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Use of γ-aminopropyl-coated glass surface for the patterning of oligonucleotides through oxime bond formation

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Abstract—The present work reports on the preparation of glass surfaces coated with NPPOC-protected aminooxy groups and their use for the patterning of oligonucleotides on glass slides and in capillary tubes. The method involves the use of surfaces coated with amino groups using (γ-aminopropyl)triethoxy silane and subsequent grafting of the aminooxy groups by using the activated ester **1**. The NPPOC-protected aminooxy groups on the surfaces can be cleaved upon irradiation. The free aminooxy groups so obtained are subsequently reacted with aldehyde-containing oligonucleotides to achieve efficient surface patterning.

The design of micro-arrays of DNA, carbohydrates and peptides is the subject of intensive research as they represent a powerful tool for high-throughput screening of biomolecules with applications in genetic analysis, molecular diagnostics, and drug discovery. Similarly, micro-fluidic systems in which the synthesis, purification, and analysis are accomplished on chip are of significant interest. The 'lab on a chip' devices along with the micro-array technologies are expected to make feasible more rapid, multi-parametric and economical analysis with minimal sample volumes.

The control of surface chemistry is crucial to achieve efficient immobilization of biomolecules for preparation of such micro-devices. One of the most challenging tasks is to carry out the immobilization of biomolecules without perturbing their intrinsic properties. Two major approaches are available for surface immobilization and patterning of biomolecules on surfaces. The first approach involves the direct on-surface synthesis (in-situ),[1] whereas the second approach involves the immobilization of prefabricated oligonucleotides on the support.[2] The latter approach is more widely used to prepare low to medium density micro-arrays on account of its flexibility. Moreover, this approach utilizes 'spotting techniques' and hence benefits from the use of automated robots. The surface immobilization of biomolecules by later approach can be achieved by chemical reaction between surface-bound reactive groups and functionalized biomolecules.[3] Several strategies including maleimide/thiol,[4] 'click chemistry' involving alkyne/azide,[5] hydrazide/aldehyde[6] have been reported for covalent attachment of biomolecules on open surfaces. However, the patterning inside the micro-channels for 'lab on a chip' preparation is more tedious and therefore only few methods have been reported to date. A photo-generated-surface-bound aldehyde group has been described earlier for surface immobilization of proteins through the formation of Schiff base.[7] Another method is based on the photo-crosslinking of surface-bound benzophenone derivatives with biomolecules through C–H insertion.[8] Recently, an elegant method based on the use of ultraviolet-light emitting diodes (UV-LED's) has been proposed for in-situ synthesis of DNA in capillaries.[9]

In this context, we have investigated the use of oxime bond formation for the covalent attachment of the oligonucleotides on glass surface. In our earlier works, we have demonstrated the efficiency of oxime linkage for functionalization of planar glass surfaces and inner wall of glass capillary tubes.[10] Others have also reported the surface immobilization of oligonucleotides on gold surfaces or polymeric particles using oxime bonds.[11] Recently, we reported the efficient surface patterning

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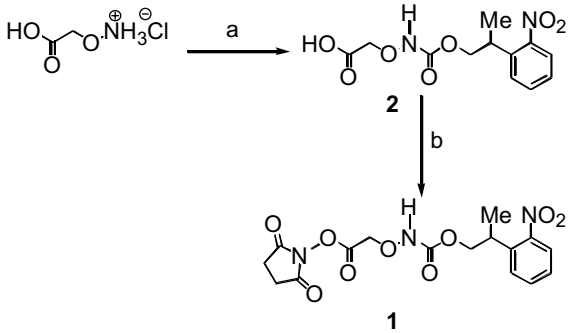
of oligonucleotides in capillary tubes as well as on glass surfaces through oxime bond formation by using surfaces grafted with reactive aminooxy groups masked with photo-cleavable protected group, 2-(2-nitrophenyl) propyloxycarbonyl group (NPPOC).[12] NPPOC groups were removed by brief irradiation to unmask the reactive aminooxy group on surfaces and subsequently reacted with the aldehyde-containing oligonucleotides. The masked-aminooxy moieties were grafted on the glass surfaces by using a triethoxysilane derivative bearing the NPPOC-protected aminooxy group. The triethoxysilane derivative was prepared in four steps from 11-bromo-1-undecene. In the present work, we report an alternative method for grafting NPPOC-protected aminooxy moieties on the glass surfaces. The current strategy involves coupling reaction of activated ester **1**, bearing the NPPOC-protected aminooxy group, with surface-bound amino groups. The preparation of surfaces coated with amino groups using (γ-aminopropyl)triethoxy silane (APS) is well documented in literature.[13,14] We report that current approach allows also an efficient surface immobilization and patterning of oligonucleotides on glass slides and in capillary tubes.

The activated ester **1** was prepared in two-steps from carboxymethoxyamine hydrochloride (Scheme 1). The aminooxy moiety was protected with photolabile NPPOC chloride derivative and *N*-hydroxysuccinimide was introduced next using the standard procedure.[15] Compounds **1** and **2** were characterized by NMR and mass analysis.[16]

The oligonucleotide immobilization was accomplished first on glass slides and then in capillary tubes.[17] The glass slides were activated by treatment with sodium hydroxide solution. The slides were then dipped at room temperature in a solution of (γ-aminopropyl)triethoxy silane in toluene overnight to achieve chemisorption of amino groups on surfaces. This was followed by curing at 110 °C for 3 h to stabilize the silane layer. The silanization step was followed by overnight coupling reaction with activated ester **1** in DMF at room temperature. The photo-cleavage of the NPPOC-protecting group of the aminooxy moieties on the glass surface was next carried out by irradiation at 365 nm through a mask containing holes (diameter of the holes = 100 μm) in a 20% pyridine



Scheme 1. Reagents and conditions: (a) 2-(2-Nitrophenyl)propyl chloroformate, Na_2CO_3 10% H_2O, rt, 3 h, 90%; (b) DCC, *N*-hydroxysuccinimide, CH_2Cl_2, rt, 4 h, 67%.

aqueous solution for different time durations (10, 15, and 20 s). It should be noted that the presence of base is required for the photolysis to proceed. Oligonucleotide immobilization was accomplished by dipping the glass slides in a solution of 0.4 M ammonium acetate buffer containing oligonucleotide aldehyde (20 μM) for 2 min. The oligonucleotides containing aldehyde functionality at 5′-terminus were prepared as reported earlier.[18]

The efficiency of this method was further evaluated by investigating the ability of surface-bound oligonucleotides to hybridize with the complementary strand labeled with Cy3 fluorescent probes. After hybridization and subsequent washings, the glass slides were scanned under a fluorescent scanner. Herein, the signal intensity directly reflects the efficiency of surface immobilization by current approach. The representative scanned images from the hybridization experiments are shown in Figure 1. A pattern of fluorescent and non-fluorescent areas with the shape of the mask was visualized. The ratio signal/noise *S*/*N* was evaluated for the different time durations (10, 15, and 20 s) to 2, 3, and 4, respectively. An irradiation time of 15 s was thus found enough to obtain a good signal to noise ratio.

The strategy was used next for surface immobilization of oligonucleotides into the inner wall of capillary tubes. Capillary tubes are extensively used in many biological applications, they are inexpensive and provide higher surface-to-volume ratio. The glass capillary tubes were silanized with (γ-aminopropyl)triethoxy silane and the coupling with activated ester **1** was performed in trichloroethylene instead of DMF. DMF was found to cause the degradation of the fused capillary and hence not used. The photo-deprotection of the aminooxy moieties was carried out by irradiation at 365 nm for varying time periods (from 5 to 30 s) through a slit aperture adjusted at 150 μm. This allowed the unmasking of aminooxy moieties only at preselected portions of the tube. The surface immobilization was achieved by filling the capillary tubes with aqueous solution of aldehyde-containing oligonucleotides. The hybridization experiments with complementary sequence labeled with Cy3 were performed at 100 nM concentrations. SSC buffer was used to remove unhybridized complementary oligonucleotides and the capillary tube was scanned under a fluorescent scanner (Fig. 2). Fluorescence spots were found localized at the irradiated portion of the tube. The photo-deprotection was rapid and an irradiation time of 5 s was sufficient to obtain a measurable response.

In conclusion, an alternative protocol for the surface patterning of oligonucleotides was developed. The effectiveness of the method was verified by accomplishing oligonucleotide immobilization on surfaces of glass slides and capillary tubes. Although the present method shows a signal to noise ratio slightly less favorable than our previously reported strategy, it presents the main advantage that the NPPOC-protected aminooxy surfaces can be prepared easily from (γ-aminopropyl)triethoxy

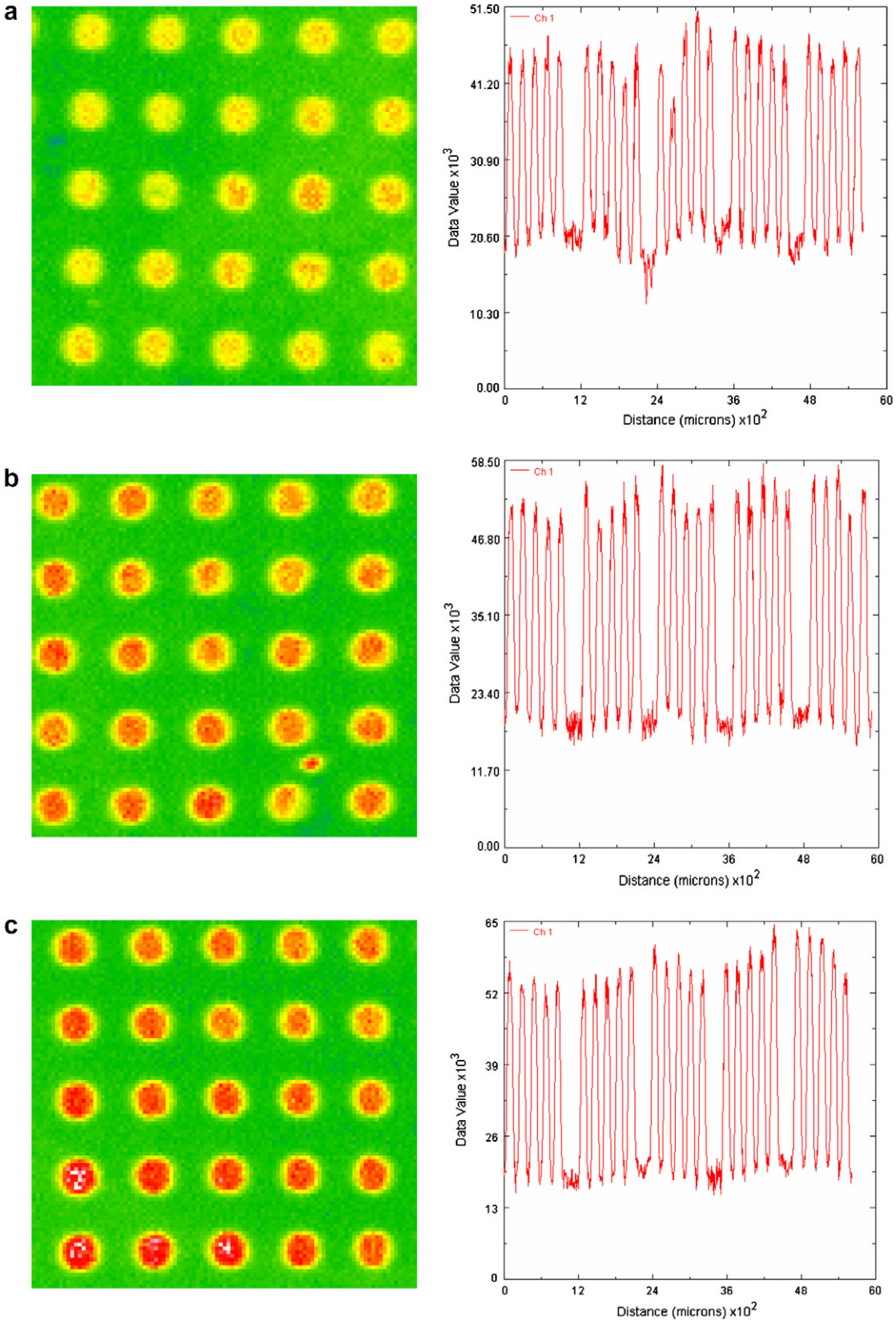


Figure 1. (Left) Scanned images of the oligonucleotide arrays on the glass slides. Arraying was achieved by irradiation through a mask (hole diameter: 100 μm) at 365 nm for (a) 10 s, (b) 15 s, and (c) 20 s in a 20% aq pyridine solution, followed by the deposition of a 20 μM solution of aldehydic oligonucleotide in 0.4 M ammonium acetate buffer for 2 min. Hybridization was carried out at 39 °C for 1 h using a 10 nM solution of Cy3® labeled complementary oligonucleotide. Fluorescence intensities are color-coded, varying from blue (low) to green, yellow, red, and then white (saturation), (right) profiles intensity for quantification analysis.

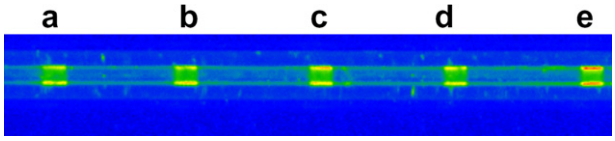


Figure 2. Scanned images of the oligonucleotides arrayed into the capillary tube. The arrayed capillary tube was obtained after following procedure: irradiation at 365 nm in 20% aq pyridine solution for various time periods; deposition of a 20 μM solution of aldehydic oligonucleotide in water for 2 min; and hybridization with the complementary strand labeled with Cy3®. Time of irradiation: (a) 5 s, (b) 10 s, (c) 15 s, (d) 20 s, (e) 30 s.

silane-coated glass. The immobilization protocol reported herein could be an efficient tool in the design of 'labs on a chip' devices.

Acknowledgments

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References and notes

1. Chee, M.; Yang, R.; Hubbell, E.; Berno, A.; Huang, X. C.; Stern, D.; Winkler, J.; Lockhart, D. J.; Morris, M. S.; Fodor, S. P. A. *Science* **1996**, *274*, 610.
2. Thibault, C.; Le Berre, V.; Casimirius, S.; Trévisiol, E.; François, J.; Vieu, C. *J. Nanobiotechnol.* **2005**, *3*, 1.
3. Beaucage, S. L. *Curr. Med. Chem.* **2001**, *8*, 1213.
4. Choithani, J.; Kumar, P.; Gupta, K. C. *Anal. Biochem.* **2006**, *357*, 240.
5. Chevolot, Y.; Bouillon, C.; Vidal, S.; Morvan, F.; Meyer, A.; Cloarec, J.- P.; Jochum, A.; Praly, J.-P.; Vasseur, J.-J.; Souteyrand, E. *Angew. Chem. Int. Ed.* **2007**, *46*, 2398.
6. Olivier, C.; Hot, D.; Huot, L.; Ollivier, N.; El-Mahdi, O.; Gouyette, C.; Huynh-Dinh, T.; Gras-Masse, H.; Lemoine, Y.; Melnyk, O. *Bioconjugate Chem.* **2003**, *14*, 430.
7. Ligler, F. S.; Breimer, M.; Golden, J. P.; Nivens, D. A.; Dodson, J. P.; Green, T. M.; Haders, D. P.; Sadik, O. A. *Anal. Chem.* **2002**, *74*, 713.
8. Balakirev, M. Y.; Porte, S.; Vernaz-Gris, M.; Berger, M.; Arié, J.-P.; Fouqué, B.; Chatelain, F. *Anal. Chem.* **2005**, *77*, 5474.
9. Blair, S.; Richmond, K.; Rodesch, M.; Bassetti, M.; Cerrina, F. *Nucleic Acids Res.* **2006**, *34*, e110.
10. Defrancq, E.; Hoang, A.; Vinet, F.; Dumy, P. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2683.
11. Boncheva, M.; Scheibler, L.; Lincoln, P.; Vogel, H.; Akerman, B. *Langmuir* **1999**, *15*, 4317.
12. Dendane, N.; Hoang, A.; Guillard, L.; Defrancq, E.; Vinet, F.; Dumy, P. *Bioconjugate Chem.* **2007**, *18*, 671.
13. Taylor, S.; Smith, S.; Windle, B.; Guiseppi-Elie, A. *Nucleic Acids Res.* **2003**, *31*, e87.
14. Trens, P.; Denoyel, R.; Rouquerol, J. *Langmuir* **1995**, *11*, 551.
15. Preparation of **1**: A solution of 2-(2-nitrophenyl)propyl chloroformate (2.2 g, 9 mmol) in dioxan (20 mL) was added drop-wise to a solution of carboxymethoxylamine hydrochloride (1 g, 4.6 mmol) in 10% aq Na_2CO_3 (25 mL) at 0 °C. The solution was stirred for 3 h in darkness and evaporated under vacuum. The residue so obtained was re-dissolved in water and the aqueous layer was washed with Et_2O, acidified with 1 N HCl and extracted from CH_2Cl_2. The organic layer was dried over Na_2SO_4 and solvent removed under vacuum. The crude product was purified by silica gel column chromatography using CH_2Cl_2/methanol (97/3, v/v) as eluent to obtain compound **2** as white powder (1.2 g, 90%). **2** (1.2 g, 4 mmol) was dissolved in anhydrous CH_2Cl_2 (15 mL) and dicyclohexylcarbodiimide (0.82 g, 4.4 mmol) and *N*-hydroxysuccinimide (0.46 g, 4.4 mmol) were added to the solution. The reaction mixture was stirred overnight, filtered and evaporated under vacuum. The crude product **1** was obtained after purification by silica gel column chromatography (Eluent: EtOAc) as white powder (1 g, 67%).
16. Characterization data for **2**: Mp: 88–90 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (d, J = 7 Hz, 3H), 3.68 (m, 1H), 4.30 (m, 2H), 4.42 (s, 2H), 7.39 (t, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.56 (t, J = 8 Hz, 1H), 7.75 (t, J = 8 Hz, 1H), 8.25 (br s, 1H), 9.45 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.3 (CH_3), 33.1 (CH), 70.4 (CH_2), 73.5 (CH_2), 124.2 (CH), 127.7 (CH), 128.0 (CH), 132.8 (CH), 136.5 (quat), 150.6 (quat), 158.2 (C═O), 172.0 (C═O). ESIMS (*m*/*z*): 297 $(M-H)^-$. Anal. Calcd for $C_{12}H_{13}N_2NaO_7$: C, 45.01; H, 4.09; N, 8.75. Found: C, 45.02; H, 4.29; N, 8.56. Characterization data for **1**: ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.39 (d, J = 6 Hz, 3H), 2.89 (s, 4H), 3.68 (m, 1H), 4.31 (m, 2H), 4.39 (s, 2H), 7.30–7.60 (m, 4H). ESIMS (*m*/*z*): 395 $(M+H)^+$.
17. For experimental protocol, see Ref. 12.
18. Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. *Chem. Eur. J.* **2001**, *7*, 3976.