

A World-to-Chip Microfluidic Interconnection Technology with Dual Functions of Sample Injection and Sealing for a Multichamber Micro PCR Chip

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ABSTRACT

This paper presents a practical world-to-chip microfluidic interconnection technology with dual functions of sample injection and sealing for a multichamber Micro PCR (Polymerase Chain Reaction) chip. After sample injection and sealing, leakage test is conducted by elevating the temperature upto 100 °C for 30 min. No leakage flows are found during the test for 10 cartridges. In conclusion, we have introduced a simple and cheap microfluidic interconnection technology for both sample injection and sealing, which provides a zero dead volume, a zero leakage flow, and biochemical compatibility. Also, this world-to-chip interconnection technology enables one or more operators to interface between the micro world and real world easily by using conventional pipettes.

1. INTRODUCTION

One of the stumbling blocks associated with the successful miniaturization and commercialization of fully integrated microfluidic systems is the development of practical micro-to-macro or 'world-to-chip' interconnects, that allow easy coupling between the micro scale in the microfluidic device and the macro scale in the real world. Most monolithic microfluidic devices (e.g. Micro PCR chips, microfluidic-based reactors and sensors) have several inlet, outlet, or vent ports, which required to be coupled to the macro environment of the real world, where pipettes, tubes and Luer fittings are normally used. To date, the most common approach to inject samples or reagents to the monolithic microfluidic devices is via the use of glued tubes. This approach has been successfully applied to world-to-chip fluidic coupling in many studies, and most-leading edge microfluidic devices are still being joined with epoxy based adhesives [1-5]. However, the use of adhesives at world-to-chip interconnects often generates following troublesome problems: leakage flow, dead volume, temperature stability, contamination and biochemical compatibility. In general, using adhesives is not recommended at all, in terms of reliability and reproducibility of the process of gluing.

In addition to the function of sample injection into the microfluidic devices, sealing of the inlet, outlet or vent ports is a critical function to succeed biochemical reaction, for example, micro polymerase chain reaction (Micro

PCR). To seal or valve the ports of the Micro PCR chip, additional micro components such as microvalves have to be incorporated and the microvalves have to address the following issues: (1) The valves have to be able to withstand the internal pressure generated during thermal cycling upto 100 °C. Evaporation and air expansion are easy to occur at elevated temperature, resulting in a failed PCR. (2) The valve materials should be compatible to PCR solution, because valves will be in direct contact with PCR solution. (3) And also, the valves should be opened easily after PCR and allow PCR solution to flow into downstream for further detection and analysis [6].

Previous approach for sample injection and sealing of a single-chamber chip, which has only one inlet and outlet, is relatively simple but cannot be applied for multi-chamber concept. Samples are loaded into the inlet port, which is micromachined to fit into the shape of pipette tips, by using conventional pipette tips. Then, the inlet and outlet ports are sealed by applying an optical tape on the top of the chip [7-8]. In the case of multi-chamber devices, however, since multiple ports are exposed, it is not easy to align the pipette tips to each hole and load samples repeatedly, resulting in a failed sample loading. Even the samples are successfully loaded, during applying the optical tape on the top of the chip, the overflows of the samples around holes may flow through the surface between the tape and chip, resulting in a cross-contamination.

To address above issues, we have proposed a microfluidic interconnection technology for both functions of sample injection and sealing, which provides zero dead volume, zero leakage flow and biochemical compatibility. In addition, the technology is simple and cheap to fabricate, and is easy to interface with the real world by using conventional pipette tips.

2. DESIGN AND FABRICATION

World-to-Chip Interconnection Concept

Fig. 1 shows schematic views of the dual mode microfluidic interconnection technology for sample injection and sealing. Plastic fittings, which contain pipette tip guides and a rubber sheet, are set to fit conventional pipette tips for PCR sample injection. As shown in Fig. 1(b), the pipette tips can be easily aligned to the inlet holes of the chip by the help of the pipette tip guides. Without the pipette

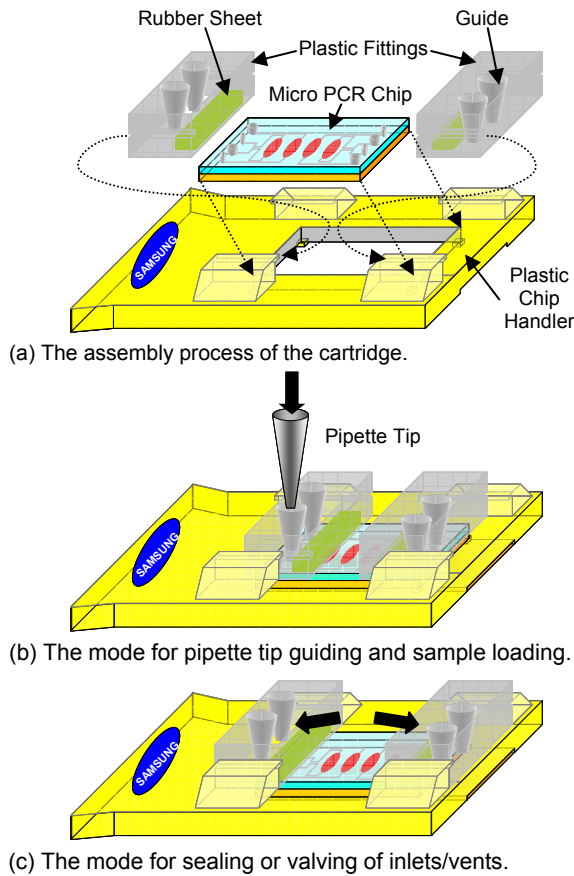


Figure 1. Schematic views of (a) the assembly process, (b) the sample loading mode, and (c) the sealing mode.

tip guides, it will not be easy to align the pipette tips to each hole and inject samples repeatedly. Once samples are injected to microchambers, the plastic fittings with the rubber sheet can be slid to the position of the sealing mode to seal the inlets/vents without dead volume. Fig. 1(c) shows that the plastic fittings can be placed in the mode for sealing or valving of inlets/vents and the fittings are also slid back to the mode for pipette tip guiding and sample injection reversely. In order to resist the internal pressure during thermal cycling, the rubber sheets are designed with appropriated materials and dimensions. In the guiding mode the sheets are not pressurized to assist an ease sliding of the fittings, but in the sealing mode the sheets are designed to be pressurized, resulting in successful sealing during thermal cycling.

Cartridge

As shown in Fig. 1, a cartridge consists of a 4-chamber Micro PCR chip, two plastic fittings and a plastic chip handler. The cartridges are assembled as following: First, the Micro PCR chip with a dimension of 13 mm x 6 mm x 1mm is inserted to the handler, which is mass-producible by a conventional plastic injection molding method. The user-friendly designed handler can keep the chip information such as a serial number and a logo. Second, the plastic

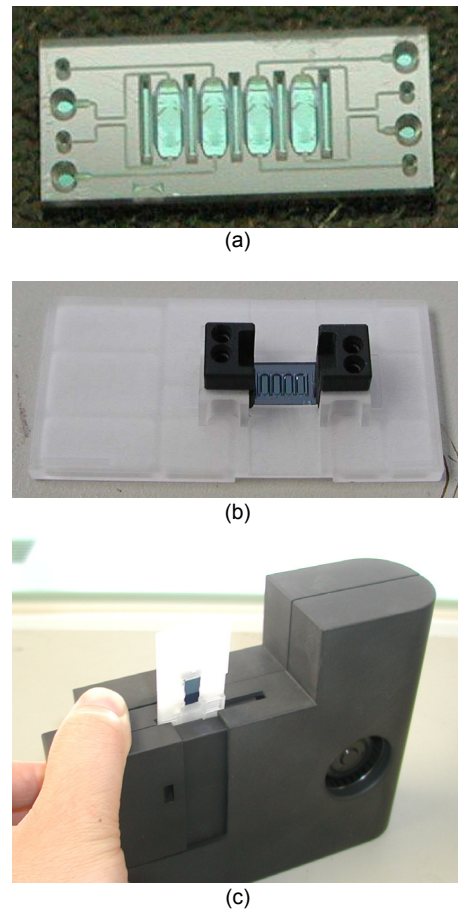


Figure 2. Photographs of (a) the 4-microchamber Micro PCR chip, (b) the cartridge, and (c) the prototyped module.

fittings with the rubber sheet are assembled with the handler, as shown in the dotted lines in Fig. 1(a).

PDMS material as the rubber sheet is selected for biochemical compatibility and the fittings are designed to withstand at least 8.1 Psi. If sealing or valving using the rubber sheet fails, the PCR sample will be pushed out of the microchamber, resulting in a failed PCR. The amount of pressure required to prevent degassing is estimated by Chiou et al. to be ~3.1 Psi [9]. In worst case, the presence of air bubbles between the sealing rubber sheet and the inlets/vents will cause an additional internal pressure drop of 3.7 Psi at 94 °C [6]; therefore, the rubber sheet has to withstand at least the total pressure of 6.8 Psi. The pressure P on the rubber surface is

$$P = E \frac{\delta L}{L}, \quad (1)$$

where E is Young's modulus, L is a total length of the rubber, and δL is an elongation length. The elongation length between two modes for sample loading and sealing is designed to be 0.15 mm, where the original length of the rubber sheet is 2 mm. By using (1), the sealing pressure of 8.1 Psi is calculated, when the Young's modulus of the PDMS is 750 kPa. Therefore, the plastic fittings are capable of resisting at least 8.1 Psi, which is enough to seal the inlets/vents of the Micro PCR chip during thermal cycling.

Module

The plastic cartridge packaged with the 4-microchamber silicon micromachined PCR chip can be inserted in each Micro PCR module as shown in Fig. 2. The module has the silicon-based microheater/sensor, a cooling fan, an optic unit for light excitations and emissions, an embedded microprocessor and a computing circuit board. Each module is capable of independent thermal cycling and in-situ monitoring of the Micro PCR chips. Independent PID (proportional integral derivative) control by the embedded microprocessor in each module can give an excellent temperature accuracy of less than 0.5°C for PCR.

3. RESULTS AND DISCUSSION

Evaluation of the World-to-Chip Microfluidic Interconnection Technology

Once the cartridges are assembled with the PCR chip, they are ready for sample loading, sealing, and thermal cycling. The world-to-chip microfluidic interconnection technology with dual functions of sample injection and sealing for the multichamber Micro PCR chip has been successfully dem-

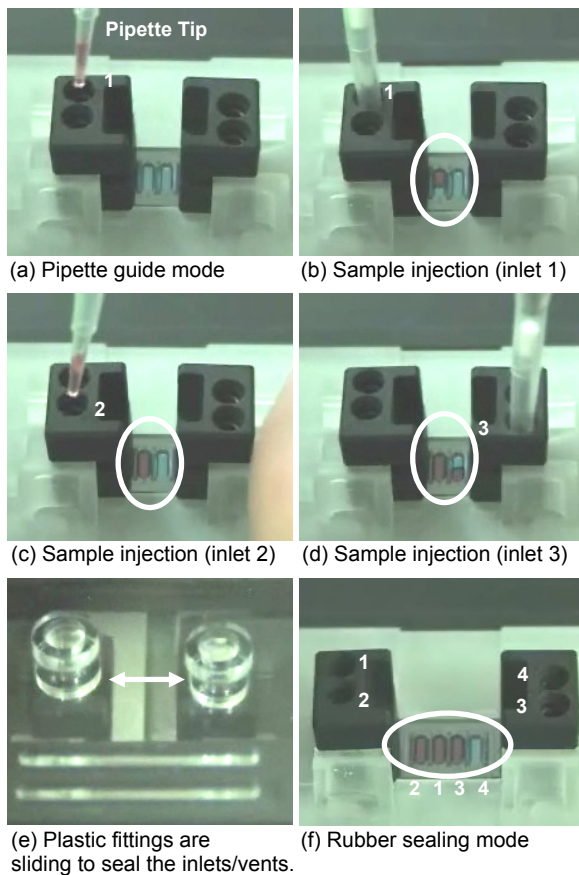


Figure 3. Demonstration for the practical world-to-chip microfluidic interconnection technology (only microchamber 1, 2, and 3 are injected, as shown in (f)).

onstrated as shown in Fig. 3. To help an easy understanding, the liquids are mixed with a red-color dye and only three microchambers are filled with the fluids.

A pipette tip (Eppendorf, $10\ \mu\text{L}$ S) is easily inserted into the microchamber 1 by the help of the pipette tip guide 1 (Fig. 3(a): pipette guide mode). And then the fluid of $1\ \mu\text{L}$ is loaded by pushing the pipette's head. Due to the SiO_2 surface coating of the microchannels and the microchambers, the fluid can be automatically loaded by a capillary effect. In term of bubble formation, dead volume zone is of importance in the microfluidic devices. When the fluid flows in the microfluidic channels, an abrupt change in the crosssectional area will make the fluid to feel an abrupt pressure drop, resulting in an avoidance of fluid pass. Therefore, the fluid can flow an easy flow pass, which will form the air bubble, so-called in the dead volume zone. In this demonstration, no bubbles inside of the microchambers are generated during sample injection into the inlet 1, 2, and 3 (Fig. 3(b)-(d): sample injection). Once the samples are injected, the pipette guide and sample injection mode is switched to the sealing mode by sliding the plastic fittings. To ensure the exact positioning of the fittings, a simple device is incorporated in sliding the fittings between two modes. The device is capable of guiding the pipette tips, in addition to sliding the fittings from the injection mode to the sealing mode (Fig. 3(e): sliding of the plastic fittings to seal the inlets/vents). Three microchambers 1, 2, and 3 are successfully filled with the fluids and the microchamber 4 is in blank without the fluids (Fig. 3(f): rubber sealing mode).

The process of sample injection and sealing is reversible, so that the fittings can be slid back into the pipette guiding mode from the sealing mode. Therefore, users can pull out the PCR products for further investigation after PCR thermal cycling. The Micro PCR chip is capable of performing the real-time PCR, since the module has the optic unit on it, so can directly detect the amplification signal without transferring the fluids into detection chamber. In this paper, however, performing of the real-time PCR and the quantitative PCR is out of scopes and still in the progress, which will be reported in a soon.

Evaluation of Sealing and Thermal Cycling

If sealing or valving using the PDMS rubber sheet fails during the thermal cycling, the PCR sample will be pushed out of the microchambers, resulting in a failed amplification. The previously mentioned issues, such as the leakage flow, biocompatibility, and sealing/opening reversibility, are critical to success the PCR amplification [6]. The leakage test is conducted by elevating the temperature upto 100°C for 30 min in a convention oven, after sample loading and sealing. No leakage flows have been found during the test, except for only two microchambers from 10 cartridges.

In addition, the Micro PCR chips have been thermal cycled and the PCR products have been analyzed by Bioana-

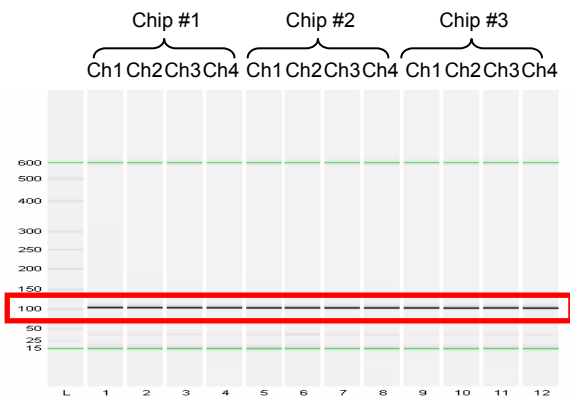


Figure 4. A gel electrophoresis result showing the percent CV of PCR yield of 3 Micro PCR chips (12 microchambers) is less than 6.7%.

lyzer 2100. To achieve the same PCR yield in each microchamber, temperature uniformity is of great importance. The temperature uniformity between the microchambers is measured less than 0.5 °C and the % CV of PCR yields is less than 6.7 %, as shown in Fig. 4.

4. CONCLUSION

In conclusion, we have introduced the simple and cheap microfluidic interconnection technology for both sample injection and sealing, which provides a zero dead volume, a zero leakage flow, and biochemical compatibility. Also, this world-to-chip interconnection technology enables one or more operators to interface between the micro world and real world easily. Thus, the new world-to-chip microfluidic interconnection technology with dual functions of sample injection and sealing developed in this work will have tremendous applications in various bio/chemical microfluidic systems.

5. ACKNOWLEDGEMENT

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