Direct Poly(dimethylsiloxane) Surface Functionalization with Vinyl Modified DNA

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Poly(dimethylsiloxane) (PDMS) remains the most employed polymer for numerous biotechnological applications[1] because of its chemical inertness, transparency, hydrophobicity, and molding properties. Moreover, its low toxicity and ease to use in standard laboratory conditions grant it great popularity for rapid and cost-effective development of protein,[2] DNA,[3] and cell[4] biochips or separation assays.[5] Concerning DNA immobilization on the PDMS surface, extensive investigations using micro-contact printing[6] (μCP) or chemical modifications[7] have already been performed and were successful but not straightforward, that is, based on multistep protocols. Indeed, PDMS surface activation requires either strong oxidative conditions[8] to transform the hydrophobic polymer into a glass like surface (silanol groups) or deposition of intermediate layers,[9] subsequently functionalized. Therefore, the benefits of using such fast processes and a simple polymer are thwarted by the use of complex protocols. An interesting approach is then to combine in a unique step the PDMS polymerization and its surface modification. The PDMS polymerization process involves Si—H functions and CH_2═CH_2 residues in the presence of a platinum based catalyst. This reaction, called hydrosilylation,[10] allows the formation of a highly cross-linked three-dimensional silicon polymer with very high efficiency. To our knowledge, only a few groups[11–13] have attempted to take advantage of the chemical process involved during the PDMS polymerization.

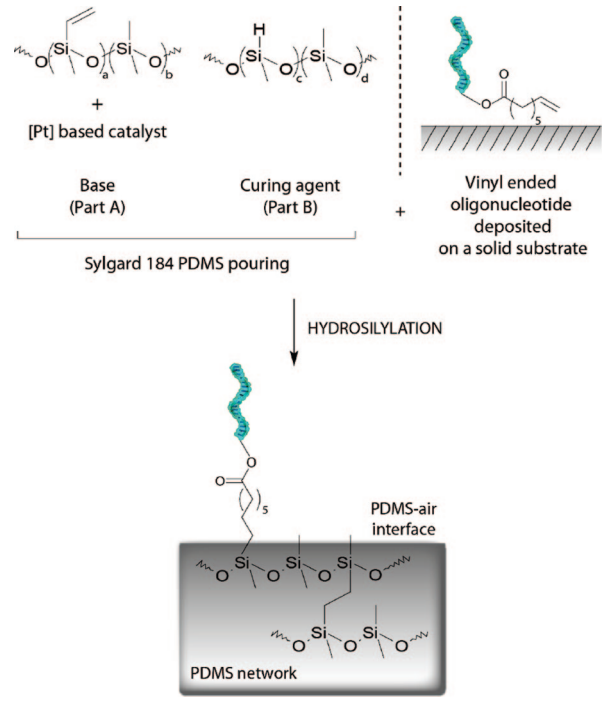

Figure 1. Hydrosilylation reaction during PDMS Sylgard 184 polymerization and interaction with vinyl ended oligonucleotide deposited on a solid substrate.

Recently, a direct method for the PDMS surface arraying with proteins, called "macromolecules to PDMS transfer", has been reported[14] by our group. This technology has the obvious advantage to produce, in one step, PDMS microfluidic devices directly integrating protein spots. The immobilization of the biomolecules was believed to be related to both an entrapment and a covalent linkage phenomenon. The active proteins were then strongly attached to the surface but without any specific orientation. We are reporting here a new path to the functionalization of Sylgard 184 PDMS surfaces with oriented oligonucleotides. The main idea was to produce, directly during the oligonucleotide synthesis, a DNA probe molecule containing at one end a vinyl function which could be integrated in the catalytic cycle of the PDMS polymerization. For this purpose, a 20-mer DNA sequence modified at its 5′-end with hexenoic acid was synthesized by Eurogentec (France). The integration of the vinyl moiety of the DNA probe within the hydrosilylation reaction mechanism[15] (Figure 1) involves a first step of oxidative addition of a silane and a vinyl function with the platinum based catalyst, followed by a reductive elimination, regenerating the catalyst. This mechanism leads then to the covalent grafting of the DNA probe to the PDMS structure.

To obtain spots of DNA probes immobilized using this mechanism, solutions of 5′-vinyl DNA were arrayed (as 1.3

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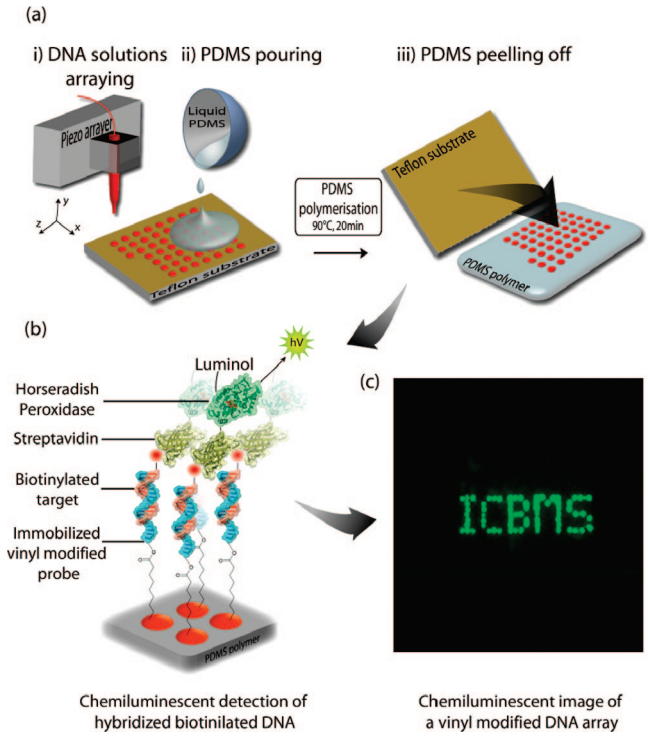

Figure 2. (a) Overview of the "macromolecules to PDMS transfer". (b) Schematic representation of the chemiluminescent labeling of DNA probe/target hybridization. (c) Chemiluminescent image of a hybridized 5′-vinyl DNA microarray.

nL drops) at the surface of a 3D Teflon master (Figure 2a). Liquid PDMS was then poured onto the Teflon substrate, covering the 5′-vinyl DNA spots, and cured at 90 °C for 20 min. Peeling off the polymer generates a PDMS microarray exhibiting spots of orientated DNA probe at the PDMS−air interface. The ability of these immobilized probes to be hybridized with complementary oligonucleotide was studied using a chemiluminescent labeling of a biotinylated target sequence (Figure 2b). A special arraying pattern was easily produced for this purpose by spotting our institute logo ICBMS with 5′-vinyl DNA (25 μM) out of a 625 spots matrix. The chemiluminescent image obtained after hybridization with a complementary biotinylated target sequence (1 nM), and its labeling using peroxidase−streptavidin conjugate is presented in Figure 2c. An intense and specific signal was obtained from the interactions between the immobilized 5′-vinyl DNA probe and the target sequence, while no detectable signal was obtained from the nonspecific interactions with the bare PDMS surface.

As a control experiment, 5′-modified DNA molecules were spotted directly onto already polymerized PDMS and hybridized with complementary probes. No measurable signal was obtained, suggesting the importance of the interactions between PDMS and biomolecules during the polymerization process. Obtaining such a PDMS DNA array with a so straightforward protocol was only possible because of the use on the one hand of the "Macromolecules to PDMS transfer" technique and on the other hand of 5′-vinyl DNA probes. Indeed, most of the current technologies for DNA based biochips rely on different separated steps for the preparation of the chip which are cost intensive and time-consuming.

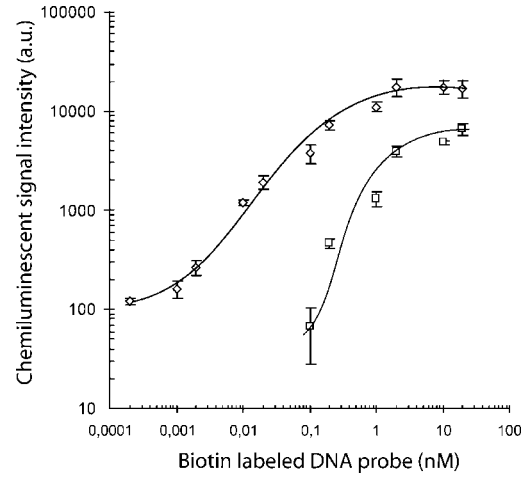

Figure 3. Analytical performances of biochips prepared with immobilized (◇) 5′-vinyl DNA probes and (□) unmodified DNA probes. Biotin labeled DNA were incubated 60 min at 37 °C in buffer containing BSA 1% and 0.1% Tween 20. Error bars are the standard deviation of four experiments, and curves are provided as a guide for the eyes.

The spos characteristics were previously described for protein arrays.[14] Particularly, their specific area was shown to be highly dependent on the composition of the spotting solution. The use of carbonate buffer (Na_2CO_3, 0.1 M, pH 9) instead of water as spotting carrier was shown to increase the specific area of the spot and then lead to higher spot signal. Indeed, the salt charged spotting solution crystallizes during the drying step, leading to highly textured surfaces. Thus, during the PDMS pouring and drying steps, these surfaces were used as masters to produce PDMS replica entrapping the biomolecules and having a high specific area. A similar effect was experienced for the present 5′-vinyl DNA spots (AFM images shown in the Supporting Information). A drastic 940% increase of the spot chemiluminescent signal, related to this increased specific area, was recorded when using carbonate buffer instead of water as a spotting carrier.

To demonstrate the importance of the vinyl residue in the actual DNA immobilization reaction, unmodified DNA sequence probes were immobilized using the "macromolecules to PDMS transfer". After hybridization with the corresponding target sequence, a specific signal was obtained, demonstrating the possibility of trapping accessible unmodified DNA strand during the PDMS polymerization and underlining the importance of the interactions between DNA and PDMS, mainly due to Van der Waals interactions and hydrophobic effects.[16]

Nevertheless, as can be seen in Figure 3, the analytical performances of the biochips prepared with either the 5′-vinyl DNA or the unmodified DNA were really dissimilar. The vinyl based biochip exhibits a broad detection range from 0.2 pM (3.75 mol in 25 μL) to 1 nM with a detection limit three decades lower than using the biochip prepared with unmodified DNA. This difference of hybridization ability is believed to be related to (i) the high reactivity of the vinyl modification toward PDMS chains during polymerization and

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(ii) a possible better orientation of the DNA probe molecules thanks to the preferential immobilization through the vinyl residue. Finally, the specificity of the immobilized probe was assessed using a fully non-DNA sequence. No signal was recorded when the noncomplementary biotin labeled strand was incubated with immobilized DNA, demonstrating the absence of nonspecific adsorption of target sequence on 5′-vinyl DNA spots.

In conclusion, we have demonstrated that vinyl modified DNA molecules can be easily transferred and grafted at the PDMS−air interface using the "macromolecules to PDMS transfer" procedure. Such modification at one end of a DNA molecule greatly enhances the target hybridization ability when compared to the unmodified DNA probe. This difference of reactivity arises directly from the interactions of the vinyl function with the catalytic cycle during PDMS polymerization.

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Supporting Information Available: Experimental details and protocols and AFM images of 5′-vinyl DNA spots in water and carbonate buffer (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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