

# Capillary phenomenon-based pump-less biochip for uric acid determination

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In this reported study, the authors developed a uric acid biochip that does not require extra driving force. Although liquid flow is performed in a very narrow gap (2  $\mu\text{m}$ ), the capillary force is generated in this microfluidic device, which was fabricated from poly(methyl methacrylate) (PMMA) using a micromachining technique. The authors have fabricated a 2  $\mu\text{m}$  pillar array through hot-embossing and thermal bonding from a Si master mould. To increase the capillary force, the authors modified the PMMA surface by ultraviolet irradiation followed by poly-L-lysine coating. Electrodes were integrated into the microfluidic device to permit successful electrochemical measurement of uric acid with good reproducibility. This device also solves the problem of expenditure because of its potential scalability for mass production; although it is driven solely by capillary action, it does not require microfluidic pumps or any other additional power supply. The device is suitable for in situ analysis, diagnosis and house calls.

**1. Introduction:** Point-of-care-testing (POCT) allows fast and convenient analysis with high diagnostic accuracy at a patient's bedside and has recently attracted attention for medical device development [1–4]. POCT permits self-checking by patients in daily life, thereby contributing to the early detection and prevention of adult diseases. Blood glucose sensors for diabetic patients are an example of such technology that has become commercially successful.

The uric acid (UA) level in the blood is an important diagnostic indicator for gout, which is a common adult chronic disease [5, 6]. This level is greatly affected by lifestyle factors such as diet and exercise and alcohol consumption. It is expected that in addition to regular health checkups daily measurement of UA may permit early detection and prevention of gout. Therefore the demand for a POCT test chip for UA detection is expected to increase.

To accurately analyse the blood component, the plasma must be separated from blood. In previous studies, the plasma had been separated using the centrifugal force, dielectrophoresis and pump pressure for detection of a molecular marker in a biochip [6–10]. However, conventional devices that utilise extra driving force require a power source and pump. Therefore conventional devices are considered to be unsuitable for on-site analysis. We had previously developed a plasma-separation chip that utilises capillary force [11]. In our device, liquid flows were driven by the capillary force during plasma-separation process. Therefore an external driving force is not necessary, and the system can be made compact for on-site analysis.

In this study, we developed a UA biochip that adds electrochemical measurements capability to the plasma-separation chip that we had previously developed and uses the capillary phenomenon for fluid flow. We designed the array channels (depth, 2  $\mu\text{m}$ ) that were prepared through a hot-embossing process and the application of a hydrophilic modification to the surface of the microfluidics device (Fig. 1). Therefore this device is suitable for on-site analysis, because it does not require extra driving force. Although electrochemical measurements provide an inexpensive and fast response, they are often used in biochips.

## 2. Experimental

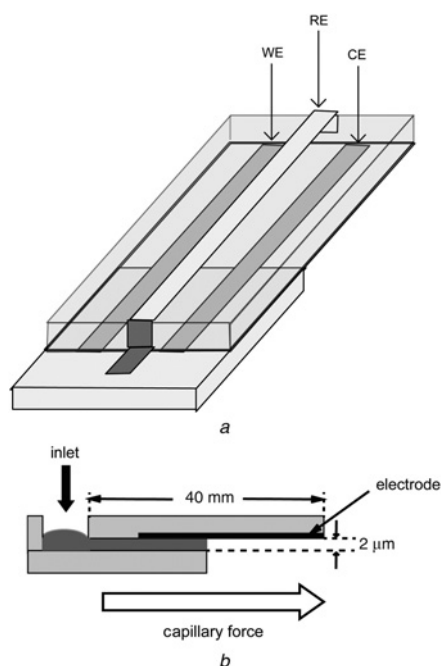
**2.1. Reagents:** UA and poly-L-lysine (PLL, molecular weight 30 000) were purchased from Wako Pure Chemical Industries,

Ltd (Osaka, Japan, <http://www.wako-chem.co.jp/english/>); all the reagents were of pure grade. Carbon ink (Jelcon CH-10) was purchased from Jujo Chemical Co. Ltd, Japan (<http://www.jujo-chemical.co.jp/e/index.html>). Every sample solution was prepared using Millipore water.

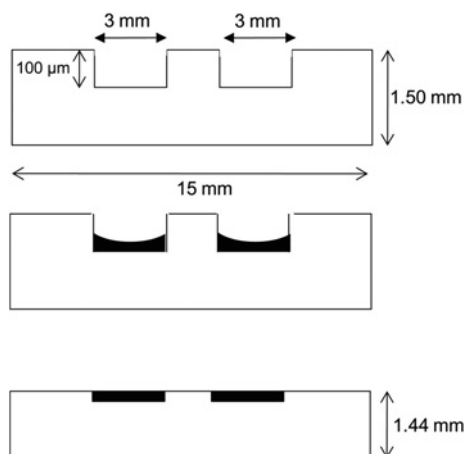
**2.2. Electrochemical measurement:** Phosphate-buffered saline (PBS) solution (0.1 M, pH 7.4) was used for the detection of UA. Electrochemical experiments were performed using a three-electrode system and an electrochemical analyser (BAS Co. Ltd). Measurements of linear sweep voltammetry (LSV) were conducted at 100 mV/s.

**2.3. Fabrication of a microchannel device for UA detection:** The devices were fabricated from polymethyl methacrylate (PMMA). The PMMA pattern was prepared as detailed in a previous report [11]. The device consisted of two PMMA sheets. In one sheet, a working electrode and counter electrode (3 mm wide, 40 mm long) were fabricated by casting and drying a carbon paste. Fig. 2 shows the fabrication process for the lid with embedded electrodes. First, two channels (40  $\times$  3 mm) were fabricated using a dicer (blade with 200  $\mu\text{m}$ ). Next, carbon ink was then injected into the channel and dried under vacuum for 12 h at 20 Torr. After drying, because of shrinkage, the level of the carbon ink was lower than the PMMA surface. Therefore the PMMA surface was polished using a rotational sander (EJ-200IN, Engis, Japan) for 2 h at 150 rpm. Finally, a very flat electrode surface was obtained with a roughness of 100 nm (data not shown).

Surface wettability was enhanced by surface modification to permit the UA solution to enter the device. The PMMA surface was modified using ultraviolet (UV) irradiation and PLL to obtain a hydrophilic surface. The conditions for UV irradiation were 14 mW/cm<sup>2</sup> and an irradiation distance of 2 cm (USHIO DENKI, Japan). PLL solution (1 mg/ml) was prepared by dissolving poly-L-lysine hydrobromide (average molecular weight: 84 000) in ultrapure water. PLL solution (100  $\mu\text{l}$ , 0.1%) was applied dropwise to the PMMA device and allowed to dry. Finally, an Ag/AgCl electrode was placed at the device's inlet.



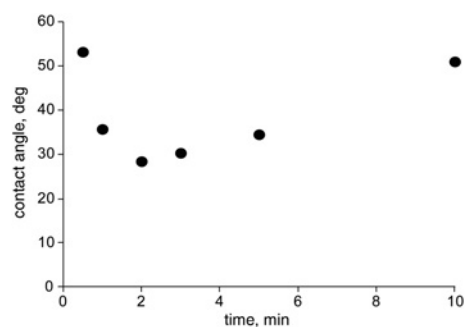
**Figure 1** Schematic representation of the UA detection device  
a Experimental setup  
b Cross-section of the UA detection device



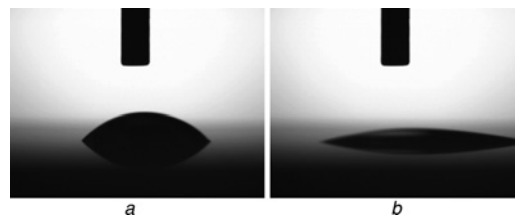
**Figure 2** Fabrication process for the cover of the device

**3. Results and discussion:** UV irradiation was performed to bond the lid to expose patterns. The UV irradiation modified the PMMA surface to expose OH groups and direct bonding was conducted at room temperature to prevent deformation of PMMA. The water contact angle was measured to confirm the wettability of the PMMA surface after UV irradiation of the PMMA surface (Fig. 3). The initial PMMA surface had a water contact angle of  $70^\circ$ , whereas after irradiation, the introduction of  $-OH$  group resulted in a reduced contact angle and an increased hydrophilicity. However, the water contact angle began to increase again after  $>5$  min of UV irradiated, probably because of an increase in surface roughness. Therefore we observed the highest hydrophilicity upon UV irradiation for 2–3 min and we used a UV irradiation time of 2 min for the device in this study.

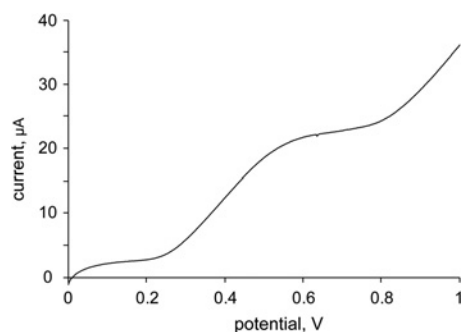
Next, direct bonding was conducted at room temperature at a contact pressure of 45 N for 10 min. At this low process temperature, deformation of the PMMA was prevented. Then, to increase the hydrophilicity of the PMMA surface further, we applied a PLL coating [12, 13]. When a 0.1% PLL was applied onto the



**Figure 3** Contact angle of the PMMA surface under various conditions after UV plasma irradiation



**Figure 4** Contact angle image of the PMMA surface  
a After plasma irradiation  
b After adsorption of PLL



**Figure 5** Linear sweep voltammograms of UA in PBS at a scan rate of 50 mV/s and UA concentration of 9 mg/ml

PMMA surface, the water contact angle decreased from  $30^\circ$  to  $12^\circ$  (Fig. 4). Thus, a hydrophilic PMMA surface was obtained by UV irradiation and PLL coating.

**3.1. Electrochemical measurement of UA:** Electrochemical measurement of UA was performed using the fabricated device. UA ( $5 \mu\text{l}$  of a 9 mg/ml solution) was applied into the inlet of the device. After dropwise application, the UA solution quickly flowed into the channel by capillary force, and the flow spread to the outlet of the device within 10 s. Fig. 5 shows the results of using the LSV measurement. Peak oxidation of UA was detected in the vicinity of 400 mV. The oxidation peak of UA was  $5.448 \pm 0.323 \mu\text{A}$ . The device provided reproducible measurement with low variation.

**4. Conclusions:** In this study, we developed a novel biochip that was driven by capillary force and had an integrated electrochemical measurement system. Although the driving force was the capillary force, the device can be easily used anywhere without pumps or tubes. To increase the capillary force, we applied a hydrophilic treatment to the material surface. The sample only requires 30 s from the time of application to the inlet of the device to the end of the electrochemical measurement.

Future application of this device for POCT or in situ diagnosis in the medical field can be expected.

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## 6 References

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