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Electrochemically assisted micro localized grafting of aptamer in a micro channel engraved in fluorinated thermoplastic polymer Dyneon THV

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The surface modification of the bottom of a micro channel of new family of microfluidic chip materials, fluorinated thermoplastic polymer (Dyneon THV), was performed for the first time through the local electrochemical carbonization of the polymer followed by the adsorption of azide-bearing diazonium moieties and the covalent linkage of alkyne-bearing aptamer through the click chemistry reaction.

Fluoropolymers are promising material for microfluidics[1-3]. Their extreme inertness to chemicals and solvents and their ultimate hydrophobicity and oleophobicity make them attractive for digital microfluidics. They can also be used for cell-based microfluidics due to their antifouling properties and biocompatibility. Dyneon THV is a new class of such fluorinated material that is well suited for droplet and organic solvent microfluidics[3]. This thermoplastic fluoropolymer is a terpolymer of tetrafluoro ethylene ($F_2C=CF_2$), hexafluoro propylene ($F_2C=CF-CF_3$) and vinylidene difluoride ($H_2C=CF_2$). It has a very low surface energy, a hydrophobic character and a high resistance to chemicals. Furthermore, its low melting temperature ($T_M \approx 165°C$) makes its use in microchip fabrication processes simple and fast. However, as most perfluorinated materials, this emerging substrate lacks chemical reactivity. Indeed, the aliphatic carbon and fluorine atoms that constitute the THV backbone make difficult its derivatization by conventional chemical methods. Nevertheless, the surface functionalization of microsystem materials is of prime importance to control the interfacial chemical properties (hydrophilic/hydrophobic character) of the microchannel. In addition to create patterns, surface chemistry control allows monitoring the flow of the solvent for the design of nonmechanical passive valves[4], avoids potential physical adsorption of undesired species etc.

The introduction of chemical functionalities on fluoropolymers can be achieved by different processes[5]. Chemical[6] or electrochemical[7] activations yield either an irreversible aggressive transformation (reduction of carbone-fluorine bonds or strong base-promoted dehydrohalogenation) or a reversible supramolecular self-organisation on the surface[8]. Irradiation or plasma processes have also been used [5,9-12]. Only one study related to the plasma-based surface modification of THV is referenced so far in the literature [12]. Such an activation process allowed the formation of a thin brominated polymeric film on the THV surface under the influence of plasma, i.e. generation of brominated surface functionalities, in order to successfully achieve further chemical activation through *click* chemistry reaction. This unique example allowed the successful functionalization of the entire surface of the flat material but it is not adequate for the local micro functionalization and micro patterning of restricted areas in the material, such as along or within micro channels of a microsystem. Indeed, the local micro patterning within micro channels of a microsystem combined with the use of specific ligands such as antibodies or aptamers is now highly desired to create specific capture zones in microfluidic channels for analytical or diagnosis purposes. The ability to create "islands" of a chosen molecule on the surface of a substrate and especially within micro channels, without masking or elaborated physical constrains patterning, fits well with the use of scanning electrochemical microscopy SECM designing[13]. Within the last ten years, it was shown that polymers might be functionalized locally by SECM by generating reactive species at the tip that are prone to react with the material surface[14-18]. For example, it was demonstrated that SECM permits the localized reduction of fluoropolymers by the electrogenerated radical anion of a redox mediator. Reduction of polytetrafluoroethylene by an electrogenerated radical anion had been reported and results in its carbonization allowing further selective post-decoration of the carbonized patterns with metals[19], organic[20] and polymeric entities[21].

In the present paper, we provide a general route to built-in functional areas patterning within a perfluorinated microfluidic system. We first present the unprecedented local patterning of a Dyneon THV flat substrate by reductive carbonization assisted by SECM and extend this process to the patterning within an engraved micro channel. To this end, we adopt a three-steps approach: *1)* SECM-assisted carbonization of the Dyneon THV, *2)* introduction of azide groups by auto grafting of 4-azidobenzenediazonium on the carbonized area *3)* covalent linkage of

alkyne-bearing molecules on the particular spot, through the Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC) reaction (see scheme 1).

In a first step, the surface of a flat plate of Dyneon THV was carbonized using a 25 μm diameter SECM tip to reduce 2,2' dipyridyl in DMF solution into its radical anion at a close distance. Preliminary experiments confirmed that the reduction of 2,2' dipyridyl starts at -2.2 V *vs* Ag/AgCl, and thus a potential of -2.3 V *vs* Ag/AgCl was used for the mass-transfer controlled local patterning process. The tip was positioned at a desired close distance from the substrate surface using conventional approach curve in the feedback mode in 0.1 M KCl + 5 mM hexaammineruthenium(III) chloride aqueous solution (see approach curves in SI). The tip electrode was positioned by recording the microelectrode current, i_T, as a function of the substrate-microelectrode distance d. It is positioned at a normalized current value equal to $i_T/i_{T,*} \sim 0.5$, where $i_{T,*}$ is the current at infinite distance.

After through rinsing of the substrate, the aqueous solution is then replaced by DMF containing 2,2' dipyridyl (50 mM) and Bu$_4$NBF$_4$ (0.1 M). The system was kept under nitrogen in a polyethylene bag during the experiment. The humidity in the plastic bag was maintained at less than 30%. The tip was biased at -2.3 V *vs* Ag/AgCl while moving the electrode at scan rates of 1, 2 or 3 μm/s. As for other fluoropolymers, the local reduction performed at the SECM tip yields the material carbonization. Fig. 1A shows the traces of the carbonization of the Dyneon THV surface observed by optical microscopy. Then, the freshly carbonized surface was immersed in 4-azidobenzenediazonium (5 mM) in acetonitrile for 1 h to allow its spontaneous grafting, leading to an azide functionalization of the carbonized areas [20,22] (see SI). In a final step, the fluorescent dye acetylene-Fluor 488 was clicked through CuAAC reaction [23]. Fig. 1B&C show that the specific immobilization of the fluorescent dye was carried out successfully and provides a visual means to evaluate CuAAC reaction yield on N$_3$-modified THV substrate following its carbonization. It should be noticed that the same experiment conducted without copper catalyst led to adsorption of the dye in the carbonized zone with lower fluorescence intensity that disappears with ethanol rinsing.

The same procedure was carried out within a microchannel of 950 μm width and 150 μm height engraved in the Dyneon THV substrate. To do so, the SECM tip was placed at the bottom of the channel through control over the negative feedback current in 0.1M KCl + 5 mM hexaammineruthenium(III) chloride aqueous solution (currents such that $i_T/i_{T,*} = 0.25$ and 0.5 were chosen). The solution was then replaced by DMF solution containing 2,2' dipyridyl (100 mM) and Bu$_4$NBF$_4$ (0.1 M). The carbonization of the surface of the micro channel through the electrochemical reduction of 2,2'dipyridyl was performed at two different positions of the tip and at two different scan rates. Fig. 2A shows the fluorescent images of the carbonized areas after reaction with 4-azidobenzenediazonium and Fig. 2B after the *click* reaction with acetylene-Fluor 488.

As expected, for a given normalized distance, the decrease of the scan rate during the reduction of 2,2' dipyridyl leads to larger carbonized patterns on Dyneon thus producing larger modified zones with acetylene-Fluor 488 after *click* reaction ($\approx 30 \mu$m width at 1 μm/s and $\approx 14 \mu$m width at 3 μm/s). For a given scan rate during carbonization, lower working distance (between the tip and Dyneon surface) leads to narrower carbonized zone ($\approx 65 \mu$m at a distance

corresponding to $i_T/i_{T,*} = 0.5$ and $\approx 30 \mu$m at $i_T/i_{T,*} = 0.25$) due to the slowness of the surface reaction. Whatever the carbonization conditions used, these data allow confirming the successful specific micro immobilization of the fluorescent dye within the micro channel on the N$_3$-modified Dyneon THV substrate following its carbonization.

Finally, the patterning of an aptamer of 75 bases with sequence 5'-ATA CCA GCT TAT TCA ATT GCA ACG TGG CGG TCA GTC AGC GGG TGG TGG GTT CGG TCC AGA TAG TAA GTG CAA TCT-3' modified with 6-carboxyfluorescein (6-FAM) at the 5' end and 5-Octadiynyl at the 3' end (see SI) was successfully performed following a *click* procedure similar to that of the anchoring of acetylene-Fluor488. Fig. 3 shows an example of the fluorescent image of the pattern obtained with a tip of 10 μm diameter positioned at $i_T/i_{T,*} = 0.5$ a within the channel and moved at 1 μm/s and further derivatized with the fluorescently-tagged aptamer. The grafted oligonucleotide was stable through several rinsings with water and ethanol and upon ultra sonication. Further experiments are in progress to clearly show that the grafted oligonucteotide retains its affinity to bind the specific target molecule (i.e. diclofenac)

Conclusion

Micro localized carbonization of perfluorinated thermoplastic material Dyneon THV was demonstrated for the first time. The process was developed to create micro areas modified with specific functions or (bio)molecules. The developed process can be performed on flat plates or inside micro channels thanks to the use of a sharp SECM tip positioned in the vicinity of the substrate and the local generation of a strong reducing agent. The dimensions and resolution of the modified zones can be tuned with the tip/material distance, the tip travelling rate during carbonization and definitely with the tip size. The obtained local zones were further used for post-modification by substituted aryl functions with azide groups, thanks to the spontaneous irreversible adsorption of the corresponding aryldiazonium salt. Then, the locally modified areas with azido-aryl functions were used to anchor covalently (bio)molecules tagged with alkyne function by *click* reaction. Using this procedure, a fluorophore and a model aptamer were successfully locally and selectively immobilized on the SECM-formed patterns in Dyneon THV micro channels. Such a procedure, which can be easily extended to other fluorinated microfluidic materials, should allow building analytical microsystem integrating aptamer-based molecular concentration for separation and detection of trace amounts of pharmaceuticals emergent pollutants such as pain killers (diclofenac) and hormones (17β-estradiol).

Acknowledgments

Notes and references

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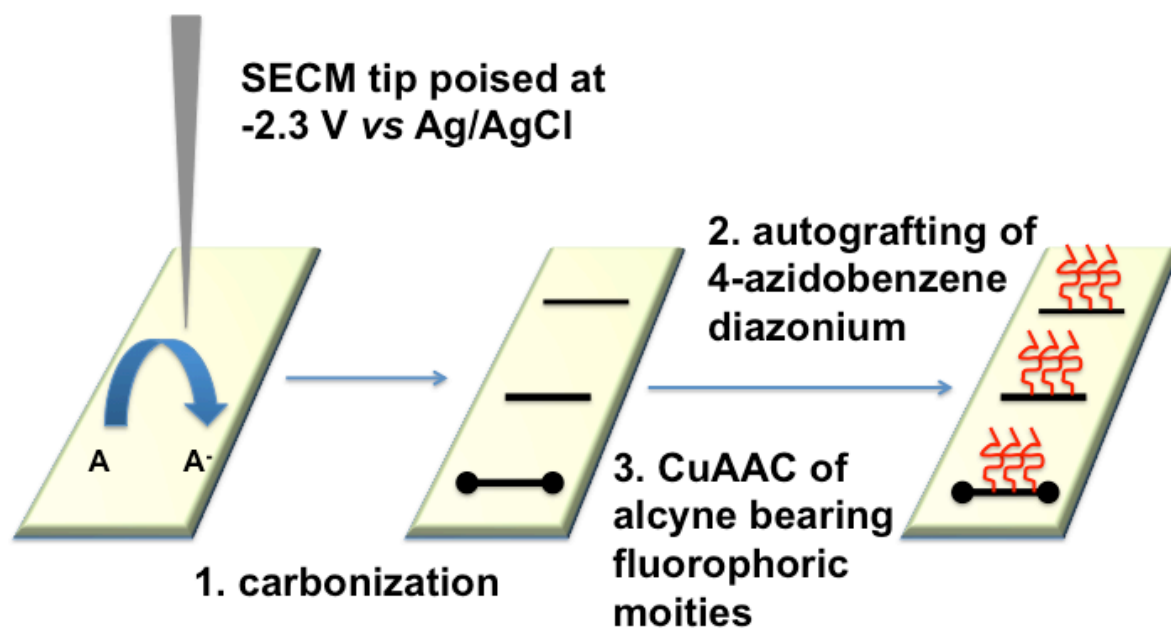[3]Université Paris Descartes, Paris, France.
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experimental details and supporting figures. See DOI: 10.1039/b000000x/

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SECM tip poised at -2.3 V *vs* Ag/AgCl

2. autografting of 4-azidobenzene diazonium

A   A·

1. carbonization

3. CuAAC of alcyne bearing fluorophoric moities

Scheme 1. Strategy developed for the local modification of Dyneon THV surface by chemical functions and/or biomolecules
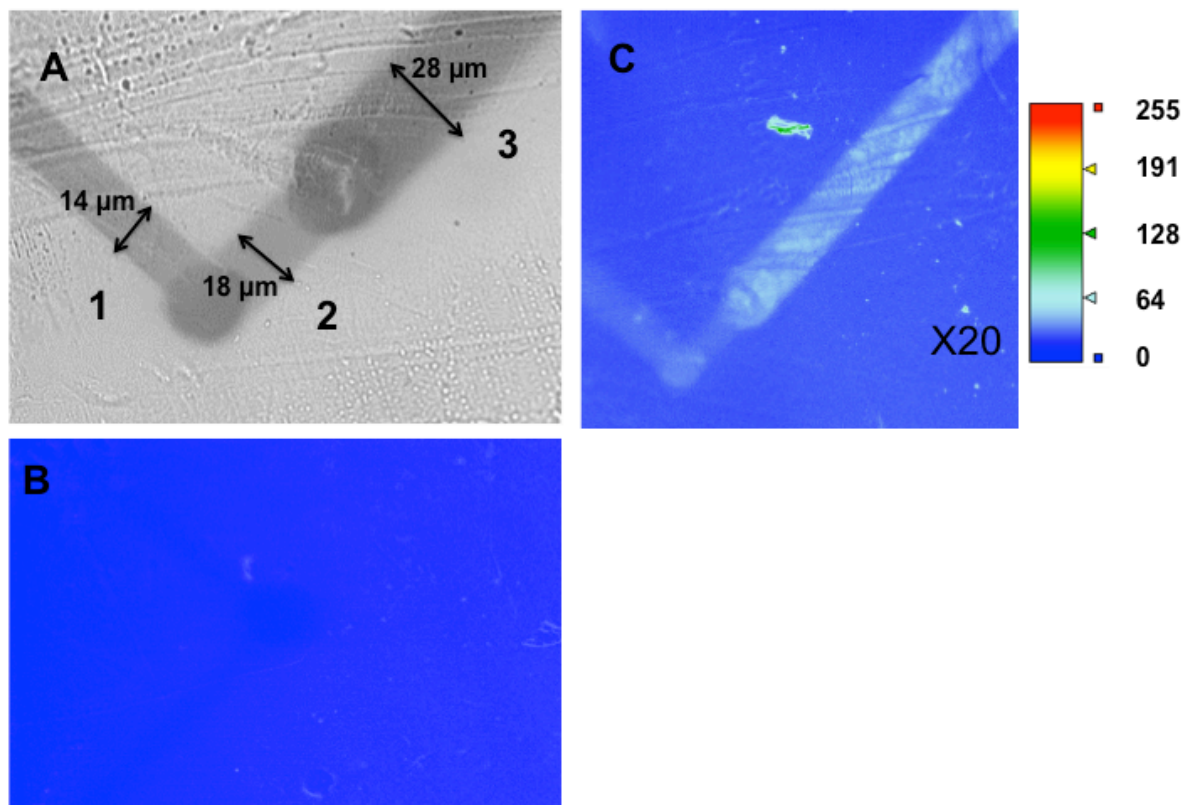
Figure 1. (A) optical image of the electro carbonized patterns of the Dyneon THV upon the reduction of 2,2' dipyridyl in DMF solution substrate by moving the tip of the SECM at 3, 2 and 1 µm/s when positioned at $i_T/i_{T,\infty} = 0.5$ (lines 1, 2 and 3, respectively). (B) Fluorescence image of the carbonized substrate after immersion in 5 mM 4-azidobenzene diazonium solution . (C) Fluorescence image after CuAAC reaction of the azide bearing patterned areas with alkyne bearing fluorophore.
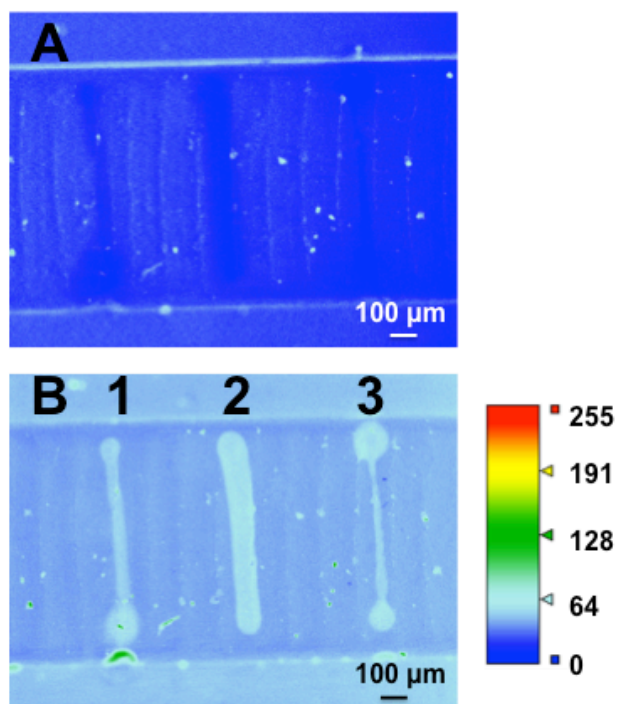
Figure 2. (A) Fluorescence image of electro assisted carbonization of the engraved micro channel after immersion in 5 mM 4-azidobenzene diazonium solution . (B) Fluorescence image after CuAAC reaction of the azide bearing patterned areas with alkyne bearing fluorophore. Pattern 1 was obtained by carbonization when moving the tip at 3 µm/s when positioned at $i_T/i_{T,\infty} = 0.5$, Pattern 2 was obtained by carbonization when moving the tip at 1 µm/s when positioned at $i_T/i_{T,\infty} = 0.5$ and Pattern 3 was obtained by carbonization when moving the tip at 3 µm/s when positioned at $i_T/i_{T,\infty} = 0.25$
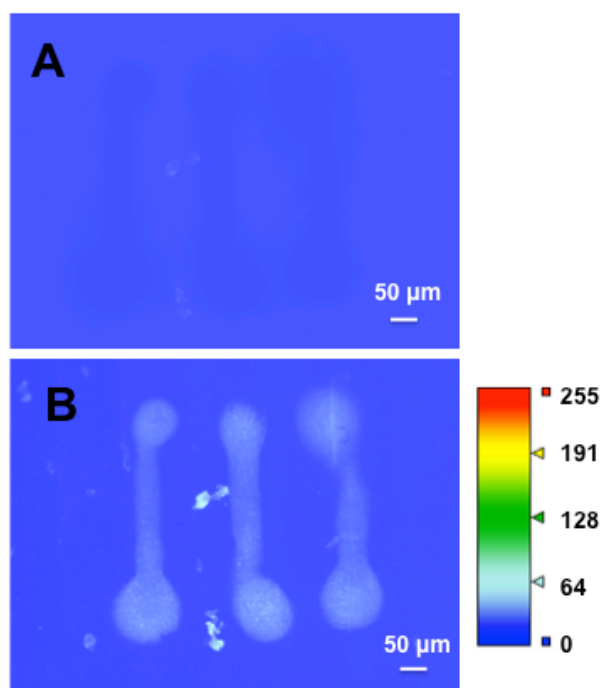
Figure 3. (A) Fluorescence image of electro assisted carbonization of the engraved micro channel after immersion in 5 mM 4-azidobenzene diazonium solution . (B) Fluorescence image after CuAAC reaction of the azide bearing patterned areas with alkyne bearing aptamer. The three patterns were obtained by carbonization when moving the tip at 1 μm/s when positioned at $i_T/i_{T,\infty} = 0.4$