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Development of a sticker sealed microfluidic device for in situ analytical measurements using synchrotron radiation

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ABSTRACT

Shedding synchrotron light on microfluidic systems, exploring several contrasts *in situ/operando* at the nanoscale, like X-ray fluorescence, diffraction, luminescence, and absorption, has the potential to reveal new properties and functionalities of materials across diverse areas, such as green energy, photonics, and nanomedicine. In this work, we present the micro-fabrication and characterization of a multifunctional polyester/glass sealed microfluidic device well-suited to combine with analytical X-ray techniques. The device consists of smooth microchannels patterned on glass, where three gold electrodes are deposited into the channels to serve *in situ* electrochemistry analysis or standard electrical measurements. It has been efficiently sealed through an ultraviolet-sensitive sticker-like layer based on a polyester film, and The burst pressure determined by pumping water through the microchannel (up to 0.22 MPa). Overall, the device has demonstrated exquisite chemical resistance to organic solvents, and its efficiency in the presence of biological samples (proteins) is remarkable. The device potentialities, and its high transparency to X-rays, have been demonstrated by taking advantage of the X-ray nanoprobe Carnaúba/Sirius/LNLS, by obtaining 2D X-ray nanofluorescence maps on the microchannel filled with water and after an electrochemical nucleation reaction. To wrap up, the microfluidic device characterized here has the potential to be employed in standard laboratory experiments as well as in *in situ* and *in vivo* analytical experiments using a wide electromagnetic window, from infrared to X-rays, which could serve experiments in many branches of science.

Introduction

Microfluidic devices operate with small amounts of samples through micrometer and/or sub-micrometer channels¹. As the main characteristics, these devices show reduced size, low weight, and high throughput. These characteristics have attracted tremendous and widespread attention by the synchrotron community^{2,3} interested in compatible instrumentation for *in situ* and *in vivo* experiments. To date, reported applications of these devices on synchrotrons, involving studies on protein crystallography^{4,5}, advanced materials⁶, electrochemistry^{7,8}, catalysis⁹⁻¹¹, *in vivo* experiments (eukaryotic and bacteria cells)^{6,12,13}, established a paramount experimental contribution for drawing a picture of these complex systems, providing precious information about electronic, structural, and chemical properties in real-time, *in situ* and/or *operando*. However, developing a small, versatile, and compact

microfluidic device able to operate in synchrotron beamlines, especially at nanoprobe where the X-ray beam size is only hundreds of nanometers, has remained a challenge due to sample environment requirements. Arguably, limits should be established on such devices. It is crucial to define what is the good compromise between the selected material and the reliability of the microfabrication process when the device is to be applied in experiments that cover a wide window of the electromagnetic spectrum (X-rays, Infrared or visible light)¹⁴.

In the last two decades, poly(dimethylsiloxane), commonly called PDMS (an elastomer), has been the most applied material as substrate and sealing layers in microfluidic devices^{15–17} due to its remarkable physical and chemical properties such as biocompatibility, flexibility, transparency, and low toxicity^{1,18,19}. Nevertheless, PDMS presents some disadvantages that should be considered: it is permeable to small molecules due to its porosity, which can affect the devices throughout²⁰, and it shows low chemical resistance to many organic solvents²¹. Hence, this elastomer can lead to non-accurate results in biological assays and it is restricted to analyses in aqueous media. Similar limitations are also found in poly(methyl methacrylate) (PMMA), another popular polymer substrate for the production of microfluidic chips. In addition, PDMS devices might reach millimeters of thickness, which is non-compatible with X-rays measurements due to the lower transmission coefficient for X-rays when compared to commonly applied polymers in microfluidic. For example, a millimeter thick cyclic olefin copolymer (COC) based device attenuates, at 12.4 keV, seven-fold less than PDMS²², being more discrepant at lower energies. A similar proportion is observed by comparing polyester to PDMS.

An insightful approach to minimize the drawbacks mentioned before, consists in drastically changing the sealing process by selecting a sealing layer transparent to X-rays and chemically resistant, which leads to well known polymer films such as polyester, polypropylene, polyimide, and polycarbonate (supplementary Information). In addition, few works have proposed using ultraviolet (UV) sensitive adhesive, like the Norland Optics Adhesive (NOA), as substrate or adhesion layer^{23–26} in microfluidic devices. NOA is a thiolene based polymer that attracted attention due to exhibiting interesting properties like low cost, optical transparency in the visible range, high chemical resistance to strong solvents (acetone, hydrochloric acid, toluene)^{24,27}, and hydrophobicity²⁶ becoming a great candidate for applications in synchrotron beamlines^{28,29}.

Motivated by the relevance and impact of synchrotron studies for diverse areas as well as by the challenges in manufacturing microfluidic devices for such applications, we have developed the microfabrication of a three-electrode microfluidic device based on a new sealing method, which is compatible with X-rays (in reflection mode), infrared, and visible light. The device has been designed with only two main parts; a glass substrate, and a thin polymer employed as a sealing layer. We have used a UV-sensitive adhesive to promote adherence between polyester and glass. It is worth noting that the microfabrication involves well-established and scalable techniques and the resulting device presents satisfactory bonding strength and high chemical stability in both organic and biological media. The approach adopted in this work converged to a multifunctional microfluidic device.

Results and discussions

Microfabrication procedure

Initially, 3 cm x 3 cm glass squares were cut, cleaned up using piranha solution at a temperature of 60 °C, and then used as a substrate. The microchannels were prepared in a cleanroom using a geometry defined by the photolithography mask. In total, four lithography masks or channel geometry, were prepared: 100 μm (C1) and 100 μm plus a reservoir (res) with 300 μm (C2), 200 μm (C3), and 200 μm plus a reservoir(res) with 600 μm (C4).

Prior to the microchannel patterning, a layer of 60 nm of chromium was evaporated on the clean glass, working as a protective layer that improves the isotropic corrosion of the glass. To prepare the channels, the trenches were exposed to a wet chemical etching³⁰ using a solution at HF 49%, NH₄F 1.38 M, and HCl 38%, and hydrofluoric acid (HF) 20%, resulting in smooth channels. After the glass corrosion, using a second photolithography mask, three electrodes were patterned, and then gold was deposited on the channels by magnetron sputtering. After the deposition of the three gold electrodes, the device was drilled to enable connecting the inlet and outlet tubes. The sequence of the channel and electrode preparation is shown in Figure S1 (supporting Information).

The most important and delicate step of this work is the sealing process, once the sealing layer is a thin film. To succeed, an adhesive composed of NOA and a polyester film was prepared, herein this stack is also called *sticker*, which simplifies the sealing process. The sealing steps are summarized in Figure 1. Firstly, a polyester film with 12 μm of thickness was fixed with double-face adhesive tape on a glass frame to ensure mechanical stability (Fig 3a). Thus, the stack glass-frame/polyester was transferred to the spin-coater and a thin film (up to 1 μm) of NOA was prepared on the polyester film at 5000 rpm for 35 seconds. Then, the device was attached to the NOA side as shown in Figure 1 (b-c). Finally, a cure was done exposing the device to ultraviolet light ($\lambda=365\text{ nm}$) for two minutes at room temperature. After the curing step, the device was kept at 50 °C for 12 hours to improve the adhesion (Fig. 3d,e). In order to access the three gold electrodes, the spare piece of the polyester film was cut-off from the sealed device (Fig 1f). In the last step, the glass was drilled in the backside, and the inlet and outlet connections (silicone tubes) were welded using PDMS support, which gives high mechanical stability to the connections. All the sealing steps were carried out into a cleanroom.

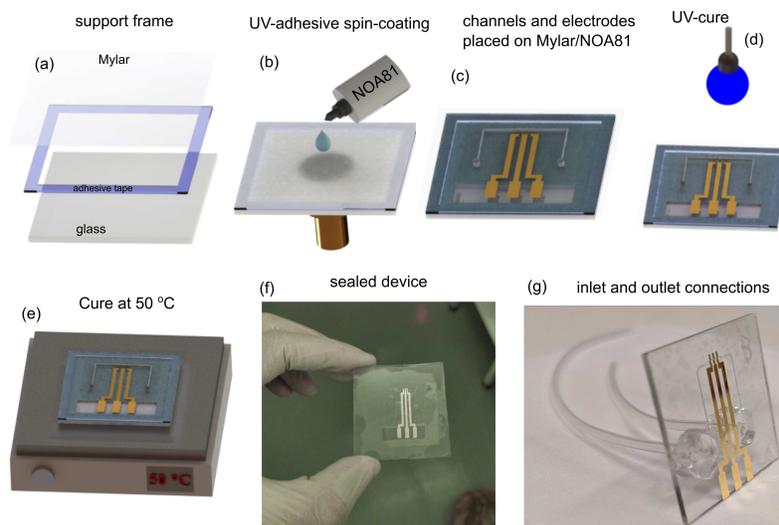


Figure 1. Sequence of the sealing process (a) Glass frame used as a support for the polyester film (b-c) Spin-coating process of the NOA on the polyester film, and the procedure to attach the device to the "sticker" (d-e) UV-cure (2 minutes) and the thermal annealing at 50 °C (12 hours) employed to improve the adhesion between the device and sealing layer (f-g) Shows the device completely sealed, and after bound the inlet and outlet connections, respectively.

Inspection of the channels and electrodes

Using glass as a substrate for microfluidic devices has some advantages: glass is inert to organic solvents, supports high temperatures, and might exhibit biocompatibility. However, the corrosion process could lead to

microcracks, microtextures, and an enlargement of the channel due to its isotropic corrosion mechanism. Thereby, prior to the sealing process, optical microscopy and profilometry were employed to investigate how precise the glass corrosion was. As it can be seen in Figure 2, the micrographs, from (a) to (d), show small defects on the channel border, which had no impact on the sealing process. In addition, concerning the channel geometry, it can be observed a channel patterning with high resemblance to the lithography mask, pointing out the good precision of such process. It is also possible to observe that the gold electrodes do not exhibit any large discontinuity or faults. In order to confirm the uniformity of the electrodes, the electrical resistance was measured for all devices, which revealed an average of $28 \pm 1 \Omega$, a value acceptable for gold thin films with about 100 nm of thickness³¹. Nevertheless, profilometry along the A-B line profile for all cells revealed channels with a slight deviation in width from the original lithography mask, as shown in Figure 2 (e-f). Usually, such deviation is due to the high intrinsic isotropic corrosion of the glass liquid-phase etching. In addition, the channel depth exhibited a linear behavior following the mask width, as shown in Figure 2 (f), hence the etching rate is significantly different in both dimensions.

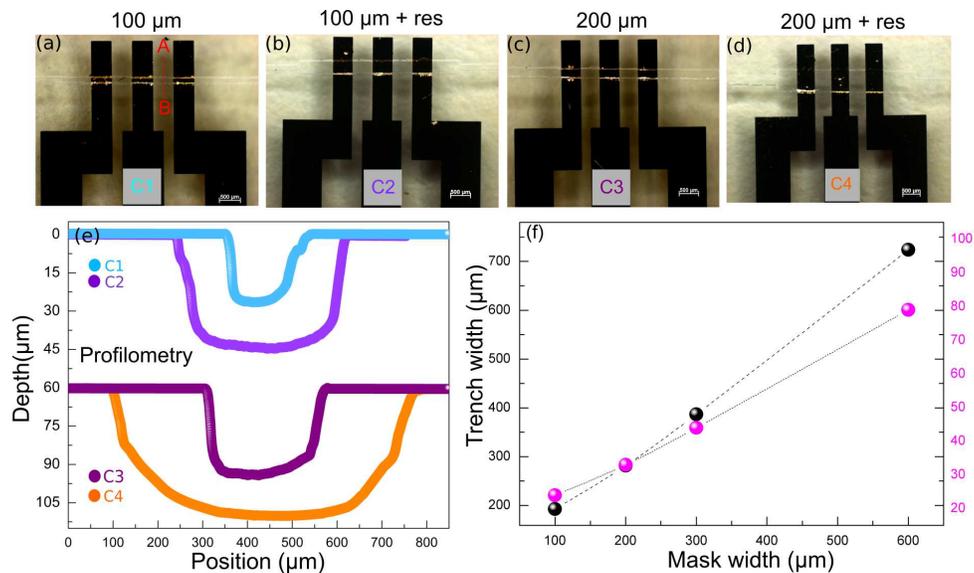


Figure 2. Characterization of the channel width and gold electrodes. (a-d) Optical microscopy of all the devices designed (C1-100 μm , C2 $\mu\text{m}+\text{res}$, C3 200 μm , and C4) 200 $\mu\text{m}+\text{res}$. (e) Profilometry measurements showing the channel depth and width. The red line A-B corresponds to the line where the measurements were taken (f) Dependence of the trench width and trench depth with the lithography mask width.

Sealing strength by bursting pressure and electrochemistry tests

For the new sealing process proposed in this work, which combines a sub-micron layer of UV-adhesive and a ten of micron of the sealing layer, it is expected an inherent limit pressure (flow rate threshold) denominated bursting pressure. Hence, the bond strength is a fundamental and critical parameter in microfluidic devices where the burst pressure strongly depends on the surface energy of the liquid-interface, wettability, and channel geometry. Initially, to check the efficiency of the sealing process, a syringe pump was used to inject water at 50 $\mu\text{L}/\text{min}$, and the flow through the channel was observed placing the device on an optical microscope (see video Supporting Information). Yet, no leak was observed, which revealed devices completely sealed (See the video in the supplementary material) and arguably ready to operate.

In order to determine the burst pressure, deionized water was pumped into the microchannels at about 25 $^{\circ}\text{C}$.

The water was driven through the channel using high-performance liquid chromatography (HPLC) pump whilst the same pump gave the pressure for each inlet flow rate, while the outlet was kept open to the atmosphere. In Figure 3 (a) it is shown the results of pressure measurements for the four devices geometry (C1, C2, C3, C4). The volumetric flow rate was varied from 30 to 340 $\mu\text{l}/\text{min}$. As it can be seen in Figure 3, the measurements reveal a burst pressure at about 0.22 MPa (or 33 psi), clearly identified due to drastic pressure drops for C1, C2, and C3. The C4 device has the larger reservoir (600 μm) and was intentionally preserved because the flow rate achieved exceeded our requirements.

In microfluidic devices the pressure versus flow rate is governed by the Hagen–Poiseuille's law³² $Q=R_H\cdot\delta P$, where Q is the volumetric flow rate, δP is the pressure difference, and R_H is the hydrostatic resistance of the device, which is inversely proportional to the channel cross-section area as described elsewhere³². Therefore, it is expected linear dependence of the pressure with the volumetric flow rate in sealed devices, where the slope gives the hydrostatic resistance of the channel geometry. Hence, narrower channels lead to higher R_H values, as shown in Figure 3.

The nature of the interface is an important characteristic interfering on the sealing process, limiting the maximum flow rate (or pressure) through the device. For example, it is found bursting pressure varying from 0.096 to 0.94 MPa for PDMS/glass device, reaching to 4.5 MPa in PDMS/PDMS devices^{18,33–35}. On the other hand, mediated by NOA, a high pressure tolerant (12 MPa) device was obtained in glass-polymer-glass³⁶. For comparison, in this work the polyester/glass device tolerated a pressure of 0.22 MPa, unfortunately, no data about the sealing interface described here was found for a comparison.

To investigate the performance of the microfluidic device in electrochemistry experiments and accessing its electrical measurement functionality, as designed, the reversible redox reaction $\text{Fe}^{3+}/\text{Fe}^{2+}$ was characterized by cyclic voltammetry (CV) at scan rate of 20 mV/s from 0.5 V to -0.5 V. Prior to the CV experiments, the channel was filled with an electrolyte consisting of 10 mmol L^{-1} of potassium ferricyanide ($\text{K}_3[\text{Fe}(\text{CN})_6]$) and potassium ferrocyanide ($\text{K}_4[\text{Fe}(\text{CN})_6]$) supported in 0.1 mol L^{-1} of potassium chloride (KCl). The potential was swept using the central electrode as a working electrode, the right electrode as a reference electrode, and the left electrode as a counter electrode. Figure 3 (b) shows the electrochemistry response for the devices. The CV shape changes from C1 to C4, exhibiting prominent oxidation (positive) and reduction (negative) current peaks for C2 and C4; the two cells with a reservoir. The transient voltammograms typical of macroelectrodes were reached by both the electrodes, reflecting a semi-infinite linear diffusion profile³⁷. The average peak-to-peak separations were found to be lower than 90.0 mV,³⁸ implying the occurrence of reversible systems that are likely because of the high conductivity and active area of electrodes, which favor the charge-transfer kinetics. In addition, for C1 (see inset in Fig. 3c) and C3 the sigmoidal-shaped voltammetric responses are characteristics of ultramicroelectrodes by exhibiting steady-state currents driven by radial diffusion.³⁷ These systems are preferred for the ultrasensitive affinity-based and enzymatic detection of biomolecules due to their higher signal-to-noise ratio when compared with electrodes bearing linear diffusion dominant behavior.³⁹ In complement, Figure 3 (c) shows a continuously cycling using the cell C4, where it is observed a stable response over the three cycles measured.

In this sense, the approach employed in this work imposes to the electrodes a curved geometry on the channel, as a consequence, for each cell the volume and the electrolyte-electrode contact area are different. Summarizing, small channels, even on the reservoir, have a smaller volume and consequently small exposed areas. Therefore, the results obtained from the electrochemistry measurements are in agreement with studies related to electrochemistry

using microelectrodes, where it is described the dependence of the CV hysteresis (or shape) with the diffusion zones geometry close to the electrode surface⁴⁰. Our findings are also important to evaluate the microfabrication procedure in terms of contamination due to the microchannels to be attached to the NOA/polyester sticker during the sealing process. Thus, the presence of the redox peaks in the CV indicates that the NOA does not cover the electrodes.

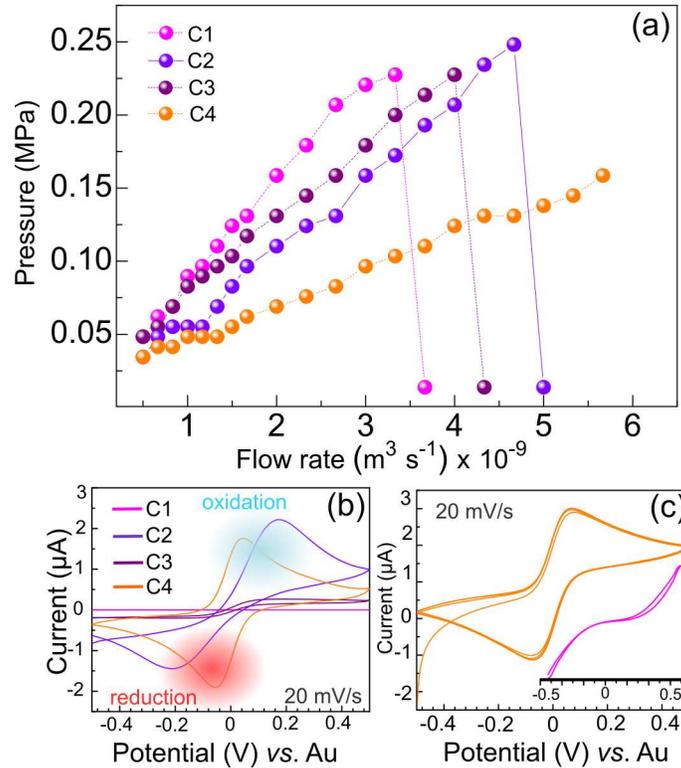


Figure 3. Pressure tolerance and electrochemical tests. (a) Determination of the burst pressure for the devices. At each flow rate, the pressure required is obtained using an HPLC pump. The pressure limit (bursting) is clearly observed for channels with $100 \mu\text{m}$ (C1), $100 \mu\text{m}+\text{reservoir}$ (C2), $200 \mu\text{m}$ (C3). Intentionally C4 ($200 \mu\text{m}+\text{reservoir}$) was kept sealed. (b) Cyclic voltammetry obtained at 20 mV/s (c) Electrochemical stability of the device accessed through cyclic voltammetry by varying the potential from -0.5 V to 0.5 V (three cycles). For clarity the voltammetry for C1 is zoomed and shown as an inset in (c).

Chemical resistance in organic solvents and biological fluid

To investigate the chemical stability of the polyester-glass devices, those chips were compared to the PDMS-glass chip by measuring the swelling ratio in the presence of organic solvents (hexane and n-propanol). For these tests, the PDMS based devices were fabricated as described in the literature^{41–43}. Briefly, channels (500 μm -width and 30 μm -depth) were engraved on PDMS by soft lithography, whereas the bonding step with a planar slide of glass was based on oxidation by an oxygen plasma. In practice, each solvent was pumped into the devices at a flow rate of 50.0 $\mu\text{L min}^{-1}$. The mass of the filled devices (C4) was continuously monitored. The values of the swelling parameter ($S = \frac{m}{m_0}$) were calculated by dividing the gradually measured masses (m) by the reference or initial data, i.e., the mass recorded right after filling the device with the solvent (m_0)⁴⁴. Assuming a linear fit, the PDMS-glass device shows a swelling rate of $5.03 \times 10^{-2} \text{ min}^{-1}$ ($R^2 = 0.98$) in the presence of hexane as depicted in Figure 4 (a). Conversely, this rate was only $2.88 \times 10^{-3} \text{ min}^{-1}$ ($R^2 = 0.99$) for the polyester-glass chip. According to Figure 4 (b), S was measured as 7.51 ± 0.01 (PDMS-glass) and 1.31 ± 0.01 (polyester-glass) after an exposition to hexane for 120 min. In addition, the swelling ratios in the presence of n-propanol for 60 min were 1.56 ± 0.01 (PDMS-glass) and 1.03 ± 0.01 (polyester-glass). These results suggest our device provides high chemical resistance to organic media. It is noteworthy that the polyester-glass chips described herein have met important requirements aforementioned, demonstrating a swelling of 17-fold lower than similar devices based on PDMS/glass.

To explore the multifunctional characteristic of the polyester-glass device, its chemical inertia was probed in the presence of biological samples (under flow) as well. Conversely to the observed for PDMS-glass devices, our mylar-glass chip further exhibited low adsorption of proteins. In practice, protein-enriched solution of bovine serum albumin (BSA) 10.0 mg mL^{-1} in potassium chloride (KCl) 1.0 mol L^{-1} was pumped into the devices for 2 h at 10.0 $\mu\text{L min}^{-1}$. Such protein was chosen as biomolecule model by accounting for 60% of the total proteins in blood plasma³⁷. Afterwards, the protein concentrations were quantified through a conventional method, i.e., Bradford assays, which were performed according to the official protocol using spectrophotometer and 96-well polystyrene plates (Kasvi, model K30-5096P)⁴⁵. The protein concentrations were determined as $8.1 \pm 0.8 \text{ mg mL}^{-1}$ ($n = 3$) and $9.8 \pm 0.9 \text{ mg mL}^{-1}$ ($n = 3$) for the samples exposed to the PDMS-glass and mylar-glass devices, respectively. While the original protein concentration was decreased by 19% in the first case, the latter chip resulted in a percentage drop of only 2%. These tests were once again performed on the polyester/glass device type C4.

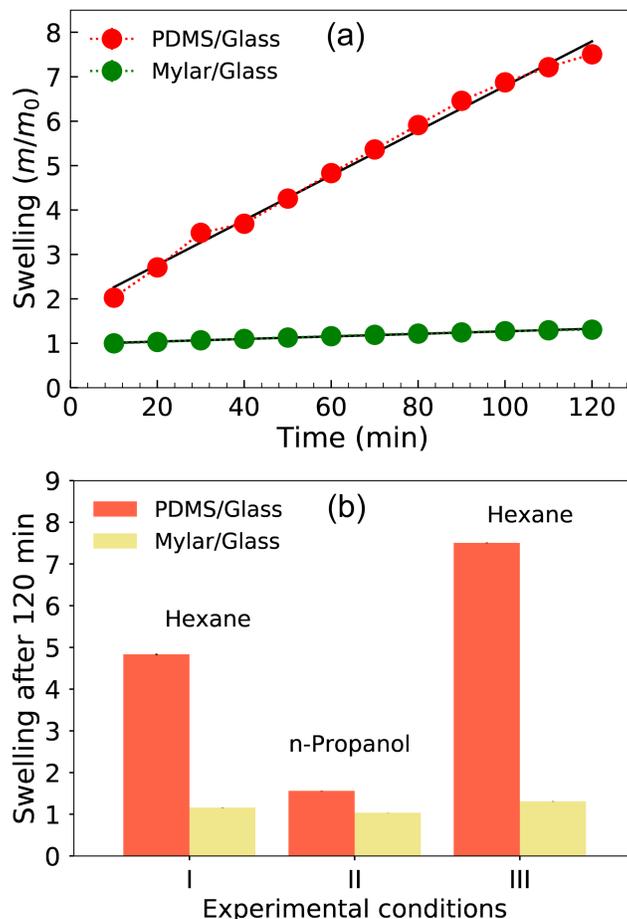


Figure 4. Values of S for the PDMS/glass and polyester/glass devices. (a) Data obtained after exposition to hexane for 120 min and (b) specific results associated with expositions for 60 (I,II) and 120 min (III) to hexane and n-propanol as highlighted, the error bars mean confidence intervals for $\alpha = 0.05$ ($n = 10$)

Nanofluorescence imaging on the microfluidic device

Carnaúba is a state-of-the-art nanoprobe beamline at the SIRIUS/LNLS⁴⁶. Its main characteristic is to provide a full coherent X-rays beam with spot on the sample varying from 30 to 600 nm, depending on the experimental station, allowing studies using cutting-edge X-rays-based techniques at nanoscale. One of the prerequisites for *in situ* experiments at the Carnaúba is to work with a small amount of samples (solid or liquid) and optimized sample holders, and as previously mentioned, microfluidic devices have risen as a great solution for complexes sample environments.

The transparency of the device to synchrotron X-rays, important to advance in experiments involving the techniques mentioned above, was tested acquiring 2D nano-XRF images on the central electrode (WE) by scanning the microfluidic device (Fig. 5a) about the nanobeam, over an area of 200 μm x 200 μm at steps of 5 μm , recording a full fluorescence spectrum (Fig. 5b) at each step (0.1 s per pixel). Thus, the elemental contrast was obtained by selecting the correspondent energy range. The experiments were carried out at the endstation Tarumã, at the Carnaúba beamline, where a Kirkpatrick-Baez (KB) mirrors focused the X-Ray beam at about 600 nm x 600 nm. The X-ray energy was selected by a four bounces crystal monochromator (Si(111)), adjusting the undulator phase.

Accordingly, the results are shown in Figure 5. An optical micrograph of the device oriented as mounted in the experimental station is shown in Figure 5 (b). The magenta rectangle indicates the region of interest (ROI), from where the nanofluorescence maps were acquired. Zoom over the ROI is presented in (c), where is possible to observe microfaults in the right and left side of the electrode.

To optimize the fluorescence counts (Fig 5b), the excitation energy was set to 13.657 keV for Au, and 9.748 keV for Cr. Chromium is the gold's adhesion layer (buried layer) with 30 nm of thickness. Prior to the experiments, the channel was filled with water. The nano-XRF maps for Au- L_{α} (Fig. 5e,f) over circles 1 and 2, which corresponds to the channel boundary in both sides of the electrode, clearly reveal the same feature observed by optical microscopy. Additionally, some very intense spots likely come from the lift-off process, which left a few flakes of gold/chromium on the electrode surface. Figure 5 (g-h) shows fluorescence maps obtained into the channels. As a main result, the maps clearly show a high contrast on the gold Au- L_{α} and for the Cr- K_{α} , despite the lower energy of the latter.

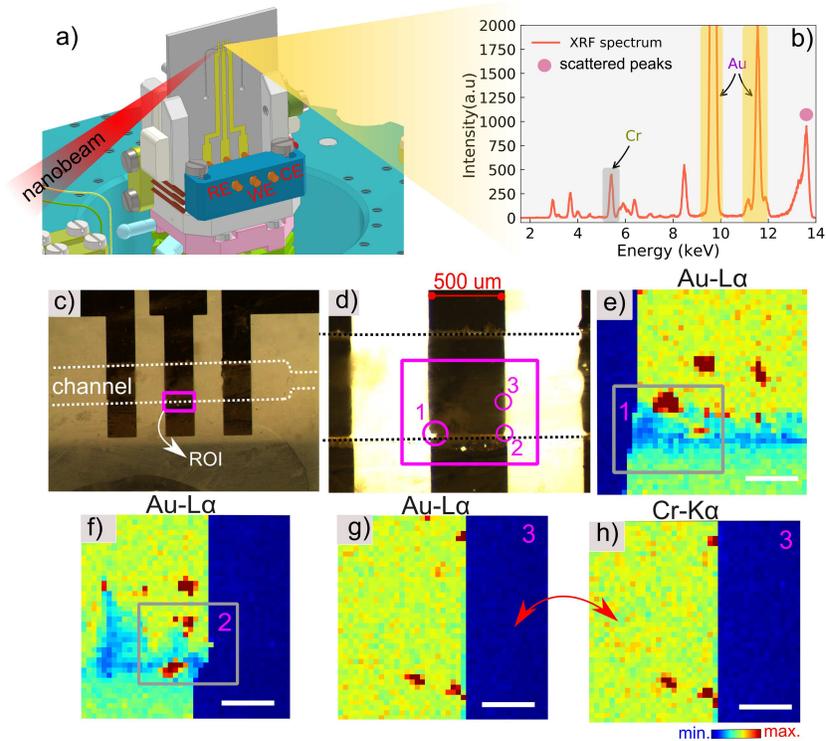


Figure 5. Nanofluorescence experiments on the microfluidic device filled with water. (a) Represents a 3D schema of the microfluidic sample holder. (b) Fluorescence spectrum collected at the central electrode. (c-d) Optical micrography of the channel and electrodes highlighting the ROI (magenta rectangle) scanned by a nanobeam of about 600 nm x 600 nm, from which the fluorescence spectrum was obtained. (e-f) Shows the Au- L_{α} (9713 eV) fluorescence maps about the circle 1 and 2 on the electrode border. (g-h) Au (L_{α}), and Cr- K_{α} (5.4 keV) maps on the channel previously filled with water.

Besides the characterization of the device by observing the fluorescence of gold and chromium, Nano-XRF was employed on a electrode covered with a silver/silver chloride (Ag/AgCl) film. Firstly, the Electrodeposited silver layer on a gold electrode was obtained by applying a negative potential at the desired gold electrode in a solution containing silver ions. Then, to form the AgCl, commercial sodium hypochlorite was pumped ($50 \mu\text{L}^{-1}$) through the channel. Figure 6 shows the integrated spectrum (top) obtained over an area of $50 \mu\text{m} \times 50 \mu\text{m}$ nm at steps of 500 nm (pixel size) as seen in the bottom panel. In addition, it can be observed minor peaks on the spectra associated with the glass substrate or contaminants. The 2D hyperspectral image (bottom) displays the AgAg/Cl film on the gold electrode obtained by selecting the energy corresponding to the Ag-L β (3.150 keV) as shown in the spectrum. The image reveals, with nanometric resolution, large islands of Ag on the electrode likely related to the mechanism of the nucleation process. The success in obtaining high fluorescence contrast, even at low energy (Ag-L β), relies on the polyester film transparency, once at this energy the attenuation is about 20 % (Supporting Information, Fig S2). It is worth noting that this modification of the gold electrode provides an excellent internal reference of potential, being extremely important for future applications of the microfluidic cell with electrochemical reactions with precisely controlled applied potential, for example *in situ* electrocatalysis experiments. In this imaging experiment the X-ray beam spot was about $200 \times 500 \text{ nm}^2$ ($E=12.759 \text{ keV}$). The results shown in this section reinforce the multifunctionality of the device presented in this work, and open a broad path for further experiments using nanoprobe beamlines.

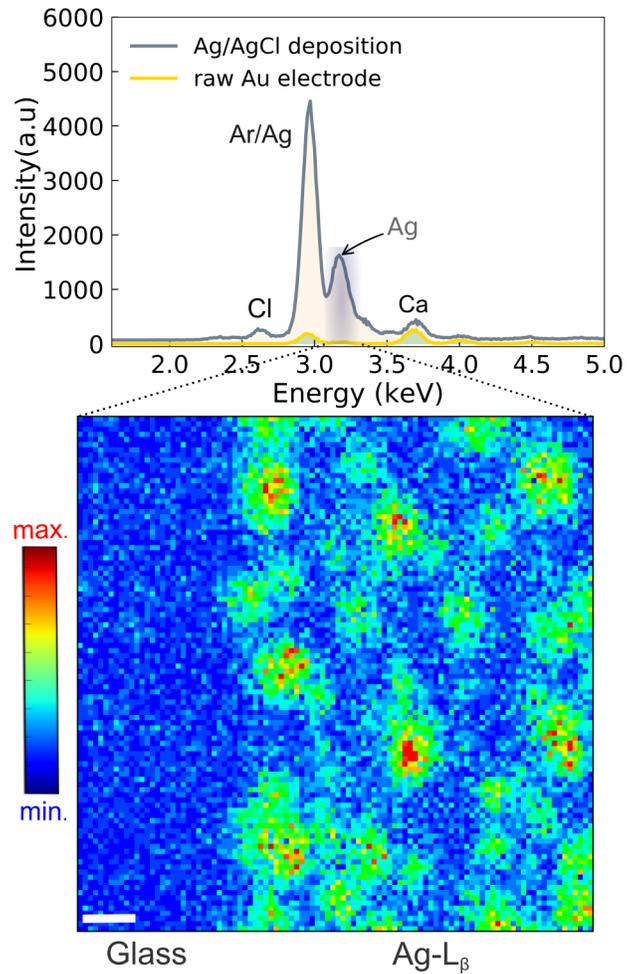


Figure 6. Preparation of Ag/AgCl film on a gold electrode (top) Fluorescence spectra comparing a raw gold electrode with a modified one. It is perceptible the presence of the Ag and Cl after the electrodeposition as well as spurious lines (Ar and Ca) (Bottom) Shows the 2D nanofluorescence map on the modified gold electrode revealing the sites where the Ag nucleated. The scale bar (horizontal) corresponds to 5 μm .

Conclusions

In conclusion, we described a successful new glass-based microfluidic device sealing process using a thin polymer, optimized for X-ray and/or optical measurements. The characterization reveals a versatile device, exhibiting remarkable chemical resistance, which can work as an electrochemical cell at room pressure but can also support high pressures when a volumetric flow ratio is needed. The device has demonstrated to be very efficient in reflection mode, showing high transparency in X-ray fluorescence experiments, proving to be suitable for *in situ* electrochemistry, and *in vivo* experiments. Thus, our results show that it will be possible to exploit, besides *ex situ* experiments, advanced multi-techniques available at synchrotron beamlines.

Materials and methods

The following chemical reactants were used during the different steps of the microfabrication process. A mix of sulfuric acid H_2SO_4 (Merck), water and hydrogen peroxide (H_2O_2 /Merck) was the standard glass cleaning solution, acetone was used for the lift-off of chromium and gold after sputtering deposition. Chromium mask etchant was purchased from TECHNIC. Photolithography process used AZ4620 photoresist and hexamethyldisilazane (HMDS) were purchased from Microchemicals GmbH (Ulm, Baden-Wurttemberg, Germany). A buffer solution of hydrofluoric acid (HF 49%, NH_4F 1.38 M and HCl 38%) and hydrofluoric acid (HF) 20% was purchased from Synth. Anhydrous potassium ferricyanide ($K_3[Fe(CN)_6]$) and trihydrate potassium ferrocyanide ($K_4[Fe(CN)_6]$) were purchased from J. T. Baker and Mallinckrodt, respectively. Potassium chloride (KCl) was purchased from Sigma-Aldrich. Hexane, n-propanol, BSA, KCl, and Bradford reagent were purchased from Sigma-Aldrich. The solutions were prepared in deionized water (purified in the Milli-Q system).

As sealing layer was used commercial polyester polymer called Mylar®. Metal deposition was performed by DC magnetron sputtering (AJA International inc.). The size end depth of the channels was checked using a profilometer (Dektak 150) with a vertical resolution of 1 Å. The channels morphology and the quality of the electrodes were characterized using a stereoscopic microscope (Zeiss Discovery V12), and Agilent 34410A multimeter was used to measure the sheet resistance of the gold electrodes. A potentiostat/galvanostat (Autolab 302N) was used in the electrochemical tests. Spectrometer Thermo Scientific Multiskan for protein adsorption quantification. Microfluidic experiments have been done using a syringe pump (New Era Pump Systems, Inc., Farmingdale, NY, USA).

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Conflict of interests

All the authors declare no conflict of interests

Contributions

All of the authors discussed all of the content and revised the manuscript. I. Neckel outlined, supervised the project and wrote the manuscript. L. de Castro, A. Gobbi, M. Piazzetta, R. Lima and R. de Oliveira, F. Calleo, V. Teixeira were mainly responsible for the microfabrication, chemical resistance tests, and fruitful discussions about the experiments. R. Vicente was responsible for the electrodeposition experiments. H. Tolentino and D. Galante allowed and supervised the experiments at the Carnaúba beamline.

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