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A disposable microfluidic biochip with on-chip molecularly imprinted biosensors for optical detection of anesthetic propofol

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ABSTRACT

This paper presents a disposable microfluidic biochip with on-chip molecularly imprinted biosensors for optical detection of anesthetic propofol. So far, the methods to detect anesthetic propofol in hospitals are liquid chromatography (LC), high-performance liquid chromatography (HPLC), and gas chromatography–mass spectroscopy (GC–MS). These conventional instruments are bulky, expensive, and not ease of access. In this work, a novel plastic microfluidic biochip with on-chip anesthetic biosensor has been developed and characterized for rapid detection of anesthetic propofol. The template-molecule imprinted polymers were integrated into microfluidic biochips to be used for detecting anesthetic propofol optically at 655 nm wavelength after the reaction of propofol with color reagent. Experimental results show that the sensitivity of the microfluidic biochip with on-chip molecularly imprinted polymers (MIPs) biosensor is 6.47 mV/(ppm mm^2). The specific binding of MIP to non-imprinted polymer (NIP) is up to 456%. And the detection limit of the microsystem is 0.25 ppm with a linear detection range from 0.25 to 10 ppm. The disposable microfluidic biochip with on-chip anesthetic biosensor using molecularly imprinted polymers presented in this work showed excellent performance in separation and sensing of anesthetic propofol molecules. While compared to large-scale conventional instruments, the developed microfluidic biochips with on-chip MIP biosensors have the advantages of compact size, high sensitivity, high selectivity, low cost, and fast response.

1. Introduction

Propofol (2,6-di-isopropylphenol) is an intravenous anesthetic widely used in induction of anesthesia, total intravenous anesthesia, and sedation of intensive care unit patients. For total intravenous anesthesia, propofol is unique and not replaceable. The effect of anesthesia depends on concentration of propofol in brain correlated to concentration of propofol in blood that could be affected by several factors, such as blood loss, volume replacement, and metabolism. Nowadays, target control infusion (TCI) applies pharmacokinetic programs for clinical anesthetic injection (Leslie et al., 2008; Schnider and Minto, 2007). Although concentration of propofol in blood can be detected by high-performance liquid chromatography and/or gas chromatography (Teshima et al., 2001; Cussonneau et al., 2007; Miekisch et al., 2008), these methods are time-consuming and not easy for access. Clinically, the distinction of anesthesia depth must be obtained immediately for diagnostics and treatment during operations, and the distinction of anesthesia depth will not only be used to know the anesthesia condition of patients, but also for further control of anesthesia dose. Besides, it will enable surgery to be completed under the safest condition. To achieve effective blood concentration and avoid adverse effect produced by excessive or insufficient propofol, clinically, we need a more convenient access to monitor blood concentration within desired clinical concentration range from 1 to 10 μg/mL.

Molecular imprinting is a newly developed technique that provides molecular assemblies of desired chemical structures and properties. In the presence of a template molecule, functional monomers are polymerized and immobilized complementarily to this molecule. After polymerization, the template is removed by solvents. As a matter of fact, molecular imprinting method is quite simple and easy to perform in a tailor-made fashion, and all we need are functional monomers, templates, solvents, and cross-linking agents. Then, polymerization is followed by the removal of template. During these processes, a number of functional monomers are assembled in an orderly fashion with their functional groups placed at the desired sites within the cavities of the desired size. Therefore, molecular imprinted polymers with binding sites, with specific shape and functional group recognition, will enjoy high selectivity and high sensitivity sensing the

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target molecular compound (Sellergren, 2001). In addition, MIP technology has the advantages of high selectivity, high sensitivity, low cost, and robustness, whereas several researches have used these MIP materials to realize molecular imprint to conduct separation or sensing materials (Thoelen et al., 2008; Bossi et al., 2007). Times before, MIPs had been realized with optical sensing techniques in biosensing for several analytes, such as Ca^{2+} ion detection in serum, atrazine detection, and glucose detection (Caglar et al., 2006; Piletsky et al., 1995; Malitesta et al., 1999).

The performance of propofol imprinted polymer material has been extensively investigated using HPLC and spectrophotometer systems (Petcu et al., 2004, 2009). However, the propofol imprinted polymer was fabricated in bulk forms, and propofol MIPs have not yet been used as on-chip propofol biosensors with microfluidic systems. Also, the detected signals of MIP from spectrophotometer systems are incapable to reach high accuracy and high sensitivity at low concentrations.

The objective of this work is to develop a disposable microfluidic biochip with on-chip molecularly imprinted biosensor, with the advantages of compact size, high sensitivity, high selectivity, low cost, and fast response, and it is for accurate measurement of propofol concentrations. In this work, molecularly imprinted anesthetic biosensors are integrated with disposable microfluidic biochips.

2. Design and materials

The optical microfluidic system includes a microfluidic biochip, MIP biosensors, laser diodes, photodetectors, and a control circuit. As shown in Fig. 1(a) and (b), it is found with a schematic illustration of microfluidic biochips with on-chip molecularly imprinted

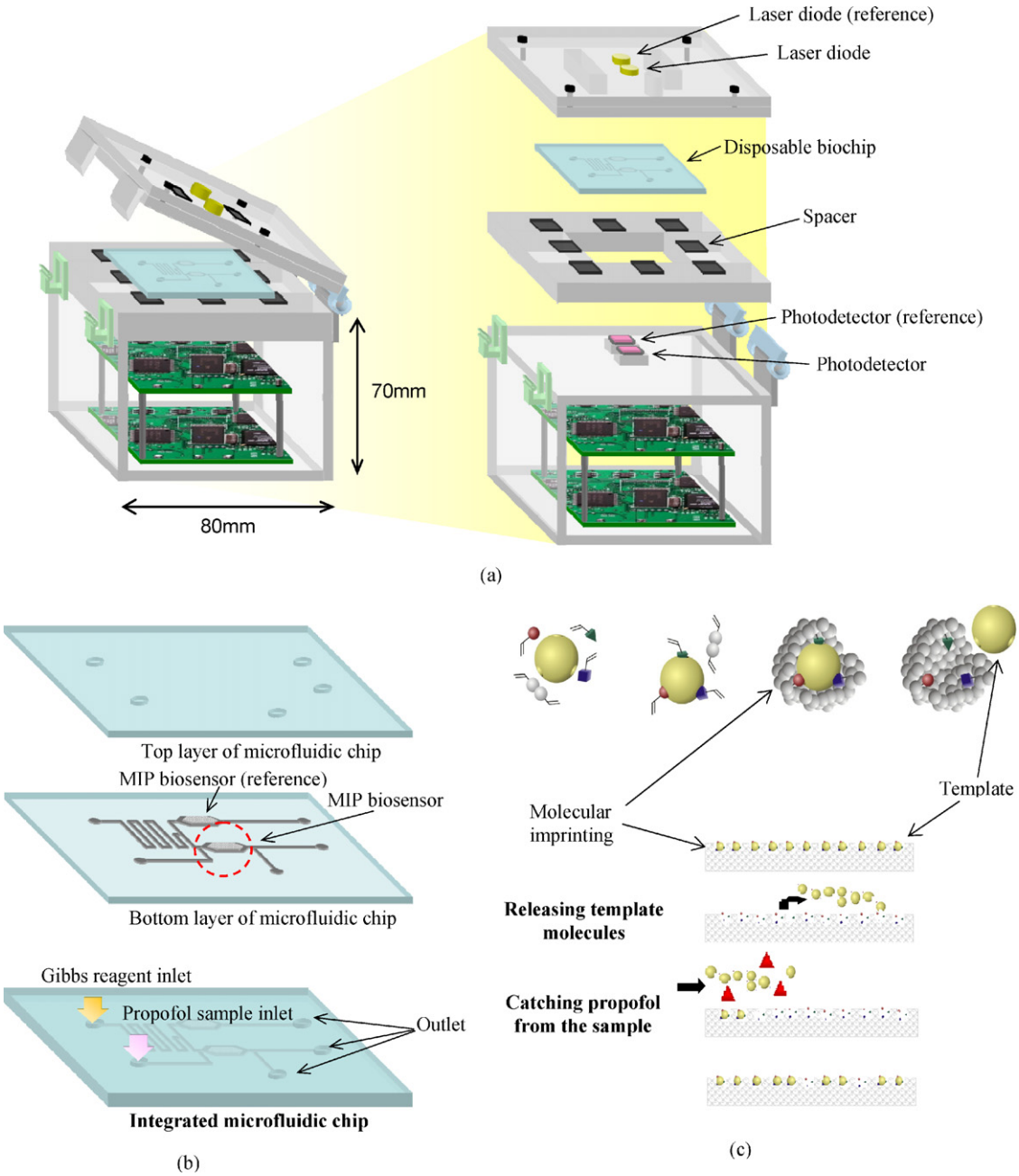


Fig. 1. Schematic illustration of the microfluidic biochips with on-chip molecularly imprinted biosensors for anesthetic sensing and the working principle: (a) the whole microfluidic system, (b) the microfluidic biochip, and (c) the working principle of molecular imprinting.

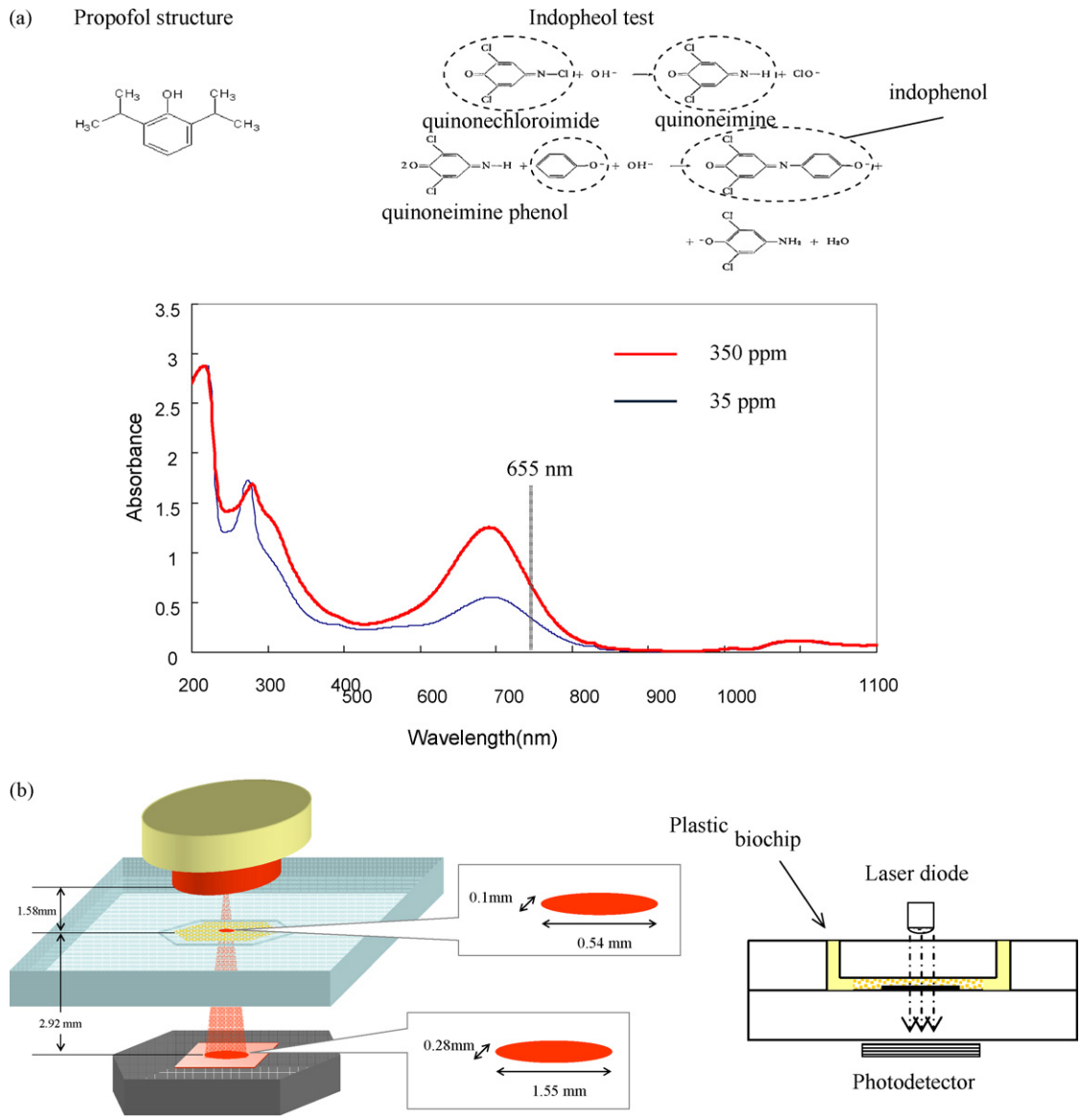


Fig. 2. Optical sensing principle with molecularly imprinted polymer for anesthetic propofol detection: (a) optical absorption spectrum of propofol with color reagent and (b) schematic drawing of optical detection of analyte samples.

biosensors for anesthetic sensing, and the microfluidic biochip was designed to precisely transport samples to the biosensor microchambers. The microfluidic chip is a two-layer structure. The fluidic inlets and outlets are on the top layer. The microfluidic channel and MIP biosensors are on the bottom layer. The integrated microfluidic chip is shown at the bottom of Fig. 1(b). When propofol separation from the sample solution by MIP biosensors is done, a color reagent was injected into the biosensor microchambers to react with propofol molecules. The working principle of the molecularly imprinted biosensors is shown in Fig. 1(c). First, template molecules are imprinted on the sensing film. Then, the specific cavities are formed by releasing template molecules with solvent. The designed cavities have the ability to catch analyte molecules from sample in the microfluidic chamber.

The chemical structure of propofol and the chemical reaction of quinonechloroimide with phenol are shown in Fig. 2(a). First, quinonechloroimide will change to quinoneimine molecules in alkaline solution. Quinoneimine molecules will then react with phenol structures to form indophenols. As seen, chemical structure of propofol can be applied to Gibbs's phenol tests for optical reaction (Svobodovfi et al., 1977). Having reacted with Gibbs reagent (color reagent), the solution will change from yellow color to blue color as the color reagent was premixed with methanol and bicarbonate buffer. On the other hand, the optical sensing principle with molecularly imprinted polymer (MIP) is shown in Fig. 2(b), which can be used to detect propofol optically at 655 nm wavelength after reaction of propofol with color reagent (Gibbs and bicarbonate buffer). At the end, the optical transmittance of mixed solution at 655 nm, being a function of the concentration of propofol solution, was detected by laser diodes and photodetectors.

For molecular imprinting, methacrylic acid and 4-vinylbenzeneboronic acid are often used as functional monomers, while ethylene glycol dimethacrylate and divinylbenzene are often used as cross-linking agents (Holthoff and Bright, 2007). In this work, 2,6-dicholorimideqinone hydrochloride (Gibbs reagent), ethylene glycol dimethacrylate (EGDMA), methacrylic acid (MAA), and 1,1′-azo-bis(cyclohexanecarbonitrile) (ABCHC) were purchased from Sigma–Aldrich. MAA was used as a monomer, EGDMA a cross-linker, and ABCHC as an initiator for polymerization. The inhibitor in MAA and EGDMA was removed before use, and ABCHC

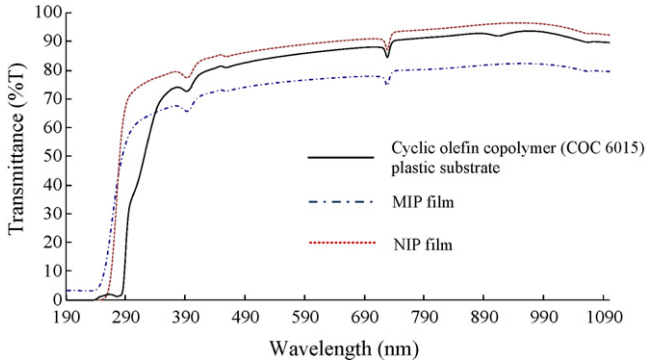


Fig. 3. Optical transmittance spectrum of a MIP film and a COC plastic substrate.

recrystallized before use. In this work, cyclic olefin copolymers (Topas® COC 6015, T_g: 150 °C) were chosen as chip substrates, and they were made into 4 in. plastic wafers by injection molding. Due to COC contains high optical transmittance, low water absorption, and good resistance to several chemical solvents (Khanarian and Celanese, 2001), COC has been used for protein chips and disposable lab-on-chip applications (Sohn et al., 2005; Do et al., 2008). The optical transmittance of COC substrate, MIP film, and NIP film, as shown in Fig. 3, has been characterized using a conventional UV–vis spectrophotometer (maker: JASCO, model: V-630, double-beam spectrophotometer with single monochromator). COC substrate and MIP film with good optical transparency are suitable for this study. From the material characterization, MIP film shows the decrease in optical transmittance compared to NIP film.

3. Fabrication

Fabrication steps consist of the fabrication of microfluidic biochips and MIP biosensors, and then the encapsulation of device as summarized is found in Fig. 4(a). A plastic material such as cyclic olefin copolymer (COC) was chosen as the substrate. In this study, 4 in. COC wafers were made by plastic injection molding techniques, and MIP biosensors were fabricated on the COC substrate. The fabrication process of MIP membranes involves three steps, including combination, polymerization, and extraction. A monomer, methacrylic acid (MAA), was used to mix with template molecules. On the other hand, MIP was made of propofol:MAA:EGDMA:ABCN upon the weight ratio of 1:4:30:0.41. A blank 4 in. COC plastic wafer was prepared and 25 μm thick spacer was attached on the COC surface. A photolithograph mask was put on the spacer to form a gap between the COC wafer and the mask. Then the mixture of MIP solution was injected to fill the gap. A UV aligner was applied to perform the polymerization of MIP solution by UV exposure. The required UV energy for polymerization of 25 μm thick MIP film was 17 J/cm^2 (20 mW/cm^2, 850 s). After polymerization of MIP by UV, the imprinted sites were formed as it released template molecules from MIP by methanol solvent. MIP film was patterned on a COC plastic substrate after the above procedure. In Fig. 4(b) and (c), it shows the SEM picture of the fabricated non-imprinted polymer and molecularly imprinted polymer. Theoretically, the size of the propofol molecule is around 1–2 nm. Some of the voids on MIP film surface are much large than the propofol molecule. Probably, propofol molecules were gathered together and were imprinted in the cavity as shown in Fig. 4(c). The microfluidic channel was fabricated with a hot-embossing mold on a 4 in.

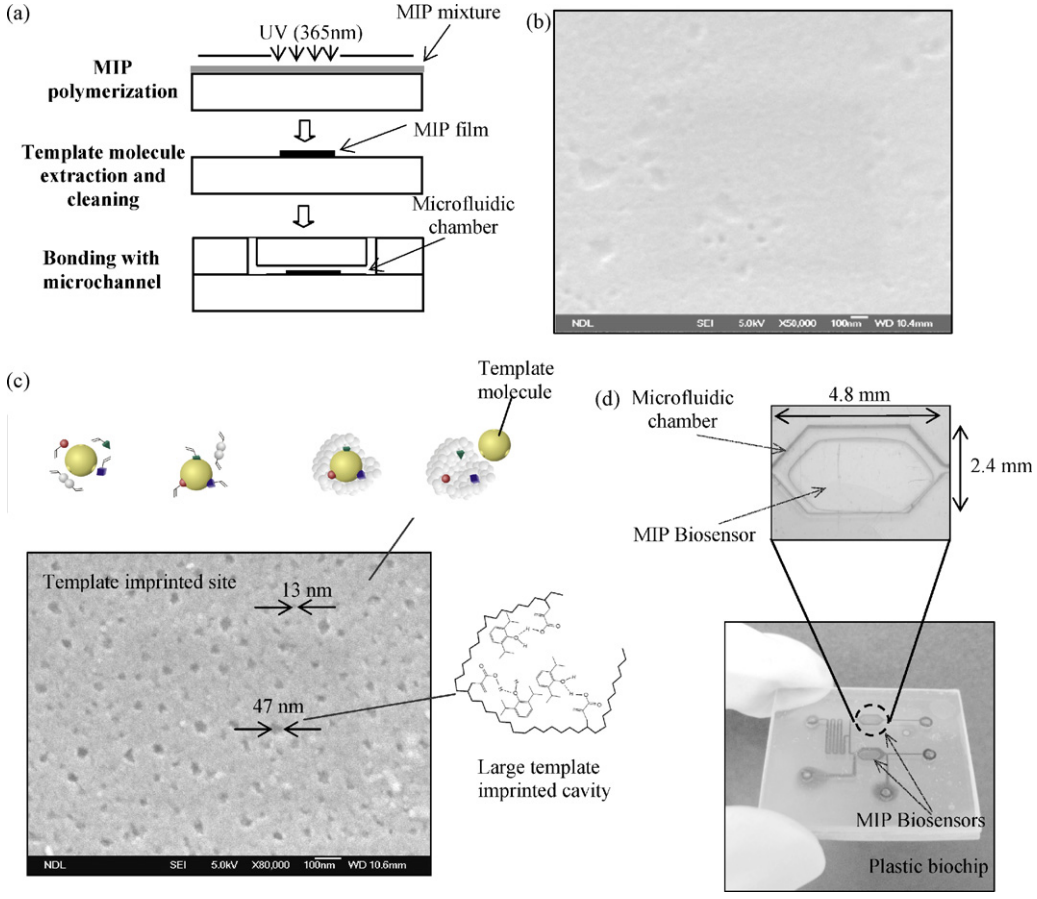


Fig. 4. Microfabrication of the microfluidic system with on-chip molecularly imprinted anesthetic biosensors: (a) microfabrication steps, (b) SEM picture of the fabricated non-imprinted polymer, (c) SEM picture of the fabricated molecularly imprinted polymer, and (d) photograph of the fabricated microfluidic chip.

blank COC wafer. 2100 SU-8 negative photoresist with UV photolithography techniques was used to pattern microfluidic patterns on a silicon wafer. A mold with patterns of microfluidic channels for hot embossing was got by cast molding PDMS on the SU-8 patterns (Narasimhan and Papautsky, 2004). During hot-embossing processing, the high temperature (170 °C) above the glass temperature (150 °C) of COC was applied with pressure of 0.5 MPa and it was kept for 5 min. Then, the heating temperature (125 °C) and bonding pressure (2.5 MPa) were applied to COC microchannel and COC wafer with MIP biosensors with the hot-embossing machine. Afterwards, the MIP biosensor was integrated with a plastic microfluidic chamber with a 100 μm depth using thermal bonding process. The fabricated device is shown in Fig. 4(d).

4. Experimental results and discussion

The binding experiments were performed by immersing the polymer films (film size is 12 mm × 12 mm and 25 μm in thickness) to contact with a known molarity propofol solution. The remaining propofol concentration in solution was got after 60 s and then mixed with the color reagent. The absorbance of the mixture was measured by a conventional UV–vis spectrophotometer at the wavelength of 594 nm. The amount of propofol molecules absorbed on the polymer films was determined using a calibration curve. The specific binding tests were performed by the characterization of absorption ratio of propofol molecules on MIP and NIP surfaces. The specific binding with different material surfaces is defined by Eq. (1). The specific binding of MIP to NIP can be as high as up to 456% with the concentration of 1 ppm. The specific binding of MIP to COC is 685%. According to the experiments, the binding capacity of the MIP films is 0.0075 μg/mm^2. Binding on the MIP films is a dynamic chemical reaction, which can be explained by Eq. (2). Binding affinity K_D can be defined as Eq. (3). B_{Bound} is the absorption amount on the film. $B_{Unbound}$ is the free binding site. C is the concentration of the analyte. Binding affinity of the MIP films K_D = 17.289 μmol/L. Specificity (imprinting factor) of the MIP films is 456%.

$$\text{Specific binding} = \frac{\text{Absorption}_{MIP}}{\text{Absorption}_{NIP}} \quad (1)$$

$$\text{Guest} + \text{Binding site} \underset{k_2}{\overset{k_1}{\longleftrightarrow}} [\text{Guest–Binding site}] \quad (2)$$

$$\text{Binding affinity, } K_D = \frac{k_2}{k_1} = \frac{B_{Unbound} \times C}{B_{Bound}} \quad (3)$$

The experimental setup for anesthetic concentration measurement using the designed biochip reader is shown in Fig. 5. The microsystem for propofol sensing was composed of 655 nm laser diodes, photodetectors, and a control circuit.

In the experiments, the microfluidic system was connected to a power supply and a PC-based DAQ system for real-time continuous data recording. Furthermore, different concentrations of propofol samples were prepared mixing 98% propofol with methanol solvent, and the color reagent was made of 0.45 mm Gibbs reagent, bicarbonate buffer, and methanol based upon the weight ratio of 1:1:1. The microsystem was connected to a LabVIEW-based DAQ system as it characterizes the dynamic response of propofol molecule that released and color reagent reaction, and its signal was detected by laser diodes and photodetectors. In the experiment, the propofol sample was injected into the plastic biochip using a syringe pump at a flow rate of 10 μL/min. For voltage of the photodetector, it was measured by the DAQ system, and increased immediately when the liquid sample filled the detection chamber. Then, the syringe pump was turned off to keep propofol sample in the detection chamber for 60 s so that the separation of propofol molecules was carried out by MIP films, and the liquid sample was removed with air injected into the microchamber. The voltage of the photodetector was decreased to the original value when the sample was removed completely. Afterwards, the color reagent was injected into the detection chamber and reference chamber

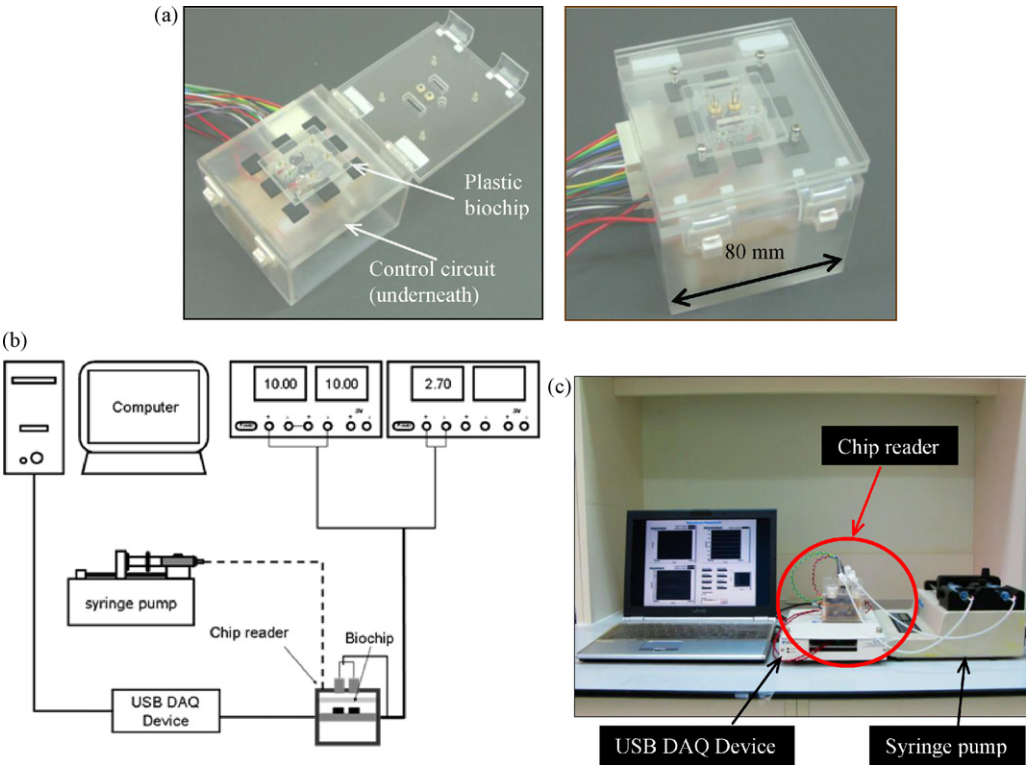


Fig. 5. Experimental setup for optical anesthetic concentration measurement: (a) biochip reader with microfluidic biochip, (b) schematic illusion of experimental setup, and (c) photograph of experimental setup.

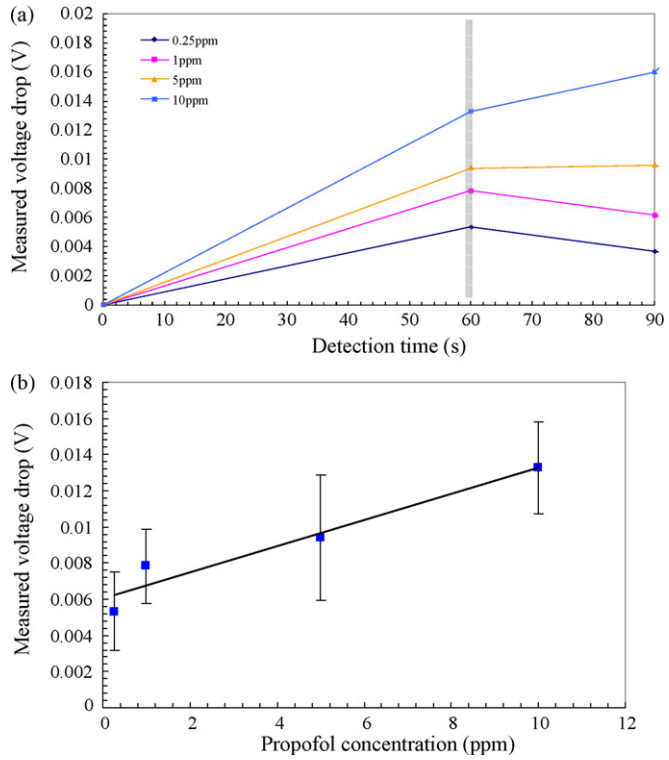
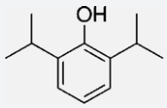
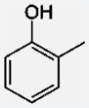
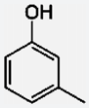


Fig. 6. Measurement results: (a) dynamic measurement results of anesthetic propofol sample at different concentrations (propofol release and color reaction), and (b) chip-to-chip measurement results of anesthetic propofol sample at different concentrations at 60th second.

(empty without MIP film) by a syringe pump. Next, the pump was turned off when the color reagent had filled both of the microchambers, and the propofol molecules, captured by the MIP film earlier, began to release and reacted with the color reagent. Both of the light detection devices that were monitoring the reaction within detection chamber and reference chamber were recorded by DAQ system concurrently. As for the voltage drop, it was obtained by subtracting the detected signal with the reference signal that was related to the propofol concentration. From the dynamic measurement results, the concentration of sample using the microfluidic system can be determined within 60 s, as shown in Fig. 6(a). The chip-to-chip measurement results at different concentrations at 60th second show linear relation between 0.25 and 10 ppm, as shown in Fig. 6(b), while the sensitivity of the microsystem with on-chip MIP biosensors is 6.47 mV/(ppm mm^2). When measured with a conventional UV–vis spectrometer, the lowest concentration is around 0.2 ppm. It is not easy to control mixing time of sample and color reagent while loading the mixture into the cuvette manually. Also, it is not easy to get the accurate response time by the conventional UV–vis spectrometer. But using the developed microfluidic systems with on-chip MIP biosensors, it is easy to load the sample and mix with the color reagent precisely in the microfluidic chamber. The reaction can be monitored in real time. So, the response time of the developed microfluidic system can be measured precisely. Because the separation and sensing area of on-chip MIP films is well defined by microfabrication process, it is more precise to quantify the amount of captured propofol molecules than the conventional analysis procedure.

When blood testing is performed in the future, materials that impact light absorbance, such as blood cells, should be filtered out with a microfilter. Only the analyte and blood plasma can enter the chip. Only molecules with specific functional groups can be separated by the MIP layer. Thereafter, only phenolic molecules can react with the color reagent. There should also be a certain degree of specificity when performing blood testing. The large background from blood could be eliminated by the procedure mentioned above during blood tests. Cresol-type structures would be in competition with propofol molecules, as shown in Table 1. Because cresol is toxic, propofol-type anesthetic does not compete with cresol in actual use. However, in the human blood, propofol molecules easily combine with human serum albumin (HAS), so inhibition may occur after combination.

Table 1
Chemical structure of propofol molecule and cresol-type molecules.

	Chemical		
	Propofol	o-cresol	m-cresol
Structure			

5. Conclusions

In this work, novel plastic optical microfluidic biochips with on-chip anesthetic biosensors using molecular imprinting technology have been successfully developed and characterized for rapid detection of propofol. The optical microsystems with on-chip molecularly imprinted biosensors have showed excellent performance in the separation and sensing of anesthetic propofol molecules. Experimental results show that the developed microfluidic system with propofol MIP biosensors can successfully detect propofol samples with concentrations ranging from 0.25 to 10 ppm, while specific binding of MIP can be as high as up to 456% and the sensitivity of the microsystem with on-chip MIP biosensors is 6.47 mV/(ppm mm^2). The specific binding of MIP to COC is 685%. Binding affinity of the MIP films K_D = 17.289 μmol/L. Specificity (imprinting factor) of the MIP films is 456%. The binding capacity of the MIP films is 0.0075 μg/mm^2.

Furthermore, the microfluidic biochips with on-chip anesthetic biosensor using molecularly imprinted polymers are found with the advantages of compact size, simple structure, low cost, ease of use, small sample volume, and fast response. In addition, optical microfluidic systems with MIP biosensors for anesthetic sensing presented in this work have showed excellent performance in the separation and sensing of anesthetic propofol molecules. As compared to large-scale conventional instruments, the developed microfluidic systems with MIP biosensors are given with the advantages of compact size, high sensitivity, high selectivity, low cost, and fast response.

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