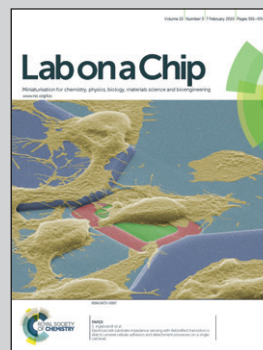


Featuring work from the Bio-Optofluidics Laboratory of Prof. Dachao Li at Tianjin University and the Additive Microfabrication Laboratory of Prof. Norman C. Tien at The University of Hong Kong, China.

Title: Inkjet-printed microelectrodes on PDMS as biosensors for functionalized microfluidic systems

A three-electrode electrochemical sensor was fabricated in a microchannel of PDMS towards functionalized microfluidics. The modification of PDMS by MPTMS improved the surface wettability and metal adhesion of PDMS, and the multilevel matrix deposition method further overcame the coalescence of adjacent droplets.

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Inkjet-printed microelectrodes on PDMS as biosensors for functionalized microfluidic systems†

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Microfluidic systems based on polydimethylsiloxane (PDMS) have gained popularity in recent years. However, microelectrode patterning on PDMS to form biosensors in microchannels remains a worldwide technical issue due to the hydrophobicity of PDMS and its weak adhesion to metals. In this study, an additive technique using inkjet-printed silver nanoparticles to form microelectrodes on PDMS is presented. (3-Mercaptopropyl)trimethoxysilane (MPTMS) was used to modify the surface of PDMS to improve its surface wettability and its adhesion to silver. The modified surface of PDMS is rendered relatively hydrophilic, which is beneficial for the silver droplets to disperse and thus effectively avoids the coalescence of adjacent droplets. Additionally, a multilevel matrix deposition (MMD) method is used to further avoid the coalescence and yield a homogeneous pattern on the MPTMS-modified PDMS. A surface wettability comparison and an adhesion test were conducted. The resulting silver pattern exhibited good uniformity, conductivity and excellent adhesion to PDMS. A three-electrode electrochemical biosensor was fabricated successfully using this method and sealed in a PDMS microchannel, forming a lab-on-a-chip glucose biosensing system.

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Introduction

PDMS-based microfluidic systems have been used extensively in the control and manipulation of different liquids^{1–6} because of their remarkable biocompatibility and easy fabrication. Electrochemical detection in microfluidics is on the rise, especially in cases where the analyte is present in a sufficiently high concentration to generate a relatively high voltage or current signal, thus allowing a comparatively simple and robust read-out. To fabricate these devices in microfluidics for electrochemical sensing, the ability to form robust microelectrodes on PDMS is supremely necessary, because the stability and durability of the microelectrodes play an important role in the performance of the system. However, microelectrode patterning on PDMS has been a long-standing worldwide technical difficulty because of the hydrophobicity of PDMS and its poor adhesion to metals.⁷

In past decades, many fabrication techniques of metal patterning on PDMS have been explored, such as chemical reduction,^{8–11} evaporation or sputtering,¹² screen printing,^{13–15}

lift-off,^{16,17} pattern transfer,^{18–21} soft lithography,^{22,23} *etc.* Chemical reduction usually needs to fabricate channels for aqueous solutions in advance, and the continuity of the metal pattern is always unsatisfying. Physical vapor deposition techniques such as evaporation or sputtering, often using lift-off, require vacuum conditions and need multiple process steps, making them complex and time-consuming. The pattern transfer procedure usually requires cleanroom working conditions, and it is nondirective. Lift-off is a popular method but requires an intermediate layer to promote adhesion and match the thermal expansion coefficient,¹⁵ while most intermediate layers are not readily removable in the lift-off process. Screen-printed electrodes are very thick, approximately 8–10 μm, meaning it is almost impossible to bond a cover lid across such a structure without having leakage around the edges. Inkjet printing, which is emerging these years, is an alternative microfabrication technique, enabling the possibility of microelectrode patterning on PDMS. It is an additive technology, meaning only the areas needed to be conductive are coated, not requiring any stamps or masks. However, there are still two obstacles to be overcome when fabricating microelectrodes on PDMS using inkjet printing. One is the inherent hydrophobicity of PDMS, meaning it is difficult for the ink droplets to disperse on its surface, which will cause a long evaporation time for the solvent and the corresponding coalescence of adjacent droplets. Besides, the poor adhesion between PDMS and metals also

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inhibits a robust microelectrode formation. If not strongly bonded to the surface of PDMS, the microelectrodes will be easily lifted off by the applied voltage, pressure or liquid immersion.²⁴

In this study, an additive method for microelectrode patterning on PDMS using inkjet-printed silver with a chemical reagent as the wettability and adhesion promoter was presented. (3-Mercaptopropyl)trimethoxysilane (MPTMS), a coupling agent of noble metal to Si-based materials, was used to modify the PDMS. The surface modification improves the surface wettability of PDMS, which decreases the evaporation time of the silver droplet solvent and effectively avoids the coalescence of adjacent droplets. Besides, the modification also provides a tight bonding between the silver patterns and PDMS. Additionally, a multilevel matrix deposition (MMD) method, which prints the sub-images of the as-designed pattern and sequentially overlaps them,²⁵ is used to further overcome the coalescence of adjacent droplets on MPTMS-modified PDMS. The resulting printed silver patterns exhibited good compactness, conductivity and excellent adhesion to PDMS. The work here was used to fabricate a three-electrode electrochemical sensor on PDMS successfully and the sensor was sealed in the microchannel of a microfluidic system, manufacturing an integrated lab-on-a-chip biosensing system.

Methods of silver patterning on PDMS by inkjet printing

Wettability and adhesion promotion of PDMS using MPTMS modification

As a stretchable and flexible material, PDMS also has an intrinsic disadvantage. The surface of PDMS is inherently hydrophobic, on which the contact angle of water is approximately 120°,^{26–30} as shown in Fig. S1(a) (“S” represents the figures in the ESI†), and Fig. S1(b) shows a hydrophilic surface for contrast. It is difficult for liquid droplets to disperse. Consequently, it would take a long time for the solvent of the silver ink to evaporate on this hydrophobic surface, which would easily lead to the coalescence^{31,32} of adjacent wet droplets caused by surface tension.

Additionally, the inherently poor adhesion^{18–21} between PDMS and noble metals also hindered robust pattern formation.

If not strongly bonded to the surface of PDMS, the microelectrodes will be easily lifted off by the applied voltage, pressure or liquid immersion.

Here, MPTMS was used to modify PDMS to promote the surface wettability and adhesion to printed silver nanoparticle droplets. It is a silane-based coupling agent of Si-based materials and noble metals, which had been used extensively to functionalize planar or cylinder receptors to absorb gold or silver nanospheres.³³ It was also used as an adhesion promoter to implement a pattern-transfer procedure,¹⁹ in which the gold film was deposited onto the silicon wafer preliminarily and modified by the MPTMS, and then transferred to the PDMS using lift-off.

The MPTMS modification of PDMS could incubate hydrophilic groups (–OH) to the PDMS to promote the surface wettability, which is beneficial for the silver droplets to disperse and thus effectively avoids the coalescence of adjacent droplets. Additionally, it also incubates coupling groups (–SH) to promote the adhesion of metals to PDMS.

Inkjet printing silver deposition

The silver patterns were fabricated by the Dimatix inkjet printer (FujiFilm, 2831 series, shown in Fig. S2(a)†). The print carriage (shown in Fig. S2(b)†) and platen can move at the left–right direction and internal–external direction, respectively. With the two parts moving simultaneously, the silver ink droplets ejected by the nozzle are capable of fabricating two-dimensional patterns only where is as-designed and do not require any stamps or masks. The fiducial camera is used for observing the pattern state on different substrates. The cartridge holder can rotate so that the drop spacing (centre-to-centre distance) of the two adjacent silver ink droplets can be adjusted. While printing, the drop spacing is decided by the as-designed pattern.

The whole system is controlled using PC-based software. While initializing the printing parameters, the drop watcher can be used at the same time to observe so that the best state of droplets could be obtained by optimizing the jetting parameters. The pattern can be designed by some commonly used computer-aided drafting (CAD) or image-editing tools. These design tools are then converted to a printer-identified image where the geometry is defined as a matrix of pixels. The inkjet printer then operates by ejecting ink corresponding to the image in an overlapping fashion to obtain a coherent pattern.

The size of the same droplet was different on varied substrates due to the surface properties. Thus, to obtain a uniform pattern, the drop spacing should be set smaller than the real drop size on the substrate to realize the overlapping fashion. However, droplet coalescence needs to be overcome while inkjet printing. Guijun Li *et al.* reported an interlacing method to solve this problem on some relatively hydrophilic substrates, such as polyimide, polyethylene terephthalate (PET) and glass.³⁴ To obtain a homogeneous pattern on the extremely hydrophobic PDMS surface, a MMD method was used here to further avoid the coalescence of adjacent wet droplets on the MPTMS-modified PDMS. Assuming that printing starts from the upper left corner and ends at the lower right, the first matrix (yellow in Fig. 1) is printed, and after the droplets in this matrix were dried, deposition continues sequentially in a series (light blue, red, pink, purple, white, green, blue and black matrix).

The MMD method prints the next matrix before the droplets in the previous matrix were dried, thus further overcoming the coalescence of adjacent liquid droplets caused by surface tension and can be used to process patterns of any geometries, but not only applicable for rectangles or squares.

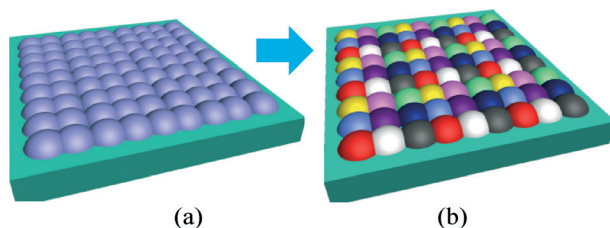


Fig. 1 Discontinuous overlapping printing to overcome the coalescence of adjacent droplets.

Fabrication procedures of silver patterns on PDMS by inkjet printing

Chemical preparation

The chemicals included PDMS (Sylgard 184, Dow Corning, USA), MPTMS (95% purity, Sigma-Aldrich, USA), 100 mm SSP silicon wafers (University Wafers, USA), ethanol (99.5% purity, Sigma-Aldrich), and hydrochloric acid (HCl) (0.1 M, Acros Organics, USA). A 10:1 (by weight) mixture of PDMS monomer/curing agent was poured onto the silicon wafer substrate contained in a Petri dish and was degassed for 40 minutes in a vacuum chamber, and then the mixture (thickness about 800 μm) was cured in an oven at 70 $^{\circ}\text{C}$ for 2 hours. After that, the PDMS was peeled off the silicon wafer and prepared for surface modification.

The MPTMS modification of PDMS

The modification of PDMS for improving its surface wettability and adhesion to silver is shown in Fig. 2.

0.5 mL of MPTMS was added to 100 mL of ethanol to obtain a 1:200 (v/v) solution. The PDMS pieces were then immersed into this solution for one hour, followed by rinsing with ethanol and drying with a compressed air stream. For hydrolysis and condensation, the PDMS samples were immersed into 0.1 M HCl for one hour, followed by rinsing with DI water and drying with an air stream. For contrast, the modification procedures without MPTMS (ethanol and HCl only) were also applied to some other PDMS samples (here we call it contrast PDMS). Then, three groups (untreated, contrast, MPTMS modified) of PDMS samples were prepared as the substrates for inkjet printing.

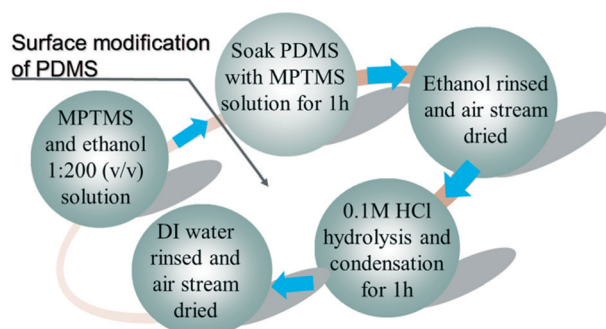


Fig. 2 MPTMS modification process for PDMS.

Silver patterning on untreated, contrast and MPTMS-modified PDMS

To move it freely, the PDMS substrate was put onto a glass wafer and the glass wafer was fastened onto the platen of the inkjet printer. First, three single lines were printed on untreated PDMS; Fig. 3 shows the droplet state on pure PDMS when the drop spacing was set to 50 μm , 40 μm , 38 μm , and 30 μm . As observed in Fig. 3, the drop size on untreated PDMS was about 20 μm . When the drop spacing was set to 50 μm and 40 μm , the three lines were still composed of single droplets, but when the drop spacing decreased to 38 μm , several single droplets had congregated with each other. Furthermore, the three lines had merged to some bigger drops when the drop spacing was set to 30 μm . The result here also confirmed the easy coalescence of silver droplets on a highly hydrophobic surface.

Next, several single lines with a drop spacing of 20 μm were printed onto the PDMS of the contrast group utilizing the MMD method, as shown in Fig. 4 (under the microscope). Here, three matrixes were printed along the vertical direction at a time interval of five minutes.

It can be clearly observed that the drop size (shown in Fig. 4, Step 1) was about 23 μm , which was slightly larger than that on the untreated surface, meaning that the surface

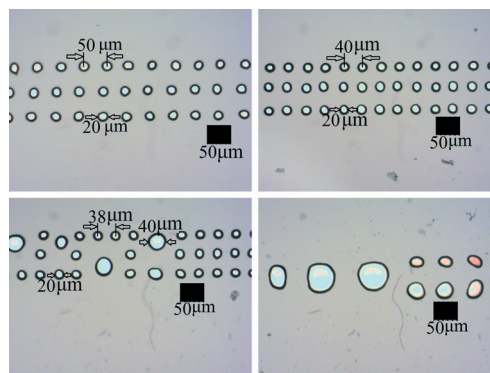


Fig. 3 Droplet state on untreated PDMS illustrating droplet coalescence when the drop spacing does not exceed the drop diameter (scale bar, 50 μm).

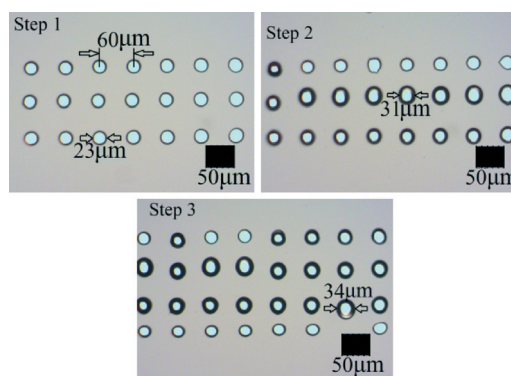


Fig. 4 MMD method printed single lines on PDMS of the contrast group (scale bar, 50 μm).

of PDMS was still hydrophobic. It could be concluded by comparing Step 2 and Step 3 with Step 1 that the coalescence of two adjacent droplets still existed on the PDMS of the contrast group (without MPTMS). Theoretically, the droplets should overlap to form a line at the vertical direction after three steps, but the result shown in Step 3 diverged with this anticipation. It confirmed that the surface wettability of PDMS without MPTMS modification has no significant change and this PDMS surface of the contrast group was inappropriate for a continuous pattern formation even using the MMD method.

Finally, a 2 mm × 2 mm pad was printed onto the MPTMS-modified PDMS using the MMD method at a time interval of 1 minute. The result is shown in Fig. 5 (under the microscope, just showing part of the pad to illustrate the detail of the MMD method). The background difference between Fig. 5 and 4 (Fig. 3) is due to parameter variation (exposure, white balance) of the CCD camera by multiple researchers during image collection.

From Step 1 in Fig. 5, it could be seen that the drop size on MPTMS-modified PDMS was approximately 40 μm, nearly twice the size on untreated PDMS. It could also be seen under the fiducial camera that the silver droplets dried quickly on the MPTMS-treated PDMS, approximately 6–8 seconds. After Step 3, the lines formed at the vertical direction, indicating that the surface wettability of the PDMS had obviously changed from hydrophobic to hydrophilic. Here, the contact angle measurement of water was also performed, and the contact angle of water on MPTMS-treated PDMS was approximately 60–70°, which also confirmed that the surface modification improved the surface wettability of PDMS. Under the microscope, the pattern exhibited good compactness and continuity, as shown in Fig. S3† (due to the vision limitation of CCD, the 2 mm × 2 mm pad was divided into 4 parts).

Adhesion test of silver patterns on PDMS

To test the adhesion of the silver patterns on the MPTMS-modified PDMS, the soaking test (water for 2 hours), blowing

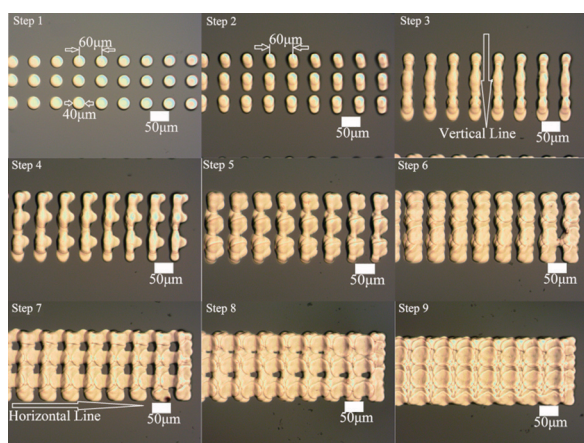


Fig. 5 MMD method printed silver patterns on MPTMS-modified PDMS illustrating the nine-step process (scale bar, 50 μm).

test (compressed air, 5 minutes) and ultrasonication test (in water for 10 minutes) were sequentially conducted. For contrast, the same test experiment was also applied to the silver patterns on O₂ plasma-treated PDMS. The result was as follows.

Soaking test

The pattern was immersed into water for 2 hours. Then it was observed under the microscope, and the result is shown in Fig. S4†. Though immersed in water for 2 hours, the pattern has no apparent change and exhibited a good stickiness on the MPTMS-modified PDMS. Additionally, the soaking test was also performed using the aqueous solution of glucose, and the soaking time was extended to one day, but the pattern was still tightly attached to the surface of PDMS.

Blowing test

To simulate the subatmospheric pressure in the microchannel of the PDMS-based microfluidic system, the pattern was blown with an air stream of 0.5 MPa (air pump, DG-10, KAMADA, Japan). After blowing for 5 minutes, the pattern was observed (shown in Fig. S5†). From Fig. S5†, the edge of the pattern exhibited good robustness and no debris fell off the PDMS.

Ultrasonication test

After that, the PDMS substrate with the silver pattern was placed into a water beaker and ultrasonicated (VGT-2000 ultrasonicator) for 10 minutes. The result is shown in Fig. S6†. Even under ultrasonication, the pattern still exhibited excellent adhesion to the PDMS substrate, which was sufficient to meet the requirement of fabricating a robust micro-electrode in the microchannel of PDMS microfluidic systems.

Contrast test of patterns on O₂ plasma-treated PDMS

For contrast, the same adhesion tests were also applied to patterns on oxygen plasma-treated PDMS, and the result is shown in Fig. S7†. Inferred from Fig. S7†, the plasma treated PDMS had more hydrophilic surface properties and was easier for the silver droplets to form coherent patterns. However, the adhesion between the plasma-treated PDMS and the printed silver was extremely weak, even failing the soaking test.

Conductivity test of electrodes fabricated using the MMD method

The conductivity of the electrode is a key factor to the electrochemical biosensing system for obtaining a reliable electrical signal. To test the conductivity of the electrode and confirm that it can be used for current measurement, a two-probe device (shown in Fig. S8†) and a digital multimeter (Tektronix DMM 4050) were used to measure the resistance of the fabricated electrode. Here, five different samples were measured, and the result is summarized in Table 1.

Table 1 Electrode resistance variability for five samples

| Sample no. | 1 | 2 | 3 | 4 | 5 | Average |
|-------------------|-----|------|----|-----|------|------------|
| Resistance (ohms) | 9.2 | 11.6 | 13 | 8.9 | 10.4 | 10.6 ± 1.7 |

From Table 1, it can be observed that the conductivity of the electrodes was good and the resistance was relatively low, which is beneficial for obtaining a comparatively larger current when applying a fixed voltage. Thus, the electrode fabricated here was very suitable for manufacturing electrochemical devices and for applications in microfluidic sensing. The sheet resistance and the resistivity of the silver patterns will be further investigated when fabricating other biosensors.

Application of inkjet printing towards fabricating integrated electrochemical sensors

Fig. 6 shows the schematic diagram of the three-electrode electrochemical glucose sensor. From left to right, the three electrodes are reference, working and counter electrode, respectively. The reference electrode was chloridized, obtaining a Ag/AgCl electrode, which provided a reference potential for the working electrode. Glucose oxidase was immobilized onto the working electrode so that the current produced by the oxidase-catalysed electrochemical reaction could be detected between the working and the counter electrodes.

To protect the other two electrodes, the reference electrode was first printed using the MMD method. After that, the substrate was removed from the printer platen and the reference electrode was chloridized. It could be seen that the color of the chloridized electrode changed from white to black. Then the substrate was put back to the platen. After alignment, the other two electrodes were printed continuously and the resulting three-electrode sensor is shown in Fig. 7.

In our previous work, a microfluidic system based on PDMS was fabricated,³ which was composed of a five-layer microstructure. This system integrated microchannels, microflowmeter, microchambers, microvalves, and negative pressure source and realized the transdermal extraction, transmission, volume measurement and storage of the subcutaneous interstitial fluid (ISF). But that system did not integrate an electrochemical sensor and was incapable of detecting the glucose concentration in the ISF. With the work here, the PDMS substrate attaching the three-electrode electrochemical sensor

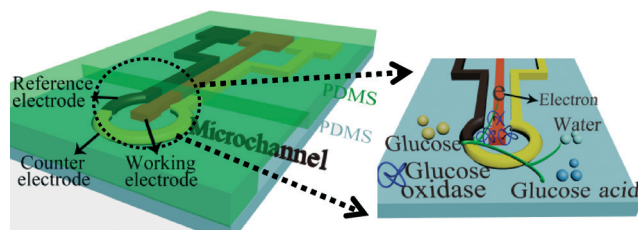


Fig. 6 Schematic diagram of the electrochemical sensor for glucose measurement and its integration into a fluidic channel.

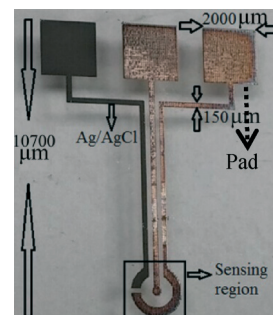


Fig. 7 Optical image of a three-electrode electrochemical sensor on PDMS after silver chloride (black) formation.

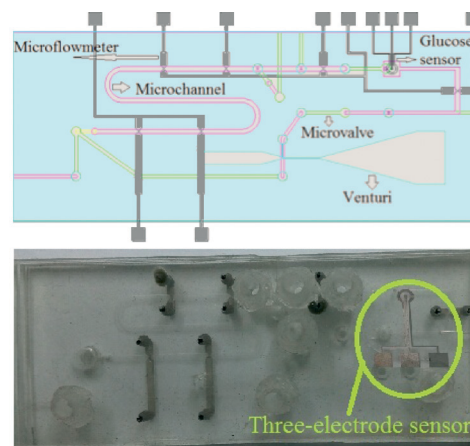


Fig. 8 Lab-on-a-chip system integrated with three-electrode electrochemical sensor. (top) Schematic diagram of the all-in-one system, different colors representing different layers. (bottom) Optical image of the complete fabricated system.

was bonded to the previous system and the sensor was sealed in the microchannel, forming an all-in-one system, as shown in Fig. 8, which is able to implement the transdermal extraction of ISF and the glucose detection simultaneously.

Conclusions

An effective inkjet printing method for metal patterning on the PDMS surface using silver nanoparticles to form microelectrodes in microfluidic channels was presented. MPTMS, a chemical coupling reagent of noble metals and PDMS, was utilized to promote the adhesion of PDMS to silver patterns and its surface wettability, which is good for silver droplets to disperse on its surface and thus effectively avoids the coalescence of adjacent droplets. The modified PDMS exhibited better surface wettability, and the drop size on the modified PDMS was approximately 40 μm and was substantially larger than that on untreated PDMS. Additionally, a multilevel matrix deposition method was used and it further overcame the coalescence of adjacent droplets on the MPTMS-modified PDMS. The silver pattern also exhibited good adhesion on the modified surface even under ultrasonication. The average resistance of the electrodes was 10.6 ohms, which was

comparatively low and suitable for obtaining a reliable current signal. A three-electrode electrochemical sensor for microfluidic sensing was fabricated successfully using inkjet printing and the integrated lab-on-a-chip system was manufactured. The system implemented the extraction, transmission, volume measurement, storage of the interstitial fluid and glucose detection in one highly integrated microfluidic chip. The work here described a new direction for fabricating microelectrodes on PDMS for functionalized microfluidics.

Acknowledgements

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