Chromatography

Focusing Review

Development of Microfluidic Components for Micro Total Analysis Systems

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Abstract
Microfluidic device is capable of reducing amount of sample consumption, shortening the analysis time, and miniaturizing instruments. Many applications of microfluidic devices have been developed not only in chemical analysis but also in medical diagnosis, and the food and agricultural industries. Electrophoresis or chromatography in a microfluidic device has improved the speed, reproducibility and separation resolution. Some of them have been integrated with complex experimental functionality on a small substrate, of which concept is known as micro total analysis systems. To build up a system, microfluidic components that carry out an experimental functionality in a microfluidic channel and novel technology to support the developments of microfluidic components are indispensable. In this review, three-dimensional (3D) fabrication by thiol-ene quick reaction, sample injection with an inkjet ejector, and molecular detection based on electroosmosis are focused briefly. The 3D fabrication realizes to make various 3D microstructures that conventional fabrication method cannot make without specific equipment. The sample injection by using inkjet technology can apply tiny amount of sample solution into multiple microfluidic channel on some precise spots, which leads to rapid analysis with high accuracy. The electroosmosis-based molecular detection indicates a possibility to develop a new portable device without any peripherals for detection or pretreatment for specimen labeling.

Keywords: Microfluidics; Three-dimensional fabrication; Sample injection; Portable analytical detection system

1. Introduction
The first microfluidic device was reported in 1979 by Terry at al. [1]. They have fabricated all the component of gas chromatograph and integrated on a silicon wafer. The device has a sample injection valve and a 1.5-m-long separating capillary column on a silicon wafer and a thermal conductivity detector mounted on the wafer. It reduced device size by three orders of magnitude and specimen volume for an analysis by less than 1 nL and achieved separation of hydrocarbon mixture in 10 s. This study has had a great impact to analytical chemistry.

Microfluidic technology has experienced an exponential growth since the 1990s. Manz et al. presented a 5 × 5 mm silicon substrate containing an open-tubular column and a conductometric detector for a miniaturized liquid chromatography (LC) [2]. They used a conventional LC pump and valves to perform high performance liquid chromatography in this work. They have tried to integrate all the experimental components including sample pretreatment, separation and detection, and proposed the concept of micro total analysis systems (µTAS) in 1990 [3]. The microfluidic scale has several advantages, such as lower consumption of reagents, separation speed and resolution, device portability, high repeatability, multiple assay etc. For this reason, many applications of microfluidic systems have been proposed so far, not only in chemical analysis but also in medical diagnosis, and the food and agricultural industries [4-8].

Since the concept of a µTAS is an integrated device of all required processes for analysis, it is important to improve existing technology, develop new material and fabrication method, and install novel technologies for making the devices. This review focuses on our recent works for miniaturization of separation methods [9-12].

2. Fabrication of three-dimensional microstructures
Three-dimensionally designed structures have been developed since around 2000 to apply microfluidic techniques in various field including separation science. The chaotic mixer is one of the most widely used three-
dimensional (3D) structure for microfluidic mixer reported in 2002 [13,14]. A 3D structure in the device has grooves similar to rifling in a gun barrel. The structures can twist flow three-dimensionally in a channel and the flow enhances the mixing efficiency. More complex 3D structure in a microfluidic baker's transformation (MBT) device is designed from chaos theory [15,16]. It can achieve realizing four processes for the baker's transformation: fusing two solutions, folding them, stretching and cutting the fluids, and fusing fluids again. Although the 3D structures achieved high mixing efficiency in microfluidic channels, their configurations were limited by the demolding process to shapes without undercuts such as cuboids or pyramids [17].

3D structures with undercuts for microfluidics can be obtained by microstereolithography [18,19]. The method employs a liquid UV-curable polymer, UV laser and a XYZ stage. The first patterns of the cross sections of a desired 3D structure is drawn by the UV laser to get a thin layer with curing UV-curable film. Subsequently the XYZ stage moves down to a position for the next layer and UV laser draw a pattern for the next cross section of the 3D structure. These two processes are repeated over and over for creating the 3D structure. Microstereolithography has been adopted to many 3D printers. 3D printers can save labor for the first stage of developing a device and reduce fabrication limitation in the field of microfluidics [20-23]. On the other hand, the minimum layer thickness of most commercial 3D printer is about 100 µm. It does not satisfy a requirement of fabrication of microfluidic devices.

To overcome these problems, we have developed a simple rapid prototype manufacturing method based on soft lithography with a UV-curable polymer for high throughput 3D fabrication [9,10]. The method adopted a thiol-ene reaction-based microfabrication. C. F. Carlborg et al. took advantage of the quickness of the thiol-ene reaction and reaction inhibition by oxygen to make a microfluidic device to rapid prototyping for 2D microfluidics [24]. They also characterized mechanical and chemical properties of microfluidic devices based on the thiol-ene reaction. The method has a great potential to bridge the gap between mass production for 2D microfluidics and that for 3D microfluidics. We combined their fabrication method and injection molding using poly(dimethylsiloxane) (PDMS) molds to extend the 2D fabrication to the 3D fabrication. It can be done by a conventional equipment for soft lithography and requires no troublesome bonding processes for multilayer lamination.

The fabrication process is schematically illustrated in Fig. 1a. Thin sheets of commercial UV-curable adhesive, Norland Optical Adhesive 81 (NOA 81), was injected into the PDMS molds obtained by softlithography. Although the NOA 81 is cured by UV irradiation, surfaces of NOA 81 sheets in contact with PDMS remained uncured [25,26]. PDMS has a high permeability of oxygen, and oxygen in the PDMS molds inhibit and delay the thiol-ene reaction. The uncured surfaces of NOA 81 could be used as a bond layer for the next sheet. The uncured surfaces are helpful to laminate thin sheets to build up 3D structures. The uncured NOA 81 were polymerized by UV irradiation for 90 s with a handheld UV black light with wavelength of 365 nm. The polymerized NOA 81 sheets with uncured surfaces can be bonded with another sheet. The next NOA sheets were put on the previous sheet and bonded in order by UV-irradiation for 30 s. The lamination process was repeated to build the objective 3D structure. The 3D structure was exposed to UV light for 20 minutes for a complete polymerization. This fabrication technique could control the sheet thickness from 4 µm to 100 µm. The minimum layer
thickness of 4 µm is higher than that of the high-end commercialized 3D printer with a minimum layer resolution of around 10 µm [22,27-31]. As for bond strength between two sheets, two NOA 81 sheets with bonding area of 1 cm² could bear load of 3.4 kg for a few minutes. The bond strength was about 330 kPa and equivalent to bond strength of the conventional PDMS-PDMS bonding method [32-35].

Three kinds of 3D structures: Menger sponges, spiral structures, and a channel-in-channel structure, were built to demonstrate our fabrication method. Menger sponge is a geometric configuration with a self-similar pattern. It has similar structure of monolithic silica in that they have large pores and small pores. Our method can fabricate level-1 and level-2 Menger sponges, in which the sizes of the structure were 90 µm³ and 810 µm³ respectively (Figs. 1b and 1c).

The structure of the spiral structure is like spiral stairs (Fig. 1d). A wall in a channel consists of 5 layers and its cross sections change their shape to rotate 180° around a central longitudinal axis of a channel. The spiral structures were embedded in a Y-junction microfluidic channel to apply in a microfluidic mixer. The flow in the channel is similar to the flow in a chaotic microfluidic mixer [13,14] or a spiral type mixer [19], which enhances the mixing efficiency.

A 3D sheath device has a large channel with a smaller channel in it and the channels are connected to two different inlets (Fig. 1e). A fluorescein solution was introduced into the smaller channel and a rhodamine solution was introduced into the large channel to visualize a flow in the device. The fluorescein solution was stably sheathed by the rhodamine solution at 5 mm far from the merged point.

We also applied the reaction to fabricate a thermopneumatic valve that is composed of several layers (Fig. 2). The chamber sealing system consists of the reaction chamber, main channel, thin membrane acting as the valving element, and the pneumatic chamber used as a valve actuator. The membrane bends into the main channel and tightly seals the reaction chamber, once the device is being heated using a temperature controlling system due to expansion of the pneumatic chamber. NOA 81 is easily deformed by weak pressure at high temperature due to its low glass transition temperature. This allows us to get the pneumatic chamber without using a thermal sensitive fluid.

Simple actuation of the membrane can be tested using a straight main channel without a reaction chamber. The fluorescent solution in the main channel was clearly observed in the channel at 25 °C. But the fluorescent intensity was getting lower with an increase in temperature at the valve part, except near the sidewall. This meant that the valve completely closed the channel at high temperature. The valve repeated closing and opening operations during a thermal cycling for polymerase chain reaction.

The valve system was installed into a chamber sealing system that encloses solution within a reaction chamber by heating a device. The self-actuated valve worked and completely closed the reaction chamber in a main channel at high temperature and effectively sealed the reaction chamber for PCR at 80°C, in contrast to the situation at 25°C showing the presence of the fluorescent solution in the reaction chamber in the main channel.

Our microfabrication method can be carried out with conventional equipment for photolithography, a portable UV black light for detection of biomolecules and stereomicroscope to build complex 3D structure. The bond strength by the method satisfied with requirements for making microfluidic devices and the minimum layer thickness of 4 µm is higher resolution than the commercialized 3D printer. The Menger sponge-like structures similar to monolithic silica, spiral structure for micromixer, channel-in-channel structure for making a 3D sheath flow, and thermopneumatic valve for control of flow in microfluidic channel were achieved by our method. Our fabrication method can meet the requirements of new 3D structures in a miniaturized separation equipment.

3. Sample injection for microfluidics-based separation

Although conventional cross- or T-shaped microfluidic channels have been contributed to sample injection in electrophoreses [36-38], such microfluidic channels require four reservoirs in a single device for separation, complicated voltage programming (sample loading and separation processes), and large samples. These disadvantages have prevented researchers from attaining higher throughput. A straight microfluidic channel is an ideal design because it
DNA samples were not cleaved by the inkjet ejector process. Although only \( \lambda \)DNA cleavage generated with the microfluidic channel were 100 \( \mu \)m each. The channel was connected to these electrodes for electrophoresis. Two platinum electrodes were inserted into the both ends of a straight microfluidic channel fabricated on a cyclic olefin copolymer (COC) substrate. A power supply was connected to these electrodes for electrophoresis. The applied electric field was 160 \( \text{V/cm} \). The diameter of the injection ports for DNA droplets and the width of microfluidic channel were 100 \( \mu \)m each. The channel was filled with 0.2% methylcellulose in a concentrated buffer solution (0.5\( \times \)TBE; 45 mM Tris-borate and 1 mM EDTA, pH 8.3).

There was a good correlation between the dropping velocity of DNA droplets and the applied voltage less than 7 \( \text{V} \); the dropping velocity was proportional to the applied voltage. Higher applied voltage than 7 \( \text{V} \) caused disruption of a DNA droplet formation the balance between the applied voltage and the surface tension. Generally, longer size DNAs are much easier to be cleaved by external forces. Although only \( \lambda \)DNA cleavage generated with the inkjet ejector process was confirmed at an applied voltage of 7\( \text{V} \); the dropping velocity was proportional to the applied voltage and frequency. The inkjet ejector eliminated the conventional sample loading process, and complex microfluidic channel. It leads to high throughput and high reproducible separation. The injection approach had good potential for realizing versatile applications to the high throughput analysis.

4. Electroosmosis-based detection for portable devices

Microfluidic techniques have miniaturized various procedures in a lab scale experiment. Microstructures control flow in a microfluidic channel, which leads to a development of microreactor, micromixer, microextractor, microdish for cell culture etc. These experimental tools have been integrated to miniaturize conventional instruments for analytical chemistry, biochemistry and medical science. However, most of microfluidic devices requires peripheral for detection process or pretreatment of samples for coloration to visualize a sample without any detector. To develop a microfluidic-based portable analytical tool, a novel detection method has been desired.

An electroosmotic pump (EOP) is a small-sized pump based on the electroosmotic flow (EOF), which was operated by low voltage without pulsation and mechanical noise. The cause of the EOF is the diffusion layers that is formed near solid–liquid interface \([40,41]\). Since counterions in the diffusion layer interacts loosely with the surface charge, they can be moved by Coulomb force under an electric potential along the liquid. This motion of the ions flows bulk liquid near material surfaces. The EOF velocity is proportional to the magnitude of the applied electric field \( (E) \) and it also depends on the zeta potential \( (\zeta) \) of surfaces. When some ionic compounds are mixed in the liquid and they adsorb onto the material surface, the EOF velocity should be changed by varying of zeta potential. The change of the EOF velocity has the capability of the use as an adsorbate sensor. The EOP can integrate two crucial functions for a portable analysis system, i.e., a pump and a sensor (Fig. 4). The sensor can detect chemical substances in an aqueous solution by evaluating the change of the EOF.

A microfabricated EOP for chemical sensing had a...
microstructure array region which divided a wide microfluidic channel into several narrow channels to increase the surface area. The width of the narrower channels was 10 µm. The microfluidic devices were made from poly(dimethylsiloxane) (PDMS) by using conventional soft lithography. The surfaces microfluidic channel were treated by a similar way in our previous study to modify with the various functional groups [42]. A microfluidic channel was flushed with 1 M HCl aq, followed by filling with a 10% (v/v) 3-aminopropyl-triethoxysilane (APTES) / ethanol solution at room temperature to introduce amino-groups on the surfaces. 20 mM ionic complex composed of benzyltributylammonium chloride (BTBA) and 4-sulfobenzoic acid in methanol was reacted at room temperature. The ionic complex was obtained by mixing BTBA and 4-sulfobenzoic acid potassium salt in water. After reaction of the ionic complex and amino groups on the surfaces, BTBA was removed by flushing a 2 M NaCl aqueous solution at room temperature.

A 30 mm-long pump generates two times higher pumping rate than a 60 mm-long pump at the same applied voltage. This is because the EOF velocity is proportional to the electric field strength according to the Helmholtz-Smoluchowski equation. The remarkable point is that the two pumps of different length indicates approximately the same maximum pressure at the same applied voltage. While the EOF is depends on the applied electric field, connecting pumps in serial improve the pumping pressure. In the situation that a long pump is regarded as series of two shorter pumps, the maximum pressure should have linear relationships to the length of pump (L), which cancel the effect of the electric field. That is why the maximum pumping pressure is proportional to the applied voltage (V).

The optimum microfluidic EOP has the short length for enhancing electric field strength and the large cross-sectional area for large pumping rate. The optimized microfluidic pump was driven at only 9 V and generated 1.6 ± 0.1 nL against the 54 mm fused silica capillary.

Surface modification of EOP with APTES improves pumping performance. The surface modified with the high APTES concentration solution has more amino groups due to an effective modification, which causes large surface charge. The pumps modified with 10% and 100% APTES solutions gave approximately the same performance. The number of amino groups on the modified surface reached the saturation point. This result suggests that the pump performance was dependent on the number of ionic functional groups on the material surfaces.

For proof of the concept, we evaluated the change of the pumping performance of the sulfo-modified EOP after molecular adsorption on the EOP surfaces (Fig. 5). While the sulfo-modified EOP without interaction to BTBA generates 4.58 nL/s, the pumping rate was reduced to 3.81 nL/s after adsorbing BTBA. Since a sulfo-group interacted with the cationic sample, BTBA and adsorption of BTBA reduced pumping performance. When the BTBA adsorbed on the surface, the electric charge derived from the sulfo groups were cancelled by the ionic interaction with BTBA and some amino groups was covered by the alkyl chain of BTBA. This interaction between of the sulfo group and BTBA reduce the surface positive charge. Although the surface modification and a material of a microfluidic EOP should be improved for more sensitive sensing, the microfluidic pump worked as the adsorbate sensor. We expect that the combination of a molecular imprinting technology will reach to development of the new type of a molecular-selective sensor [42-46].

Fig. 5. The flow rate change of the surface modified EOP by adsorption and desorption.

5. Conclusions
This review summarizes our recent work for...
miniaturization of a separation method, 3D fabrication by the thiol-ene quick reaction without expensive technologies, inkjet sample injection toward multiple analysis, and molecular detection based on the electroosmosis for onsite analysis.

The 3D fabrication adopted the thiol-ene reaction with the PDMS mold. The uncured surface of the NO81 sheet by reaction inhibition worked as a bonding layer between the sheets. It makes bonding process easier and realizes to make various 3D microstructures with undercut structure. The minimum layer thickness is thinner than the commercialized 3D printer.

The sample injection with an inkjet ejector can apply certain amount of sample solution into the multiple microfluidic channel with high accuracy. There is no cleavage of several thousand basepairs of DNA samples less than applied voltage of 7 V. This technique achieved to inject a DNA solution into three microfluidic channels sequentially and improve reproducibility of multiple assay.

The pumping performance of the EOP depends on the number of ionic functional groups on material surfaces. Pumping rate reduces after molecular adsorption on the EOP surface. Combination of this detection method and molecular imprinting technique has a potential to selective molecular detection.

Microfluidic device has been recognized as a powerful and a reliable analysis platform. Some analytical instruments based on microfluidics have been launched in recent years. The techniques introduced in this review enable to contribute to the development of new portable devices and industrial field.

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