Multi-functional silica microdot arrays by inkjet printing for biosensor applications

O. De Los Cobos[*], M. Lejeune[*], F. Rossignol[*], C. Carrion[**], C. Boissière[***], F. Ribot[***], C. Sanchez[***], J-O. Durand[****], X. Cattoen[****] and M. Wong Chi man[****]

[*]Laboratoire de Science des Procédés Céramiques et de Traitements de Surface (SPCTS), UMR CNRS 7315, CEC, 12 rue Atlantis, 87068 Limoges, France.
[**]Plateforme Cytométrie-Imagerie-Mathématiques (CIM), UMR CNRS 6101, Faculté de médecine, 2 rue du Dr Marcland, 87025 Limoges, France
[***]Laboratoire de Chimie de la Matière Condensée de Paris (LCMCP), UMR CNRS 7574, Université Pierre et Marie Curie Paris VI, Collège de France, 11 place Marcelin Berthelot, 75005 Paris, France.
[****]Institut Charles Gerhardt Montpellier (ICGM), UMR 5253 CNRS-UM2-ENSCM-UM1, place Eugène Bataillon, 34095 Montpellier, France.

ABSTRACT

Silica-based microarrays for molecular recognition are realized using a piezoelectric drop-on-demand inkjet printer. In an evaporation induced self-assembly route, a silica sol containing (3-azidopropyl)triethoxysilane is used as ink to print azido-functionalized mesoporous silica microdots arrays clickable with various alkyno-peptides, in accordance with the Huisgen 1,3 dipolar cycloaddition commonly called "click reaction". In a similar route, a second ink is formulated integrating an alkyne silylated precursor. By using a multi-printhead system, a bi-material of intercrossed azido and alkyno-functionalized network can be built. The successive reactions with clickable peptides lead to the array multi-functionalization. The success of the click chemistry, EISA and inkjet printing combination highly depends on the ink formulation, which is adjusted in regards to the viscosity and surface tension in the appropriate range for inkjet printing.

Keywords: inkjet printing, mesoporous silica, click chemistry, biosensor

1 INTRODUCTION

The IJP technology offers promising opportunities in the field of sensors fabrication thanks to its flexibility, its current resolution and its capability to build in patterned arrays of microdots that can be specifically and individually functionalized for sensing by using a multi-nozzle device. Thus, IJP has already been used to produce glucose [1], antibodies sensors [2] and DNA arrays [3,4]. However, in these previous studies, the functionalization has been achieved by introducing biomolecules directly inside the solution before printing. As a result, the biomolecules may be damaged during the ejection [5]. In contrast, in the present work, clickable mesoporous silica microdots arrays are deposited by a one-pot IJP process, allowing a safe and selective post-functionalization by copper-catalyzed azide-alkyne cyloaddition (CuAAC) click reactions.

Click chemistry is a well-known and powerful tool to covalently bind (bio-)molecules bearing terminal alkynes onto azido-functionalized surfaces [6,7,8,9,10]. Such a regio-selective and orthogonal copper-catalyzed reaction between azides and alkynes has successfully been applied to mesoporous silica with high yields [11,12].

In this work, the challenge was to integrate azide and alkyne functions in a silica sol in respect of inkjet printing criteria to produce silica-based microarrays for biosensor applications. Herein, we demonstrate the potential of combining mesoporous silica microdots arrays obtained by IJP/EISA together with click chemistry to obtain innovative multifunctional arrays.

2 EXPERIMENTAL SECTION

The following compounds (3-azidopropyl)triethoxysilane (AzPTES) [11], (3-(prop-2-yn-1-yl)thiopropyl)triethoxysilane **4** [12] and the clickable dyes **3** and **5** [13] were prepared according to published procedures. The silica source (TEOS) and the nonionic structuring agent (Pluronic F127) were purchased from Sigma-Aldrich. The hydrophobic additive (TFTS) was purchased from ABCR. The substrates consist in hydrophilic silicon wafers with hydroxylated native silicon oxide (Si-Mat, Ref SW 150 mm/p/boron).

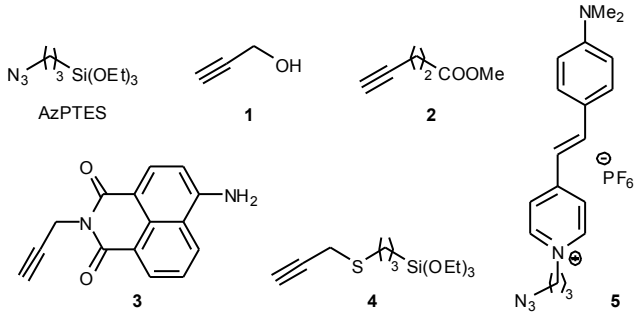

Chart 1: Compounds used in this study, namely AzPTES and **4** for inks formulation, **1** and **2** for FT-IR study then **3** and **5** for fluorescence microscopy.

The IJP equipment used was developed in the SPCTS laboratory and is a drop-on-demand type with piezoelectric printing heads [14]. The printing head displacement has a resolution of 0.5 µm, a reproducibility of 2 µm, and an accuracy of 2 µm. The current resolution achieved is ranged between 30 and 50 µm. Additional technical data are given in the corresponding patent (PCT/RF04/02150) and by the equipment manufacturer under the reference Ceraprinter L01 (Ceradrop, Ester Technopole, BP 36921, Limoges, France).

^{29}Si NMR experiments were performed on the sol in order to study the ageing time on a Bruker Avance 400 MHz spectrometer. All other measurements were realized on microdot array. 2D-Small-angle-X-Ray scattering (2D-SAXS) were performed a Rigaku S-max 3000 diffractometer. Scanning Electron Microscopy (SEM) images were achieved with a Philips XL30 at 200 kV. Transmission Electron Microscopy (TEM) images were obtained with a Jeol 2100 F apparatus at 200 kV. Confocal Scanning Microscopy (CSM) images were produced with a Zeiss LSM 510 META instrument using excitation wavelengths of 405 and 543 nm for the 4-amino-1,8-naphtalimide dye **3** and the red dye **5**, respectively. Fourier Transform InfraRed spectroscopy (FT-IR) spectra were obtained in a transmission mode using a Perkin-Elmer 100 spectrometer.

3 RESULTS AND DISCUSSION

3.1 Formulation of the inks

Based on a previous work [15], an azide-based ink formulation containing (3-azidopropyl)triethoxysilane (AzPTES, Chart 1) was tested, with the following composition: TEOS : TFTS : AzPTES : F127 : H2O : HNO3 : EtOH 1:0.05:0.10:0.006:5:0.02:20. The presence of tridecafluorooctyltriethoxysilane (TFTS) as hydrophobic surfactant was necessary in order to guarantee the reliability of the ejection due to the hydrophilic nature of the printing-head.

The aging time was adjusted regarding the silica condensed species Q_2 $[Si(OSi)_2(OX)_2]$ and Q_3 $[Si(OSi)_3(OX)]$ measured by ^{29}Si NMR, in order to reach similar proportions of Q_2 and Q_3. These conditions allowed a good control of the size of the oligomers to guarantee the self-assembly of the micelles with the condensation of the organic precursor during EISA and to avoid the clogging of the printing head by formation of a gel in the nozzle [16]. The introduction of 10 mol% AzPTES leads to a decrease in the condensation rate compared to the standard sol. Whereas the suitable aging time range for the standard sol was between 26 and 36 hours, this one was extended for the azide sol between 48 and 68 hours. Consequently, the aging time was set at 48 hours to reduce the synthesis time.

The ink exhibited appropriate rheological parameters to allow an approriate inkjet ejection: a surface tension (γ) of 22.78 mN/m and an apparent viscosity (η) of 5 mPa.s were measured, which corresponds to an ejection ratio, $Re/We^{1/2}$, equal to 4.6 according to equation (1) with the density (ρ), the nozzle radius (*r*), the Reynolds number (*Re*) and the Weber number (*We*).

$$1 \le \frac{\mathrm{Re}}{\sqrt{We}} = \frac{\sqrt{\rho\gamma r}}{\eta} \le 10 \qquad (1)$$

3.2 Inkjet printing of microdots arrays

Before the fabrication of the microdots arrays, the electric pulse applied to the piezoelectric nozzle was adjusted to get a consistent droplet ejection. This adjustment was carried out by capturing stroboscopically backlit images of the ejection with a CCD video camera, as illustrated Figure 2.

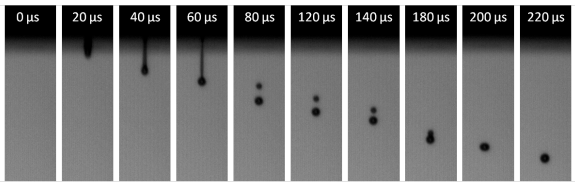

Figure 2: Visualization of the azide ink ejection.

Five or twenty-five layers microdots arrays were deposited under controlled conditions of temperature (293K) and humidity (50%), by 10 min-delayed successive deposits of droplets on a same location, this drying time allowing mesostructuration [15] onto hydrophilic silicon wafers with hydroxylated native silicon oxide. The patterns obtained (Figure 3) consisted in well-resolved microdots arrays (diameter of 96 µm spaced out of 40 µm, height of 1.5 µm) and illustrated the capability of ink-jet printing to allow the fabrication of highly resolved and complex patterns.

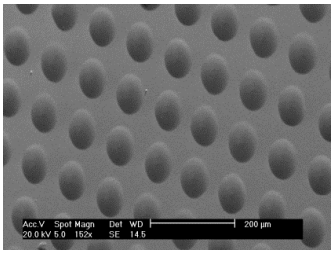

Figure 3: SEM image of a 25-layers azido-functionalized mesoporous silica microdots array deposited onto a silicon wafer by inkjet printing.

The as-obtained microdots arrays were then submitted to a mild thermal treatment (130 °C, 48 h) in order to stiffen the inorganic network without damaging the functional groups. A subsequent Soxhlet extraction in acidified ethanol was performed to remove the F127 structuring agent from the porosity [17], so that all the pores became available for the "click" post-functionalization.

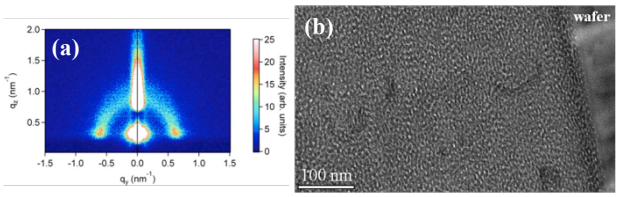

Figure 4: Structural organization of 25-layers microdots made with a 10 mol% AzPTES solution aged for 48 h (a) 2D-SAXS pattern and (b) TEM micrograph of a microdot cross section.

2D-SAXS measurements of the deposited microdots arrays (Figura 4a) revealed a diffuse diffraction ring, characteristic of a wormlike structure of the mesoporosity, which was confirmed by TEM observations (Figure 4b). Previous studies had already shown that such ink-jet printed microdots arrays made without AzPTES exhibited a centered rectangular structure [16]. The loss of regular mesostructure organization upon the addition of AzPTES could result from a too high amount of organosilane (i.e. 15 mol% overall taking into account TFTS): in fact, as observed by Matheron [18], a saturation of silica oligomers is observed as the organosilane content increases, which limits the percolation phenomenon around the surfactant and consequently the mesostructure organization. Nevertheless, considering the fact that the microdots usually exhibit an open porosity [16], a disordered wormlike structure could not be detrimental to achieve a significant sensitivity for the targeted application.

3.3 Post-functionalization by click chemistry

The presence of the azide moieties before and after the template extraction was ascertained by its characteristic band at 2100 cm^{-1} in FT-IR [11] (Figure 5B and C). To evaluate the potentiality of these microdots arrays towards the CuAAC post-functionalization reaction, three alkynes, namely propargyl alcohol **1** and methyl pent-4-ynoate **2** (Chart 1) [11], were reacted under standard conditions [alkyne, CuSO4 / sodium ascorbate catalytic system, water/t-BuOH (1:1) solvent mixture for 24 hours]. After the click reaction had proceeded, successive washings with sodium N,N-diethyldithiocarbamate in methanol and pure methanol were performed to remove the residual copper salts and alkyne remaining in the porosity. The success of the click reaction was evidenced after the reaction with propargyl alcohol by the almost complete disappearance of the azide band (Figure 5D). The remaining intensity of azide band likely corresponds to groups trapped in the silica walls and thus not accessible, an approximate anchoring of 93% being estimated from this spectrum considering that spectra C represents the total amount of azide. The functionalization of the mesoporous silica was also confirmed by the reaction with methyl pent-4-ynoate **2** (Figure 5E). This molecule exhibits a specific ester vibration band at 1730 cm^{-1} in IR [19]. As expected, both the decrease of the azide vibration intensity and the appearance of the ester characteristic band at 1730 cm^{-1} were observed even after vigorous washing. Furthermore, an approximate anchoring of 93% was also estimated in this case.

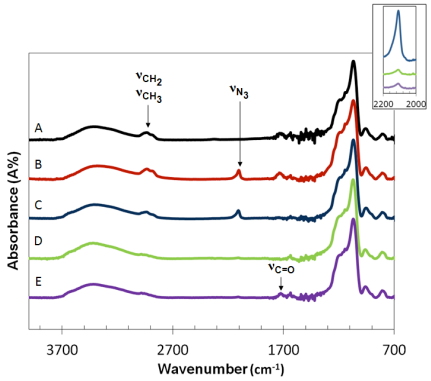

Figure 5: FT-IR analyses of mesoporous silica microdots (A) un-functionalized, (B) azido-functionalized before F127 extraction, (C) azido-functionalized after F127 extraction, (D) after "click" reaction with alkyne **1** and (E) after "click" reaction with alkyne **2** and in the right corner, the magnification of the azide band.

In order to demonstrate the specificity of the CuAAC reaction and its potential to obtain multifunctional arrays, a wafer printed with alternate rows of azide- and alkyne-functionalized microdots was obtained by the coupled EISA / IJP technique by using AzPTES and precursor **4** in silica sols ejected successively with two printheads. After thermal treatment and surfactant removal, the object was submitted to two successive CuAAC reactions: the first one was carried out with the alkyne-functionalized green fluorophore **3**, while the second one was realized with the azide-containing red fluorophore **5**. The alkyne-based fluorophore **3** only reacted with the azido-functionalized rows (Figure 6a), while the alkyne-functionalized microdots specifically reacted with **5**, without any mixture (Figure 6b). These results underline the specificity of the "click" reaction and highlight the possibility to specifically functionalize microdots in order to collect one type of fluorescent signal per row. This scheme can be extended to multifunctional microdots arrays.

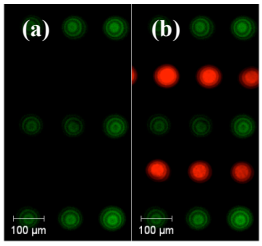

Figure 6: Confocal fluorescence micrographs of azide- and alkyne-functionalized microdots alternate rows (a) reacted only with fluorophore **3** and (b) successively reacted with **3** and **5**.

3.4 Towards biosensor applications

DNA biosensors are based on the interactions between two oligonucleotides sequences, the molecular recognition ocurring between two complementary nucleobases by hydrogen bonds. Thus, melamine with alkyne end function was synthesized and immobilized on the azido-functionalized rows of an alternate network with neutral rows, according to the standard click reaction. The as-obtained microdot array was immersed in chloroform with fluorescent labelled thymine, which exhibits a three hydrogen bond interaction with melamine [20]. After rinsing, the thymine remained on the melamine-functionalized row, as observed Figure 7b, demonstrating that the interactions were sufficient to allow selection and detection by fluorescence microscopy on the microdot array.

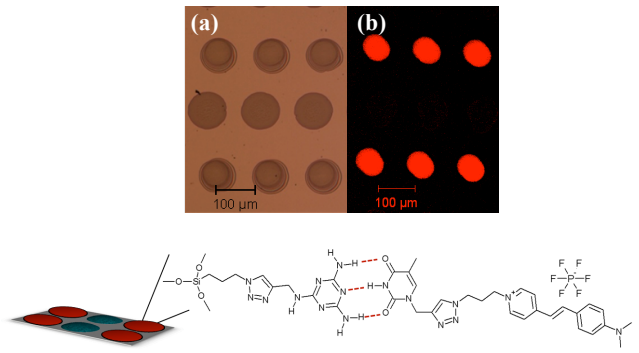

Figure 7: (a) Optical and (b) confocal fluorescence micrographs of the alternate rows after molecular recognition of fluorescent labelled thymine.

4 CONCLUSION

This study demonstrates the interest of combining IJP, EISA and CuAAC-click chemistry to get multifunctional micropatterned surfaces. In the whole process, the IJP technique brings more specifically its potential in terms of selectivity, resolution and flexibility. In particular, the capability to specifically functionalize each microdot in a one-pot process by using a multi-nozzle printhead is very promising to click afterwards a large range of molecules. With adapted biomolecules, silica-based biosensors should be accessible, for diagnosis based on oligonucleotides detection for instance.

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