



SARFUS: Study of chemical micropatterning on polycarbonate for peptide immobilization

Collaboration: O.Carion, V.Souplet, C.Olivier, C.Maillet, O. El-Mahdi, O.Melnyk (IBL, Lille, France)
J.O.Durand (Univ. Montpellier 2, France)

Abstract

A new way is described to micro pattern polycarbonate surface for biomolecular interaction studies. The Sarfus technique allows characterizing the micropatterns and reveals a doughnut effect which hopefully has a minor effect on the fluorescence image.

Introduction

Preparation of microfluidic devices widely use polycarbonate (PC) as substrate[1–3]. Due to the possibility of being used as compact discs for the high-throughput analysis platform of biomolecular interactions, its utility for bioanalysis has recently attracted much attention [1, 4–6].

In this paper, a new method for the chemical micropatterning of polycarbonate substrate is reported (figure 1). Silica nanoparticles **1** functionalized with semicarbazide groups were printed on PC by using a noncontact piezoelectric microarrayer to give micropatterns **2**. The semicarbazide groups present on the micropatterns were site-specifically ligated with unprotected peptides **3** derivatized by an α -oxo aldehyde group, to give substrate **4**: peptides linked to the micropatterns through semicarbazone bonds[7–9]. The surface between the spots was left unchanged. The use of nanoparticles of different diameters (27 to 304 nm) permitted to study the influence of surface curvature on signal strength and capture specificity. The nanoparticle layer on PC substrate was characterized by using Sarfus.

Experimental part

(See ref 10)

Results and discussion

Semicarbazide silica nanoparticles (**1**) were prepared from silica nanoparticles of different diameter (27, 82, 151, 192, 256 and 304nm) and were printed on PC slides (75 x 25 x 1 mm) by using a noncontact piezoelectric arrayer (three drops, ~1 nL total).

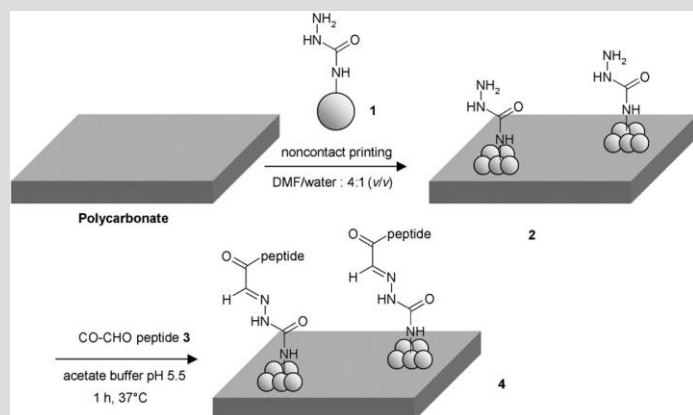


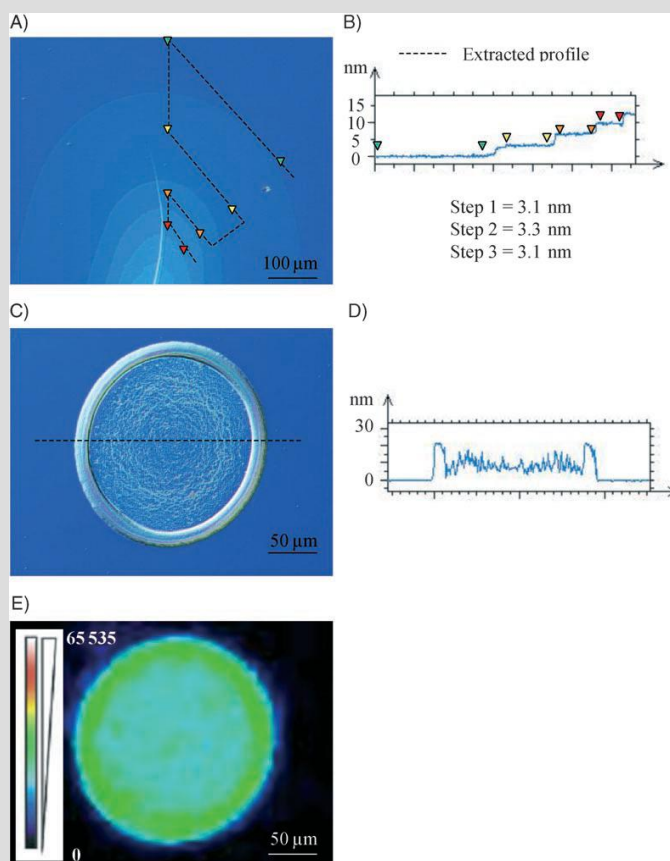
Figure 1: Chemical micropatterning of polycarbonate surface for the site specific immobilization of peptides.

The printed PC slides were incubated under cover-glass with peptides COCHO-HA or COCHO-FLAG, and then anti-HA or anti-FLAG antibodies followed by tetramethylrhodamine-labelled secondary antibodies. Fluorescence analysis (data not shown, see ref. 10) shows the high specificity of the capture of

anti-HA or -FLAG antibodies by immobilized peptides and that highest signals were obtained for 82- and 27-nm nanoparticles, probably due to a higher specific surface area.

The micropatterns formed by printing semicarbazide nanoparticles on a PC substrate were also characterized by Sarfus. For this analysis, a Surf with a polycarbonate toplayer (termed 'Surf PC') was used. Preliminary experiments demonstrated the inertia of solvent on the toplayer. Sarfus analysis showed no significant changes in the size or height of the micropattern before and after the different incubations meaning that no nanoparticles desorption occurred during the different washing and incubation steps, and that the thickness of the micropattern is mainly dictated by the thickness of the nanoparticle layer.

For Sarfus measurement, a calibration is performed from 4'-n-octyl-4-cyanobiphenyl (8CB) liquid crystal that forms spontaneously well-defined multi-layer structures with step height of 32Å (Figures 2A & 2B). Figure 2C shows the Sarfus image of a micropattern incubated with COCHO-FLAG peptide, anti-FLAG antibody and finally tetramethylrhodamine-labelled goat antimurine antibodies.



Figures 2: A) Sarfus image of 8CB drop on PC surf. B) Extracted profile along the dotted line in (A). C) Sarfus image of 27nm semicarbazides nanoparticles micropattern. D) Extracted profile along the dotted line in (C). E) Fluorescence image of the micropattern shown in C). Scale on left gives correspondence between fluorescence intensity and colors.



In Fig 2C, the spot displays a ring-like deposit along its perimeter probably due the migration of dispersed solids to the periphery of the drop during liquid evaporation. The 27 nm-nanoparticle layer inside the micropattern has a mean height of 5.3 nm. Like other optical techniques, the Sarfus technique is sensitive to the matter quantity per unit surface. Thus, Sarfus measurement of a compact particle (radius R) monolayer (compactness ratio of 0.74) would give a layer thickness of $0.74 \times R$. The result obtained in this study suggested a compactness ratio of about 0.4 (5,3/13.5) leading to a mean distance between nanoparticles close to their diameter (27nm).

By comparing both images in Sarfus (figure 2C) and fluorescence (figure 2E) mode, one can see that the doughnut distribution of the nanoparticles visualized using Sarfus has a minor effect on the homogeneity of the fluorescence image. This observation suggests that only the external layer of the micropattern is accessible to the peptide or to the antibody.

Conclusion

A new way for the chemical micropatterning of polycarbonate based on the printing of functionalized silica nanoparticles is reported. Specific captures of antibody is proved. The Sarfus technique allowed the easy characterization of an entire micropattern and the determination of the nanoparticle layer thickness.

Contribution/advantages of Sarfus

- Fast visualisation of pattern on surface
- Non contact/ non labelling technique
- Field of view (from $60\mu\text{m}^2$ to several mm^2) for statistical results
- Analyse at room temperature and atmospheric pressure

References

- [1] S. A. Soper, M. Hashimoto, C. Situma, M. C. Murphy, R. L. McCarley, Y.-W. Cheng, F. Barany, *Methods* 2005, 37, 103–113.
- [2] Y. Liu, C. B. Rauch, *Anal. Biochem.* 2003, 317, 76–84.
- [3] Y. Liu, D. Ganser, A. Schneider, R. Liu, P. Grodzinski, N. Krutchinina, *Anal. Chem.* 2001, 73, 4196–4210.
- [4] J. J. La Clair, M. D. Burkart, *Org. Biomol. Chem.* 2003, 1, 3244–3249.
- [5] J. J. La Clair, M. D. Burkart, *Org. Biomol. Chem.* 2006, 4, 3052–3055.
- [6] I. Alexandre, Y. Houbion, J. Collet, S. Hamels, J. Demarteau, J. L. Gala, J. Remacle, *Biotechniques* 2002, 33, 435–439.
- [7] Y. Coffinier, C. Olivier, A. Perzyna, B. Grandidier, X. Wallart, J.-O. Durand, O. Melnyk, D. Stiévenard, *Langmuir* 2005, 21, 1489–1496.
- [8] C. Olivier, D. Hot, L. Huot, N. Ollivier, O. El-Mahdi, C. Gouyette, T. Huynh-Dinh, H. Gras-Masse, Y. Lemoine, O. Melnyk, *Bioconjugate Chem.* 2003, 14, 430–439.
- [9] O. Melnyk, X. Duburcq, C. Olivier, F. Urbès, C. Auriault, H. Gras-Masse, *Bioconjugate Chem.* 2002, 13, 713–720.
- [10] O. Carion, V. Souplet, C. Olivier, C. Maillet, N. Médard, O. El-Mahdi, J.O. Durand, O. Melnyk, *ChemBioChem*, 2006, 8, 315–322.