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Microfabrication of a digital microfluidic platform integrated with an on-chip electrochemical cell

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Abstract
We report on an IC compatible microfabrication process proposed for a novel monolithic lab-on-a-chip (LOC) with an electrochemical cell embedded in an electrowetting on dielectric (EWOD) digital microfluidic device. The optimized process focused on the surface modification of Teflon, selective exposure for the electrochemical module and recovery of surface properties by one-step annealing at low temperature. The optimum modification time and annealing temperature were 20 s and 210 °C, respectively. The experimental results from atomic force microscope and contact angle (CA) measurement revealed the effects of surface roughness and apparent CA on the wettability for different etch times. The multifunctionality of droplet creation, merger and transportation in the EWOD microfluidic module and sensitive electrochemical detection for the redox probe were realized simultaneously. The proposed microfabrication process has many advantages of remarkable simplicity, prominent repeatability, low cost and compatibility with standard IC processes. It shows great promise for the microsystem of the microfluidic unit and detecting cell, and gives a brilliant conception for the future fabrication of monolithic LOC integrated with functional detection.

((Some figures may appear in colour only in the online journal)

1. Introduction
Lab-on-a-chip (LOC), which is also known as the micro total analysis system, can be defined as integrated micro electromechanical systems with biological and chemical detection that can carry out all stages of processes automatically. In the past decades, the research on LOC has grown rapidly in the field of microfluidics because of its portability, compactness, parallelization and low cost (Fair 2007, Lidija et al 2010, Lim et al 2010, Dutse and Yusof 2011, Jebrail et al 2012).

LOC is comprised of two basic modules denoted as ‘sensors’ and ‘microfluidics’ in terms of functionality. As to sensing technologies used in LOC research, optical related detection is the most popular and successful one (Vázquez et al 2012, Ibarlucea et al 2013). However, a complex optical system seems quite difficult to integrate into an LOC. Among other developed sensing techniques, microelectrode-based electrochemical detection is highly promising for microsystem applications due to its high performance detection, high integration on chip, simplicity, scalability and low cost (Huang et al 2008, Hwang et al 2009).

For the microfluidic device, the microanalysis system based on continuous microfluidics has been well developed. However, it faces great challenges in reliability, package efficiency and integration with circuitry (Ho et al 2011, Seemann et al 2012). The digital microfluidics, based on discrete droplet operation, can fundamentally eliminate these problems by implementing a complete set of programmable fluidic operations with a finite array of elemental components. Among various droplet-actuation forces, electrowetting on dielectric (EWOD) has emerged as an active research area.
in academia and industry, due to its advantages of speed, simplicity, compatibility with IC fabrication, reconfigurability and portability to perform on-chip chemical and biomedical protocols (Lidija et al. 2010, Pollack et al. 2011, Nelsona and Kim 2012).

Since both the electrochemical detection and the EWOD microfluidic device are based on similar components, i.e. electrodes, it implies that both modules have many compatible fabrication processes and their incorporation makes the integrated monolithic LOC possible. Up to now, some pioneers have developed preliminary work in the combination of EWOD devices and detection technology. For example, Dubois et al. 2006 reported on a powerful approach combining digital microfluidics and external two-electrode electrochemical detection for solution-phase synthesis. Additionally, Karuwan and coworkers (Karuwan et al. 2011) reported on another EWOD microfluidic device with an external three-electrode electrochemical sensing system for quantitative analysis of iodide. However, both reports were actually not integrated monolithic LOCs. In fact, there are few reports on efficient integration of EWOD microfluidic techniques and on-chip electrochemical analytical systems (Lidija et al. 2010).

The obstacles for realization of a fully-integrated LOC chip with these two modules can be addressed as follows. Firstly, the EWOD module must have a dielectric layer and a hydrophobic surface for droplet manipulation, while the electrochemical module needs a mirror-fresh electrode. Therefore, their layout, size and formation should be well designed when they are placed on the planar single chip (Chen et al 2012a). Secondly, in the crucial fabrication of the hydrophobic and hydrophilic surfaces simultaneously on a monolithic chip, it is difficult to deposit any films on the hydrophobic surface (e.g. fluoropolymers) which is essential in the EWOD module (Liu et al. 2010). Therefore, to wet the surface of the hydrophobic surface for other deposition (e.g. photosensit or selective patterning) on it and to dewet it to recover the hydrophobic properties used for the electrowetting function, would be the key issues.

Teflon® AF, a copolymer of polytetrafluoroethylene (PTFE) with 2,2-bis (trifluoroethylene)-4,5-difluoro-1,3-dioxole (Sabbatovskii et al. 2004), is a family of amorphous fluoropolymers with similar properties of superior electrical properties, good thermal stability and chemical resistance (Datta et al. 2003). Furthermore, it is distinct from other PTFEs by a large numbers of advantages, such as unmatched optical clarity, good creep resistance, high compressibility and controlled mechanical properties (Du Pont 2013). The Teflon® AF coatings or films can be prepared on various substrates (like metal, SiO2 and glass) by spin-coating (Fair 2007) or plasma-induced deposition (Baiyi et al. 2007). All these features have made Teflon® AF a very promising material in the electronics, microelectronics, optics and mechanical industries. Especially, with the development of EWOD digital microfluidics, Teflon® AF has been widely used in EWOD devices to provide hydrophobic surfaces due to its low surface energy (Baiyi et al. 2007, Paneru et al. 2010, Lee et al. 2011, Fan et al. 2011, Witters et al. 2011, Chen et al. 2012b, Kirby and Wheeler 2013, Huang et al. 2013). On the other hand, although Teflon has a low dielectric constant, it is found to be sufficient to act both as the insulator and hydrophobic layer for electrowetting when thicker coatings are carefully prepared without pinholes by appropriate temperature treatment (Seyrat and Hayes 2001). Some works have been reported on how to increase the surface energy of fluoropolymer—one of the simplest and developed methods is plasma-induced surface modification (Park et al. 2002, Inagaki et al. 2002). Besides this, plasma can be adopted to dry etching (reactive ion etch, RIE) for patterning Teflon in this work and the dewetting of Teflon can also be achieved by thermal annealing (Makohilo et al. 1998). Therefore, using low-temperature processes to fabricate such a monolithic LOC compatible with pre-CMOS IC process is worth studying as well.

In this paper, we report on an IC compatible microfabrication process of a novel monolithic LOC integrated with an electrochemical cell and an EWOD microfluidic platform. This work focuses on the critical processes: surface wetting of Teflon, selective patterning of the electrochemical cell and dewetting by low temperature annealing. The surface roughness and contact angle (CA) of Teflon dealing with different times and temperatures were investigated, as well as the potential mechanism of the apparent CA changing with etch time. The proposed microfabrication process was simple, repeatable, inexpensive and compatible. As a result, a multifunctional monolithic LOC was fabricated and demonstrated by on-chip droplet manipulation and rapid electrochemical detection for a wide concentration range of redox probe.

2. Material and methods

Teflon® AF1601solution (Du Pont, Grade 601S1-100-6, 6 wt%) was used as both the hydrophobic and the dielectric material in the EWOD device. Ferrocenemethanol (FcM, as the redox probe in the electrochemical detection) and phosphate buffered saline (PBS, 0.1 M, pH = 7.0) were provided by Sigma-Aldrich. All the chemicals were of analytical reagent grade. All experiments were carried out at room temperature.

Physical vapour deposition (PVD, ASC-4000-C4 Type L, ULVAC, Japan) was used to deposit Au/Ti metal layers for the microelectrodes. RIE-10NR (Samco International, Japan) was carried out to selectively etch and modify the surface properties of Teflon. CA measurements were estimated with the aid of a video-based optical CA measuring instrument (OCA 20, Data Physics Instruments GmbH) under ambient conditions. The probe liquid was deionized water. Using a Teflon blunt-ended needle attached to a syringe, a 2 μL water droplet was deposited on the surface and then the CA was measured immediately. Typically, CAs were measured three times on a 1 cm × 1 cm wafer piece. The average CA of each sample used in this experiment was measured at five randomly selected points. Atomic force microscope (AFM, Bruker MultiMod® 8) images were obtained in the tapping mode to characterize the surface morphology and roughness of the samples. The cyclic voltammetric experiment was performed by a CHI1030 electrochemical workstation (CH Instruments...
Inc., USA) in the integrated three-microelectrode-system electrochemical cell, which consists of gold interdigitated microelectrodes with three fingers as working electrodes (WEs), four fingers as counter electrodes (CEs) and a rectangular gold layer as reference electrode (RE).

2.1. Structure of monolithic LOC

Figure 1 shows the schematic diagram of a well-designed monolithic LOC platform. The common ITO electrode on the top plate is grounded. The bottom plate used as the working area consists of an EWOD microfluidic device and electrochemical microelectrodes. The size of a single square EWOD electrode and electrochemical cell is 1 mm × 1 mm and 480 μm × 540 μm, respectively. The hydrophilic electrochemical cell was embedded in the hydrophobic EWOD electrode with an area ratio of about 26%. The electrochemical cell is comprised of interdigitated fingers (360 μm × 50 μm) as WE or CE and a rectangular RE (340 μm × 50 μm). Herein, it is worth mentioning that gold material, instead of conventional Ag/AgCl, was used as the RE in order to simplify the fabrication process. This will prove to be practical for electrochemical detection in section 4.

2.2. General fabrication procedure of monolithic LOC

The optimized fabrication process flow of the monolithic LOC is illustrated in figure 2. Firstly, a Ti layer and an Au layer was successively sputtered on the pre-cleaned glass substrate by PVD (figure 2(a)). The thickness of the Ti/Au layers was about 300 nm. Secondely, both the electrodes of the EWOD microfluidic module and the electrochemical cell were patterned by one-step standard lithography and chemical etching (figure 2(b)). About 1.33 μm thickness of Teflon® film was spin-coated on the substrate and cured (figure 2(c)). The Teflon was then treated by RIE duration for 20 s with
100 W power at an argon flow rate of 30 sccm to wet the surface for subsequent photoresist coating (figure 2(d)). Figure 2(e) shows the selective etching by RIE for at least 120 s to expose the hydrophilic surface of the electrochemical cell. Finally, we immersed the device in acetone to remove residual photoresist, followed by low-temperature annealing at a constant temperature (210 °C) for 1 h to recover the surface properties of Teflon (figure 2(f)). For the critical process of wetting and dewetting, the effects of different modification times, selective etch rate and annealing temperatures were studied and optimized below.

2.3. Surface wetting of Teflon

Before wetting, each sample was prepared by coating Teflon on the glass substrate and cured. Since the effect of sputtering time on the surface topography and wettability of Teflon is essential for patterning and then selective exposure of the electrochemical cell, several groups by RIE with 100 W power at 30 sccm argon flow for different sputtering time (0–100 s) were experimented with. The average CA of each group was obtained from random five points. Each CA was measured three times. The potential mechanism for the change of CA after different etching times was discussed.

2.4. Dewetting of modified surface

The dewetting of RIE-modified Teflon was studied to characterize the recovery process by the CA measurement and AFM. Ten groups of different constant annealing temperatures (85–330 °C) for individual hours were carried out to study the surface recuperation of pre-modified Teflon. Before annealing, all the Teflon samples were treated by RIE for 20 s. As a comparison, considering the standard temperature process (Du Pont 2013), another ten groups of Teflon films were also tested. They were pre-modified for different sputtering times (0–100 s) and dewetted by three-standard-temperature technology (denoted as annealing at 110 °C for 5 min, then 245 °C for 5 min and finally at 330 °C for 10 min).

3. Results and discussion

3.1. Surface characterization of wetting Teflon

Figure 3(a) gives the typical curve of the CA and RIE time for wetting Teflon. Compared with the initial CA of untreated Teflon (about 122°), the apparent CA decreased by at least 10° during the first 5 s. Then it recovered to around 122° within the next 5 s and gradually increased and finally remained about 130° after 20 s. Besides, we also tried to test CA hysteresis for the all the modified Teflon coatings (insets of figure 3(a)). As a result, for all of them, the water droplets would not slide even when we turn the samples for 180°. This indicates that the modified-Teflon coatings treated by RIE possess good interfacial adhesive strength. Especially for 20 s (and over)-treated Teflon (inset (c)), when the surface is either facing up or turned upside down, water droplets on the surface always maintain the spherical shape, implying a superior interfacial adhesive strength with large apparent CAs.

Figure 3(b) shows that the etch rate of Teflon under the conditions used in this work is about 0.011 μm s⁻¹. Since the surface of coated Teflon before modification was not so smooth due to the inherently high viscosity, enough etching time (at least 140 s) should be guaranteed to remove the maximum thickness of Teflon coatings. For example, if we have firstly modified Teflon for 20 s (which would reduce the thickness to about 1.11 μm) before the selective etching process, the next etch time of 120 s is sufficient to remove the modified-Teflon coatings covered on the electrochemical cell.
roughness and heterogeneity are known to affect the apparent CA and CA hysteresis (Saeki et al. 2001, McHale et al. 2004), it indicates that RIE treatment changes both the surface chemical bonds and surface morphology of Teflon.

Herein, we would like to discuss the possible mechanism of the change of CA with sputtering time for the modified Teflon. From figures 3, 4 and table 1, the different apparent CAs after RIE treatment were attributed to the complex effects of the surface defluorination, cross-linking reaction (Sabbatovskii et al. 2004, Tasker et al. 1994) and the transition of the partially impregnating Cassie state (Cassie and Baxter 1944, Ran et al. 2008, Whyman and Bormashenko 2011, Zhou et al. 2012). The first two were induced by the radical species, while the last was generated and controlled by surface structure. In the first 5 s, the hydrophobic bonds on the Teflon surface were broken and unsaturated fluorinated chains including dangling bonds, –CF=CF2 and other active groups (Sabbatovskii et al. 2004) were generated and tended to arise the cross-linking reaction within them at the same time. Thus Teflon shows better hydrophilicity. Meanwhile, since the root-mean-square of roughness ($R_q$) is less than 1.4 nm, there is not enough time to form the hydrophobic nanorod on the surface of Teflon. Therefore, the apparent CAs decreased mainly due to the initial defluorination. When the samples were treated for next 15 s, the surface roughness gradually increased, and the effect of initial defluorination and the cross-linking reaction tended to become balanced, as well as limited by the saturated or near-saturated number of radical species on the surface. As a result, the apparent CAs gradually recovered to $122^\circ$ due to the common transition of the surface wettability to the partially impregnating Cassie state (Koishi et al. 2009).

The degree of impregnating Cassie state depends on the surface roughness, i.e. the apparent CA increased with longer RIE time in this stage. As the time went on over 20 s, the modified Teflon had more complex surfaces with additional large-scale roughness and further decomposition of chemical bonds.
Table 2. Relationship of apparent CA and surface roughness with annealing temperature.

<table>
<thead>
<tr>
<th>Group</th>
<th>Annealing temp. (°C)</th>
<th>Contact angle (°)</th>
<th>Rq (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>85</td>
<td>133 ± 0.60</td>
<td>3.15 ± 0.43</td>
</tr>
<tr>
<td>2</td>
<td>110</td>
<td>133 ± 1.06</td>
<td>3.09 ± 0.30</td>
</tr>
<tr>
<td>3</td>
<td>135</td>
<td>115 ± 1.20</td>
<td>0.719 ± 0.048</td>
</tr>
<tr>
<td>4</td>
<td>160</td>
<td>114 ± 0.85</td>
<td>0.706 ± 0.027</td>
</tr>
<tr>
<td>5</td>
<td>185</td>
<td>115 ± 0.72</td>
<td>0.708 ± 0.025</td>
</tr>
<tr>
<td>6</td>
<td>210</td>
<td>123 ± 0.35</td>
<td>0.613 ± 0.030</td>
</tr>
<tr>
<td>7</td>
<td>245</td>
<td>122 ± 0.45</td>
<td>0.530 ± 0.038</td>
</tr>
<tr>
<td>8</td>
<td>270</td>
<td>123 ± 0.60</td>
<td>0.547 ± 0.034</td>
</tr>
<tr>
<td>9</td>
<td>295</td>
<td>124 ± 0.85</td>
<td>0.558 ± 0.020</td>
</tr>
<tr>
<td>10</td>
<td>330</td>
<td>123 ± 0.28</td>
<td>0.549 ± 0.035</td>
</tr>
</tbody>
</table>

(denoted as secondary defluorination), but yet a large apparent CA (about 131°) as well as superior interfacial adhesive strength (figure 3(a)). This might be because the surface of the partially Cassie state further transforms to another unusual transition state, i.e. the so-called gecko-like state (Wang and Jiang 2007) or petal-like effect (Feng et al. 2008). In the gecko-like state, some air was sealed in the cavities created by surface roughness, which is different from the Cassie state (Cassie and Baxter 1944) where the trapped air pockets are connected to the atmosphere (open state). For a static water droplet on the surface, the trapped air will induce a large CA; while the adhesive forces between the water droplet and the surface arise from Van der Waals interactions, similar to the Cassie state. However, once the droplet was drawn (like the dynamic process during high-speed spin-coating of photoresist), the volume of the sealed air in the plasma-induced cavities would change and thus produced negative pressure. This may create a strong ‘adhesive’ force. Therefore, besides large apparent CAs, 20 s (or over)-treated Teflon possessed superior and stable interfacial adhesion induced by the synthetic effects of secondary defluorination and the gecko-like state.

As we all know, compared with apparent CA, CA hysteresis is a more important parameter to characterize the wettability of structured surfaces. Larger CA hysteresis means better interfacial adhesion. During the experiments, we tried to spin-coat photoresist on 3–60 s-treated Teflon coatings. It is found that the chip-to-chip repeatability of uniform photoresist film was worse on the 3–10 s-treated Teflon than on 20–60 s-treated ones. This indicates that the surfaces of 3–10 s-treated Teflon were unstable or metastable after short-time etching.

From the above, considering more stable and superior interfacial adhesion strength, the optimized wetting time of 20 s was appropriate and sufficient to use in the work. Thus, in our later experiments, 120 s was used as the etch time to expose the Teflon which covered the electrochemical cell.

3.2. Dewetting of modified Teflon

Before annealing, all the Teflon samples were treated for 20 s and the average CA and roughness after RIE were about 133° and 3 nm, respectively. The annealing time was 1 h for all samples. Table 2 shows the relationship of CA and surface roughness with annealing temperature in the range from 85 to 330 °C. Figure 5 gives the typical AFM images for different annealing temperatures. Obviously, the extent of recovery in both CA and roughness was different for different temperatures. At a relatively low temperature (85–110 °C) which is much lower than the glass temperature of Teflon, both the apparent CAs and surface roughness (figure 5(a)) were almost unchanged. This is because the modified-Teflon did not soften and thus its hydrophobicity did not restore (figure 5(a)). When treated at an increased temperature (135–185 °C), the surface roughness of the samples decreased to about 0.7 nm, indicating that most of the large-scale roughness hydrophobic structures fade away (figures 5(b), (c)); however, there might still be numbers of radial species existing on the surface. As a result, the CAs decreased to around 115°, which was similar to the results of inset (a) of figures 3(a) and 4(b).

For higher annealing temperatures (210–330 °C), both the apparent CAs and surface roughness recovered to the initial value just like that of the unmodified Teflon, mainly due to the rebuilt hydrophobic chemical bonds and smooth surface structure (figures 5(d)–(f)). This indicates that the procedure of wetting by RIE and dewetting by appropriate annealing is a quasi-reversible process.

In addition, considering the standard temperature process (Du Pont 2013), another ten groups of Teflon films pre-modified for different sputtering times (0–100 s) and then annealed by three-standard-temperature technology were also experimented. Consequently, no matter how long the RIE time was, the apparent CAs and surface roughness could always recover to the original state by using three-standard-temperature technology, which is consistent with the reference (Makohliso et al. 1998). Meanwhile, the surface roughness after annealing by three-standard-temperature technology was about 0.55 nm, which was similar to those in figures 5(d)–(f). Such results indicate that recovering the surface properties of modified Teflon at the temperature range of 210–330 °C is effective and feasible, comparable to the effects of three-standard-temperature technology. Among this wide and effective temperature range, a relatively low temperature (i.e. 210 °C) is obviously sufficient to recover the surface properties. Therefore, annealing at 210 °C for 1 h can be selected as the optimized annealing approaches to recover the properties of modified-Teflon.

4. Functional application

Figure 6 is the monolithic LOC with integration of the EWOD microfluidic module and the electrochemical cell prepared by the above optimized fabrication process. The platform consists of 19 EWOD electrodes with four reservoirs and three electrochemical electrodes (WE, CE and RE). The inset of figure 6 is the optical microscope of embedded interdigitated fingers as the three-microelectrode-system electrochemical cell.

Before verifying the function of the EWOD microfluidic device, the common electrode on the top plate was connected to the ground potential. The reservoir and driving electrodes on the bottom plate were connected to the common terminals of single pole double throw relays. The potential of the electrodes was switched between high potential and zero.
Figure 5. Typical AFM images of 20 s-treated Teflon samples after annealing at (a) 85 °C, (b) 135 °C, (c) 185 °C, (d) 210 °C, (e) 270 °C and (f) 330 °C for 1 h, respectively. The display range is −14.0–14.0 nm (a) and −6.0–6.0 nm (b)–(f), respectively. The scanning area is 2 μm × 2 μm.

Figure 6. Picture of EWOD device embedded with the electrochemical module.

potential through the relays. To get a high potential signal, a 1 kHz sine ac signal was generated from the function generator and then amplified to about 60 Vrms through an amplifier. The digital output signals of a data acquisition device programmed by the LABVIEW software were used to switch the relays to actuate the EWOD electrodes. Figure 7 shows the whole EWOD microfluidic procedure with embedded electrochemical cell. Firstly, two droplets were pulled out of the top and bottom reservoir, respectively (figure 7(a)). After mixing them up in the crossed electrodes (figure 7(b)), the larger droplet was transported to the electrochemical sensing cell (figures 7(c), (d)) for detection and finally transported to the waste reservoir (figure 7(e)). After detecting for each experiment the chip, and especially the electrochemical cell, was thoroughly washed by moving droplets from the clean reservoir to the waste reservoir (figures 7(f)–(h)) several times.

The FcM/FcM redox couple is close to an ideal quasi-reversible system in electrochemical detection. In the electrochemical sensing detection, the typical cyclic voltammetric responses of targeted droplet (about 0.4 μL) containing 0.1 M PBS solution (pH = 7.0) and 2.0 mM FcM were obtained at different scan rates in figure 8(a). Obviously, the redox reaction of the FcM probe gives roughly symmetric anodic and cathodic peaks at relatively slow scan rates. When the scan rate increases, the redox potentials of FcM shift slightly and the peak-to-peak potential (ΔE_p) also increases ranging from 78 mV s⁻¹ to 159 mV s⁻¹. The redox peak current increases linearly with v¹ (inset of figure 8(a)), as shown in following equations (1) and (2):

\[
\Delta E_p = \text{constant} \cdot \log \frac{v}{n} \\
I_p = \frac{nF\Delta E_p}{2RT} \sqrt{v}
\]
Figure 7. EWOD microfluidic function including two parts: (1) merging the droplet: pulling two droplets out (a), mixing (b), transport to the electrochemical cell for electrochemical detection (c), (d) and finally moving to the waste reservoir (e); (2) cleaning the chip: repeat the step of transporting a droplet from the clean reservoir to waste reservoir (f)–(h) several times. All the droplets are labelled by the yellow arrows for better visual effect. The dotted arrow (red) shows the movement direction of the droplet during different phases.

\[ I_{pa} (\mu A) = 0.108 + 0.056 \sqrt{v}, \quad R^2 = 0.9925 \]  \hspace{1cm} (1)

\[ I_{pc} (\mu A) = -0.110 - 0.041 \sqrt{v}, \quad R^2 = 0.9978. \]  \hspace{1cm} (2)

This demonstrates that the redox reaction of FeM at the working microelectrode is a quasi-reversible diffusion-controlled electrochemical process at slow scan rates.
Since differential pulse voltammetry (DPV) has a much higher current sensitivity and better resolution than cyclic voltammetry, it was used in the determination of FcM concentrations and the estimation of the low limit of detection. Figure 8(b) shows the experimental curves and relevant calibration curves for a wide concentration range of FcM in the range from 3.0 μM to 1.0 mM. From the DPV curves (see the bottom inset), it can be found that the peak current increased with increasing concentration of FcM. In addition, the calibration plot at relative low concentration (3.0–25.0 μM) is obviously steeper than that at high concentration (from 50.0 μM to 1.0 mM). This indicates that the electrochemical system is more sensitive to FcM at lower concentrations. The top inset of figure 8(b) shows that the calibration curve is linear over the range from 3.0 to 25.0 μM FcM with the sensitivity of about 3.31 nA μM⁻¹ ($R^2 = 0.9993$) and the estimated detection limit of about 0.53 μM (with a signal/noise ratio of 3). For the higher concentrations (50.0 μM–1.0 mM), the DPV currents are proportional to the FcM concentrations with a sensitivity of 0.73 nA μM⁻¹ ($R^2 = 0.9897$).

The stability and reproducibility of the electrochemical module were also investigated. The current responses for five independent measurements towards 2.0 mM FcM remained almost constant and the relative standard deviation (RSD) was less than 2.5%. The chip-to-chip reproducibility was determined in the presence of 2.0 mM FcM with five different electrodes, which yielded a RSD less than 6.7%. When stored at ambient environment for one day, the current signals decreased by 2.8%. After 7 days, 94.9% of the initial current was obtained. These data indicate the good stability and reliable reproducibility of the proposed fabrication for the electrochemical module. We also found that although all three electrodes (WE, RE and CE) are formed from the same material (gold), such a system did not result in a significant drift in potential and current in this experiment.

5. Conclusions

This study demonstrated a model system for the microfabrication of a novel monolithic LOC integrated with an electrochemical microcell and the EWOD microfluidic device. In order to satisfy the multifunction of electrochemical microelectrodes and EWOD microfluidic, the crucial processes of surface wetting of Teflon, selective etching by RIE and one-step annealing at low temperature were investigated. The optimum modification time and annealing temperature were 20 s and 210 °C, respectively. The experimental results from AFM and CA measurement revealed the effects of surface roughness and apparent CA on the wettability. The reported microfabrication process presented many advantages of remarkable simplicity, prominent repeatability, low cost and compatibility with standard IC processes. Based on the optimized process, the proposed monolithic LOC could not only create, merge and transport droplets, but show a sensitive, rapid and wide-range detection for FcM probe. The electrochemical detection of FcM showed two linear ranges. The sensor displayed a linear range of 3.0–25.0 μM FcM with a sensitivity of 3.31 nA μM⁻¹ ($R^2 = 0.9993$) and a linear range of 50.0 μM–1.0 mM FcM with a sensitivity of 0.73 nA μM⁻¹ ($R^2 = 0.9897$). The detection limit was 0.53 μM with a signal/noise ratio of 3. The proposed process shows great promise for the integration of the LOC with IC chips in the future. It deserves further study for practical bioanalysis application in our next work.

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