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# A multifunctional, plug-and-play and low-cost microfluidic connector system based on electronics standard†

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A reliable, low-cost and standardized microfluidic interface to the outside world is still a challenge for microfluidics. In this study, we utilized the electrical engineering standard a 2.54 mm pin-header connector to create a standard pin-header microfluidic connector (SPHMC) for PDMS based microfluidic devices. This connector is fabricated using universally available pin-header connectors and heat shrink tubing, thus its cost is less than 10 cents per unit. The density of the SPHMC is relatively high (ten fluidic I/O for 63.5 $mm^2$) and the connector can tolerate at least 30 psi, which is beyond the requirement of most microfluidic applications. Moreover, to resolve the issues about small volume injection and bubble removing, we modified the SPHMC and developed several new connectors and accessories. We demonstrate that our connectors and accessories can be used for parallel droplet generation, injection of rare samples and capture of circulating tumor cells.



## Introduction

Over the last 10–20 years, lab-on-a-chip (LOC) technology has demonstrated its potential to revolutionize the investigation of cell biology[1–3] and molecular chemistry.[4–6] Compared to bulkier analytical instruments, these miniaturized devices have many advantages: they enable highly multiplexed and parallel analysis, support accurate control of fluid flow by valves, and minimize consumption of samples and reagents.[7] However, the fluidic interfaces between the chip and the peripherals (*e.g.* injection pumps, fluid reservoirs and pneumatic controllers) are often neglected and limit the overall performance of these LOC devices.[7]

Traditionally, microfluidic devices have been connected with peripherals using tubes or needles.[8–11] With this strategy, when the LOC devices integrate more fluidic I/O (input/output) or control valves, the experimental setup becomes time-consuming and experimental failure due to user error is more likely to happen.[12] Another problem is that it is hard to draw up very small volumes of liquids by syringes and tubes. Thus, in some applications requiring only nano-scale volume of samples or reagents such as drug screens, most of the rare samples remain in the tubes after loading. A variety of connection systems are now available to address the issue of long-time experimental setup and high reagent waste. Commercial products including customized chip holders,[13,14] flexible fluidic breadboards,[15,16] and multi-tube connectors exist in the marketplace. However, these are costly and lack the design flexibility needed by research groups. Better manifold designs from research groups include self-aligned fit-to-flow interconnects[17] and the D-subminiature connectors,[18] which can fix a number of fluidic I/O at one time. Later, van Swaay *et al.* introduced a reusable connector that has reservoirs for holding low-volume samples (1–20 μl) to resolve the problem of injection and handing of very small volumes.[22] Although these manifold designs have developed varied techniques specific to the target application, a universally accepted standard for fluidic interfacing does not exist.[7] Actually, the lack of standardization has impeded the commercialization and widespread use of microfluidics.[19,20]

A pin-header that uses the industry standard spacing of 2.54 mm (0.1 inch) is a form of commonly-used electrical connector. It consists of one or more rows of male pins and is designed for soldering directly onto printed circuit boards (PCBs). Inspired by this standard connector, we present a series of reliable, low-cost and plug-and-play microfluidic connectors and accessories for PDMS based microfluidic devices. For the

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standard pin-header microfluidic connector (SPHMC) in our work, the solid metal pins of the electrical pin-header were replaced with corresponding metal tubes that permit fluid and/or gas passage. In addition to the pin-header, heat shrink tubing was used in our connectors to seal or connect two tubes with different diameters. Heat shrink tubing is a colourful and shrinkable plastic tube used to insulate wires and providing abrasion resistance in electrical engineering. In our connectors, we also utilized heat shrink tubing for color-coding of tubes to avoid confusion. Briefly, there are two advantages of using the standard 2.54 mm pin-header connectors and heat shrink tubing: (1) these components are affordable, costing less than 10 cents per unit. (2) The spacing between two fluidic I/O is only 2.54 mm, which can provide high-density tube packing.

We also present a number of standard components that could either work alone or cooperate with others to achieve different functions. By modifying the SPHMC, we developed a nanoliter sample injector, which can reduce the dead-volume of reagents and samples. This modification makes the connector particularly suitable for some applications that require small volumes of reagents, such as PCR and enzymatic reactions. Moreover, we introduce two accessories including a PDMS plug and a female SPHMC, which utilizes pin-headers for replication. By cooperating with a standard microfluidic connector, these accessories can simplify the process of bubble removing and increase the stability of the experimental operations. In the experiments, we demonstrated that our connector system could be used for high-throughput droplet generation, rare sample injection and fast capture of circulating tumor cells.

## Fabrication

### Design rules of inlets and outlets

In the field of electronic circuits, pin-headers are typically spaced 2.54 mm (0.1 inches) apart. Therefore, when we arrange the inlets and the outlets of the microfluidic chip, their spacing is 2.54 mm.

### Standard pin-header microfluidic connector

The fabrication of a SPHMC is shown in Fig. 1. 22-gauge hollow needles (Amazon Supply) replaced the metal pins of the pin-header (Digikey Supply) to connect the microfluidic device to macro-scale systems. Teflon tubes (0.76 mm ID) were then placed at the longer side of the needles and hooped by heat shrink tubing (Digikey Supply, 0.8 mm ID). Heat shrink tubing could shrink and seal the seam between the tube and hollow pin on heating with a heat gun. By selecting a heat shrink tubing of a different color, various Teflon tubes could be labelled to avoid confusion. This is useful when numerous transparent reagents are used. Moreover, this fabrication process is compatible with that of pin-headers in the electrical industry. For mass production of pin-headers, a pin-header block is first made through injection molding, and then several metal pins are aligned and inserted into the block by machine. Therefore, it is possible to mass produce our microfluidic connectors by replacing metal pins with hollow needles in the future.

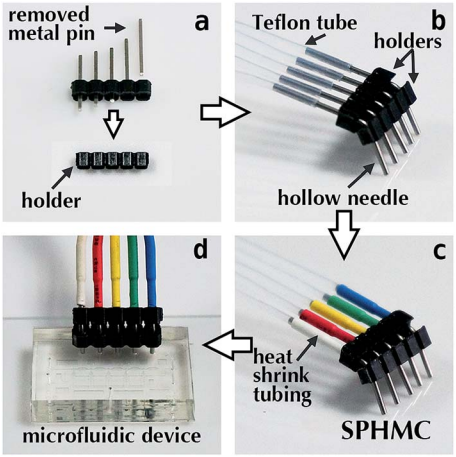


Fig. 1 Process flow for fabricating a SPHMC. Metal pins were removed from pin-header to get a holder (a). Five 22-gauge hollow needles were inserted into the holder. Another holder was fixed to reinforce the pin-header connector. Teflon tubes were then placed at the longer side of the needles (b) and hooped by heat shrink tubing (c). The SPHMC was fixed on the microfluidic device (d).

### PDMS plug, female SPHMC and nanoliter sample injector

As shown in Fig. 2, we have developed two kinds of accessories (the PDMS plug and the female SPHMC) and an advanced microfluidic connector (nanoliter sample injector). Firstly, as

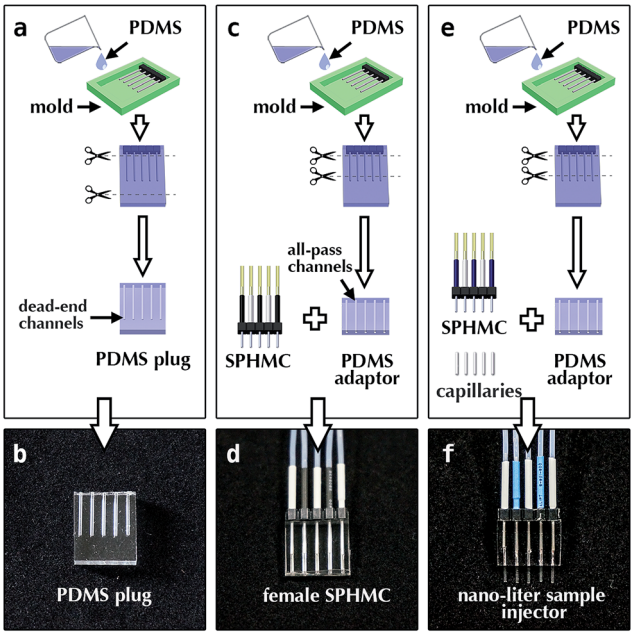


Fig. 2 Process flows for fabricating a PDMS plug, a female SPHMC and a nanoliter sample injector. Five 25-gauge hollow needles were inserted into a holder to make a mold. PDMS was poured into the mold and was cured in an oven. By cutting PDMS at different positions, we got a PDMS plug (a and b) and a PDMS adapter. A PDMS adapter was combined with a SPHMC to fabricate a female SPHMC (c and d). A nanoliter sample injector was fabricated by inserting a SPHMC into one side of a PDMS adapter and five 7 mm glass capillaries into the other side (e and f).

described in the fabrication of the SPHMC (Fig. 1a and b), five 25-gauge hollow needles (Amazon Supply) replaced the metal pins of the pin-header to make a mold. The mold was placed in a female die. Then, PDMS (Sylgard 184, 10 : 1 monomer : curing agent) was mixed and degassed. Thereafter, it was poured into the Petri dish and cured in an oven at 75 °C for at least 1 h. We carefully peeled off cured PDMS and removed the mold. By cutting PDMS at different positions, we got a PDMS plug and a PDMS adapter with different kinds of channels. Secondly, we partly inserted a SPHMC into one side of the PDMS adapter to fabricate a female SPHMC. Because the diameter of the channels inside the PDMS adapter (25-gauge 0.51 mm) is smaller than that of the needles used on SPHMC (22-gauge 0.73 mm), the PDMS plug and the PDMS adapter can provide a good seal (at least 30 psi, ESI†). Finally, the nanoliter sample injector was manufactured by fixing five 7 mm glass capillaries (300 μm ID, 700 μm OD), whose inner volume is about 500 nl, on the other side of the female SPHMC.

## Application

### SPHMC for parallel droplet generator

We demonstrated a two row SPHMC, which can tolerate 45 psi (ESI†), using a parallel, flow-focusing droplet generator made by a one-step forming method (ESI†). In our droplet generator, five independent droplet generation units were used to assemble it. Each unit had an aqueous inlet, an oil inlet, and an outlet. As shown in Fig. 3, by plugging the SPHMC into the chip, all the ten inlets can be set up at one time and the density of it is relatively high (ten fluidic I/O in 63.5 $mm^2$). After that, mineral oil was injected into the first row of the connector as the continuous phase and food dye was injected into the second row as the dispersed phase. Air pressure was exerted to drive both food dye and mineral oil. A droplet could be generated through five identical units, provided the driven pressure of the food dye and the mineral oil was maintained at 3.5 psi and 11 psi, respectively. Compared with traditional single needle connectors, the time of the setup process decreased significantly and each tube was labelled by color to avoid confusion.

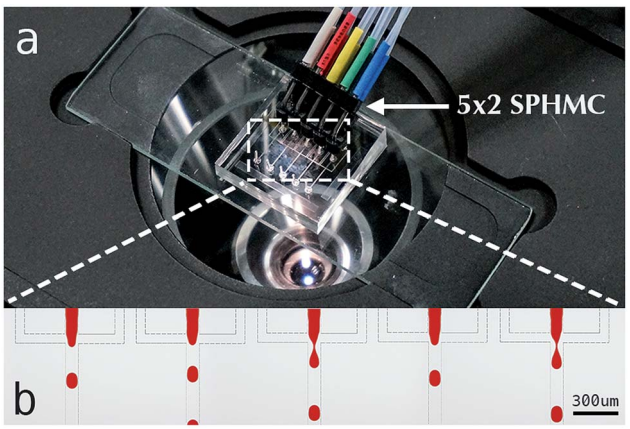


Fig. 3 Application of microfluidic pin-header connectors. A ten pin two row pin-header connector was fixed on a five-way droplet generator (a). The width of the channel was 100 μm and droplets could be generated through the device (b).

### Nanoliter sample injector for rare sample injection

Although the SPHMC described above was convenient and reliable, when it was used in some situations requiring low volumes of liquid, for example, PCR, ELISA and protein detection, most of the solution remained in the tubes. We present a plug-and-play nanoliter sample injector, based on glass capillaries, which also conforms to the standard of 2.54 mm spacing. To demonstrate this connector, we made a valve controlled microfluidic device, which required only 100 nl samples for protein assay. The capillaries of the nanoliter sample injector were first soaked in the blood sample. Since the length of the capillary (300 μm ID) is 7 mm, about 500 nl blood sample was drawn up using capillary action (Fig. 4a), and then the injector was plugged into the inlets (Fig. 4b). A pressure of 7 psi was utilized to push 100 nl samples into the chip by dead-end filling[21] (Fig. S2†). Compared to the chip-to-world interface with a small reservoir (1–20 μl),[22] the dead-volume of the samples decreased significantly. Moreover, by using glass capillaries with a lower inner diameter, the dead-volume of the samples could be further reduced.

### PDMS plug and female SPHMC for bubble removing

Besides these pin-header microfluidic connectors, we developed two 2.54 mm standard accessories: the PDMS plug and the female SPHMC, which could cooperate with a SPHMC, to quickly remove air bubbles. One of the common methods to remove air bubbles is dead-end filling, but it requires the fabrication of an additional valve to form a dead-end channel. Here we improved the dead-end filling approach and replaced the valves with our PDMS plugs. Such an improvement simplified both the microfluidic device and the control system. We demonstrated these accessories by using a circulating tumor cell (CTC) trapping device, which separate CTCs from blood cells by size. The device was based on our previous work and the gap between the dam and the bottom was 5 μm.[23]

The chip was first primed by PBS to fill channels and to remove gas bubbles. As shown in Fig. 5a, a SPHMC with no tubes was plugged into the outlets and was linked with a PDMS plug to form dead-end channels. A pressure source of 15 psi

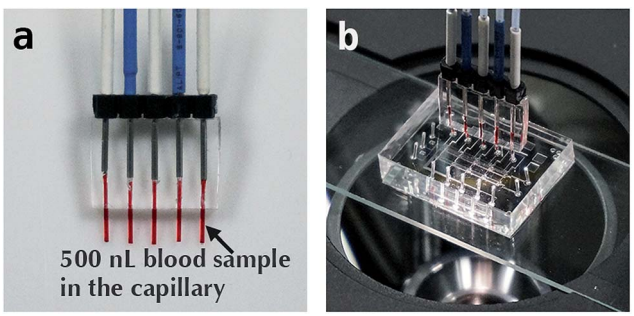


Fig. 4 500 nl blood samples were drawn up using capillary action (a). The nanoliter sample injector was plugged in the inlets (b).

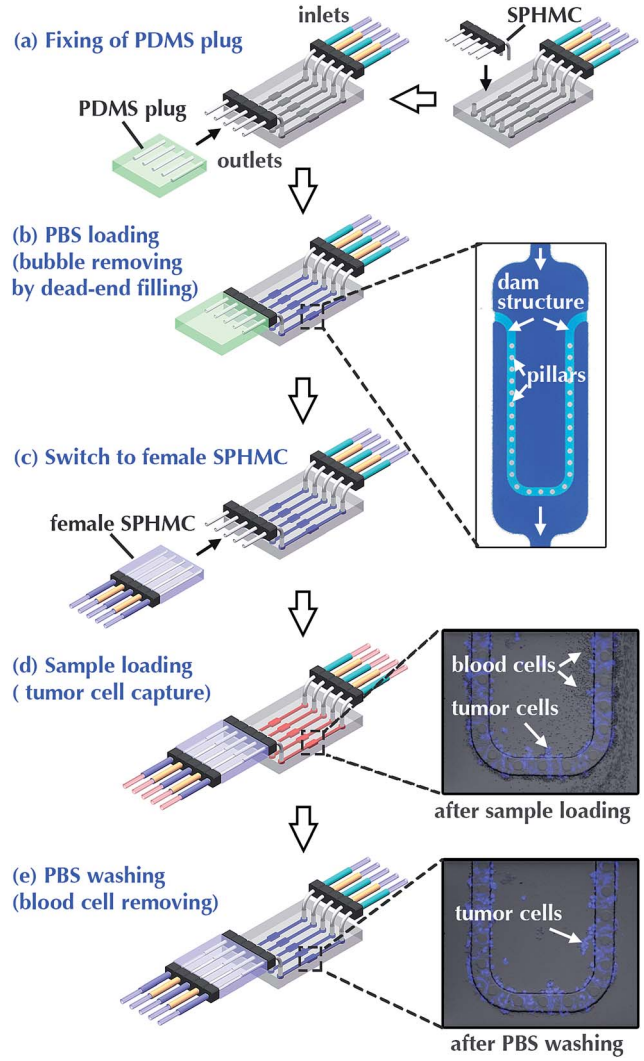


Fig. 5 Process flow for tumor cell trapping experiment. A SPHMC without tubes was plugged into the outlets and connected with a PDMS plug to form dead-end channels (a). PBS (shown in blue) was loaded into the chip and the air bubbles were removed. In our device, the main structure was a dam, which was supported by several pillars (b). After all the channels were filled with PBS, we replaced the PDMS plug with a female SPHMC (c). Blood samples (shown in red) were loaded into the chip. The results showed that tumor cells could be trapped, which were stained with DAPI, and most of the blood cells could move across the dam due to their small size (d). PBS flowed through the device and flushed remaining blood cells out of the chip (e).

was applied to force PBS (blue dye) to fill the chambers. In the process of dead-end filling, several air bubbles were trapped and were removed within 3 min (Fig. 5b, Movie S1†). Such an operation eliminates the pressure imbalance caused by bubbles and reduces the damage and death of CTCs in the later capture process. Compared to long-term injection and vacuum filling method,[24] our approach is time-saving and does not require extra equipment. After all the channels have filled with PBS, we substituted a female SPHMC for the PDMS plug rather than directly removed the male SPHMC (Fig. 5c) to avoid generation of new bubbles during fixing or removing connectors. Whole rat blood diluted in PBS and spiked with cultured MCF-7 cells was loaded into the device through a SPHMC (Fig. 5d, Movie S2†).[25] When all the blood samples had flowed through the device, PBS was used to flush the chip to remove the blood cells left in the device (Fig. 5e). The trapping efficiency was about 85%. Some of the MCF-7 cells transformed and moved across the gap due to the elasticity of PDMS. The capture efficiency could be improved by increasing the capture area of the device and by adjusting the flow rate. In summary, this improved bubble removing method reduces time consumption, simplifies chip structure and avoids introducing new bubbles during the exchange of connectors.

## Conclusion

We have illustrated a standardized, low-cost and multi-functional microfluidic connector system based on the existing standard of a 2.54 mm pin-header connector employed in electronic circuits. The manufacturing process of our standard pin-header microfluidic connectors is compatible to the current pin-header production process, so it is possible to mass-produce our standard connectors and reduce the cost. We successfully demonstrated that our connectors and accessories could simplify the experimental setup in parallel droplet generation, significantly reduce the dead-volume of samples, and quickly remove air bubbles trapped in a CTC capture device. All the connectors and accessories can tolerate at least 30 psi (ESI†), which is beyond the requirement of most microfluidic application. Furthermore, we introduce a method that is based on a reusable pin-header mold casting all the inlet holes and channels at the same time.

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