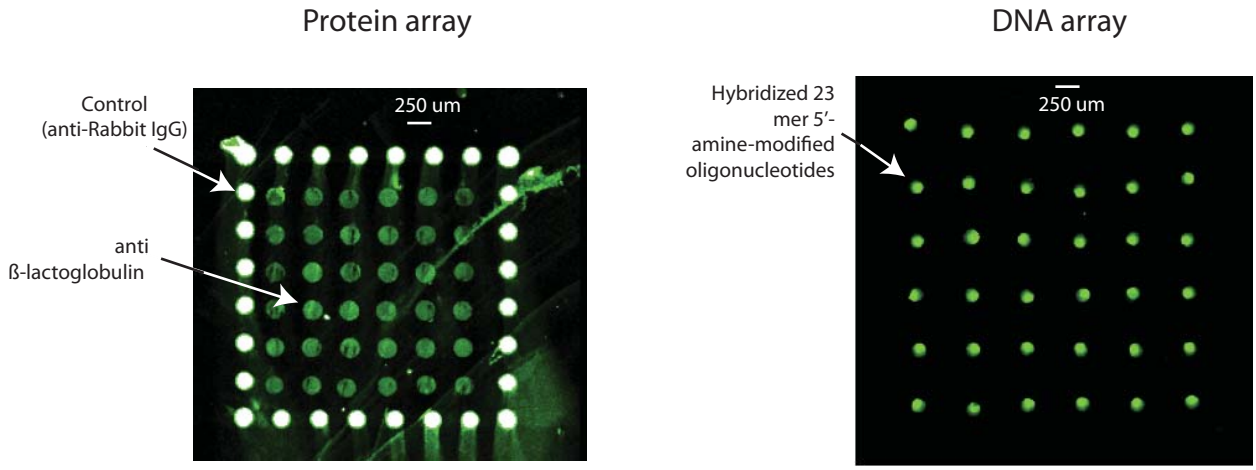

Figure 4: The results of the scanned microarrays. The results from the ß-lactoglobulin protein assay and the DNA hybridization are very promising and show homogenous spots and excellent intensity. With an optimized fluidic protocol the Biostickers will not only increase the performance of microarrays but also greatly improve and simplify the processing compared to previously demonstrated microfluidic integrations.

The results of both the protein assay and the DNA hybridization tests (Fig 4), measured with a fluorescent scanner (Scan Array, Perkin Elmer), showed excellent signal and spot homogeneity, demonstrating the potential for using the Biostickers to optimize microarrays and avoiding the need for special tools, complicated clamping, lamination or suboptimal materials.

CONCLUSION

In this first proof-of-principle we demonstrated the simple integration of a novel OSTE based microfluidic sticker to a state-of-the-art microarray surface with excellent result. Ongoing work focuses on improving the assay performance beyond non-packaged microarrays by optimizing the microfluidic flow profile and assay protocols.

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