Three-dimensional fit-to-flow microfluidic assembly

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Three-dimensional microfluidics holds great promise for large-scale integration of versatile, digitalized, and multitasking fluidic manipulations for biological and clinical applications. Successful translation of microfluidic toolsets to these purposes faces persistent technical challenges, such as reliable system-level packaging, device assembly and alignment, and world-to-chip interface. In this paper, we extended our previously established fit-to-flow (F2F) world-to-chip interconnection scheme to a complete system-level assembly strategy that addresses the three-dimensional microfluidic integration on demand. The modular F2F assembly consists of an interfacial chip, pluggable alignment modules, and multiple monolithic layers of microfluidic channels, through which convoluted three-dimensional microfluidic networks can be easily assembled and readily sealed with the capability of reconfigurable fluid flow. The monolithic laser-micromachining process simplifies and standardizes the fabrication of single-layer pluggable polymeric modules, which can be mass-produced as the renowned Lego[®] building blocks. In addition, interlocking features are implemented between the plug-and-play microfluidic chips and the complementary alignment modules through the F2F assembly, resulting in facile and secure alignment with average misalignment of 45 μm . Importantly, the 3D multilayer microfluidic assembly has a comparable sealing performance as the conventional single-layer devices, providing an average leakage pressure of 38.47 kPa. The modular reconfigurability of the system-level reversible packaging concept has been demonstrated by re-routing microfluidic flows through interchangeable modular microchannel layers. © 2011 American Institute of Physics. [doi:10.1063/1.3670368]

I. INTRODUCTION

Microfluidic devices, enabling miniaturization and integration of controlled physical and chemical microenvironments, have emerged as powerful instruments for investigating biological phenomena at cellular and molecular scales.^{1,2} In particular, the latest advancements on three-dimensional microchannel networks provide new functionality for sophisticated and versatile fluidic manipulations,³ which can be extended to a wide range of biological and biochemical investigations, such as addressing challenges of microscopic fluidic manipulations (mixing and multi-phase fusing),^{4–6} building complex microenvironments with multiple cellular, and molecular components,^{7,8} devising paper-based assays for point-care diagnosis⁹ and constructing synthetic networks for biological computing.¹⁰ Additionally, large-scale microfluidic integration—analogue to the development of modern electronic circuitry—enables digital fluidic operations (performing logic functions)^{11–14} and multi-tasking capacities (completing an intricate biological protocol)^{15,16} leading to a significantly expanded portfolio of emerging microfluidic applications.

Despite the rapid growing trend in integration of biological experiments on miniaturized substrates, several technical roadblocks persistently preclude successful translation or adaption of the promising microfluidic toolsets for solving biological challenges. Reliable system-level packaging, multilayer alignment, as well as world-to-chip interfaces are among a few long-standing problems

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in the to-be-solved list.^{17–19} For instance, a majority of microfluidic devices fabrication, deriving from soft lithography-based techniques,²⁰ exploit an irreversible packaging scheme, i.e., oxygen plasma-activated polymer surfaces forming covalent links upon intimate contacts. The plasma-based bonding offers reasonably good repeatability and adaptability; however, it is incompatible with either the standard alignment strategy or biological processing requirements. Simon and his co-workers introduced a reversible packaging technique employing web-like vacuum channels to surround and seal targeted microfluidic channels without any thermal, chemical, or optical treatment to study leukocyte adhesion under shear flow.²¹ More importantly, the technique can be applied to any single layer of soft lithography-molded devices and is capable of integrating a biological surface with microfluidic environments on demand. Magnetic forces were also used to establish reversible seal of microfluidic devices, which can be highly adaptive to a broad range of biochemically functionalized substrates.^{22,23} Recently, our group proposed an automatic packaging process using capillary bridge to self-align and self-engage two polymer surfaces with chemical activations, offering a facile solution to simultaneously align and seal multilayer microfluidic structures fabricated by photolithography or soft lithography.²⁴ Although the latest efforts have been attempted to deliver innovative solutions to address different aspects of microfluidic packaging for biological use, none of the above mentioned offers a systematic approach to overcome the challenges in bio-friendly sealing, multilayer construction and alignment, reversible packaging, and world-to-chip connectivity as a whole.

The movement towards modularizing lab-on-a-chip components (mixing, multiplexing, separation, etc.) provides a standardized user-oriented interface for chip-based fabrication and assembly while providing customization and flexibility in microfluidic designs and operations for the desired applications.²⁵ Pre-fabricated microfluidic assembly modules, resembling Lego[®] building blocks, have been developed to provide simultaneous assembly and bonding.^{26,27} The concept of plug-and-play microfluidics is attractive due to its simplicity to quickly establish connectivity.^{28,29} Recently, we have introduced a modular plug-and-play interconnection, known as fit-to-flow (F2F), to address world-to-chip microfluidic interface, analogous to the ubiquitous Universal Serial Bus (USB) architecture in computer peripherals. One scheme of fit-to-flow connections utilizes surrounded vacuum networks to secure fluidic passage from a tube assembly to microfluidic chip plug-in with excellent sealing performance.³⁰

In this paper, we have successfully extended the established F2F concept from an interfacial connection scheme to a complete packaging scheme, which facilitates the assembly of integrated three-dimensional microfluidic networks. The newly proposed F2F assembly design consists of an interfacial chip with tube connections, pluggable alignment modules, and multiple monolithic layers of microfluidic channels with interlayer vias. It allows for an easily aligned and readily sealed convoluted three-dimensional microfluidic network that provides potential reconfigurability of fluidic flow patterns. In addition, all the microfluidic components are laser-micromachined on monolithic polymer substrates to fit a standard single-layer modular design, which enables connections in a plug-and-play fashion to be mass-produced. Moreover, interlocking features in each layer, complementary to the alignment modules, permit precise, secure, yet simple alignment operations to the multilayer constructs. Figure 1(a) illustrates the F2F assembly of a three-dimensional reconfigurable microfluidic chip, comprised of multilayer polydimethylsiloxane (PDMS) microfluidic networks. As shown in Figure 1(b), an extended vacuum aspiration network seals around the entire microfluidic passages from the interfacial tubular parts to individual fluidic layers. In each PDMS layer, the surrounding vacuum network with negative suction pressure supplies adequate reversible adhesion to hold the multilayer assembly together as the positive pressure-driven flow passes through the chip.

II. EXPERIMENTAL

A. Laser micromachining

All microfluidic components, including the individual layers of microchannel networks and through-hole accesses, the world-to-chip connection with external microtube assembly, and the pluggable alignment blocks, were laser micromachined from PDMS (Sylgard 184, Dow Corning)

FIG. 1. (a) An assembled reconfigurable F2F device with world-to-chip interface, alignment modules, and 3D flow patterns (scale bar: 5 mm). (b) A close-up view of the vacuum networks which seal the fluidic channels in every layer (scale bar: 1 mm).

with different thicknesses coated on glass substrates. PDMS pre-polymer was first mixed at a 10:1 weight ratio of the base to the curing agent and following the spin-coating step cured on a hotplate at 120 °C for 10–15 min. A pulsed CO₂ laser engraver (VersaLaser, Universal Laser System) at a low power setting was used to directly produce laser-etched micropatterns on the polymeric layers, of which the etching profiles can be controlled by fine-tuning of laser engraving parameters through a graphic user interface software (e.g., CorelDraw or Photoshop). The two main adjustable parameters for laser micromachining are power level and raster speed. Detailed characterization and optimization of laser micromachining on the same desktop laser module can be found in a previous report from Liu and coworkers.³¹ In our standardization protocol, we applied two low power/speed settings (0.4 W/1.4% speed and 0.1 W/1.8% speed) to create microfluidic channels of 200 μm in height and 350 μm in width and vacuum aspiration network channels of 70 μm in height and 100 μm in width, respectively, in 400 μm thick PDMS layer. Increased power settings (0.8 W to 1.5 W) at a low speed (1.4%) are used to generate interlayer vias (350 μm in diameter) and to accurately laser-etch borders of each layer for pluggable alignment. The minimal resolution (of 30 μm) of the laser-micromachined building blocks are limited by the operational optical wavelength of the laser engraver and material properties (e.g., thermoset or thermoplastic).³¹ All the components can be processed in a standardized monolithic step for the following microfluidic assembly.

B. Fit-to-flow assembly

Figure 2 schematically illustrates the principle of 3D fit-to-flow assembly. It begins with placing a base layer of PDMS microfluidics on a planar surface (e.g., glass slides), as shown in

FIG. 2. Schematic illustration of the F2F assembling process: (i) placing the base layer on the substrate; (ii) plugging in the alignment modules; (iii) stacking additional modular microfluidic layers; and (iv) sealing a world-to-chip interfacial piece with tube connections from the top.

Fig. 2(i). In the following step, two alignment modules, each of 1.0 mm in thickness, are positioned at the diagonal corners of the microfluidic layer, of which the borders have been laser-etched to complement that of these alignment blocks, Fig. 2(ii). Two geometric designs, a stair-like and an arrow-shaped structure, with different dimensions have been incorporated to assess the precision of the alignment and the easiness to implement. Once the alignment blocks are in contact with the microfluidic base, additional PDMS layers can be subsequently stacked on top of each other using the thick alignment blocks as a reference and vice versa, Fig. 2(iii). Finally, the world-to-chip interfacial piece with tube connections (of $150\ \mu\text{m}$ inner diameter and $360\ \mu\text{m}$ outer diameter, Upchurch Scientific) is plugged in from the top to allow external injection of solutions for the intended fluidic operations and connection of vacuum networks to establish intimate seal among all layers and interconnections, Fig. 2(iv). After the completion of the layer-by-layer microfluidic assembly, external vacuum is applied on demand by a portable vacuum/pressure pump (400-3901, Barnant). Misalignments between microfluidic layers are measured based on the positioning of the interlayer alignment marks using an optical microscope (BH2-UMA, Olympus) equipped with a digital camera (EC3, Leica). Each aligning method has been tested on two identical packaging sets for 10 times each.

C. Leakage test

Blister tests were performed to evaluate the bonding performances in both the multilayer F2F configuration and the single-layer setup with an interfacial connection module. Testing fluid, a dyed aqueous solution, was injected by a syringe pump (KDS 100, KD Scientific) through the input port of the connection module, while the outlet was directed to a digital manometer (840081, Sper Scientific) from which pressure readings were recorded through a

RS232 computer interface. The syringe pump perfuses at a constant flow rate of $1.1 \mu\text{l/s}$ until a leakage spot is identified in a single-layer or multilayer assembly. The leakage test has been repeated for 5 times at each vacuum holding pressure.

III. RESULTS AND DISCUSSION

The standardized component fabrication combined with the reversible F2F packaging scheme offers modular connections and expansions of multilayer microfluidic networks, analogous to the well-known plug-and-play Lego mechanism. Furthermore, multilayer assembly utilizing alignment blocks with complimentary geometrical designs has been implemented for building three-dimensional microfluidics. The F2F microfluidic assembly enables a generally applicable method to devise stereo-microflow networks through a monolithic fabrication process of planar microchannels, which significantly reduces the technical threshold to perform the three-dimensional and large-scale integrated fluidic manipulations.

A. Assembly and alignment

An important measure of the F2F microfluidic assembly is the degree of interlayer misalignment as the device is vertically assembled using two diagonally positioned alignment modules as a reference. Misalignment of the microfluidic assembly can lead to constricted flow path or complete fluid blockage between layers. In the former case, the misalignment-induced flow constriction can result in unexpected leakage, and therefore, the layer-to-layer misalignment needs to be characterized and minimized. Figure 3 shows characterization of the alignment performance for the F2F assembly scheme where two geometrical designs (arrows and stairs) at various scales have been utilized. The average misalignments of two PDMS microfluidic layers stacked using stair-like or arrow-shaped structures with the feature size of 1.0 mm were $66 \pm 24 \mu\text{m}$ and $69 \pm 25 \mu\text{m}$, respectively. Average misalignments of the same geometrical shapes (stairs and arrows) but with the feature size scaled down to 0.5 mm were improved to $46 \pm 21 \mu\text{m}$ and $45 \pm 18 \mu\text{m}$, respectively. In control experiments where no alignment blocks were involved during the assembly process instead only using the edges of the device as guides, an average misalignment of $68 \mu\text{m}$ was obtained with an extremely wide standard deviation of $\pm 53 \mu\text{m}$. Presence of the multilayer misalignment can partially attribute to the slant sidewall

FIG. 3. Characterization of multilayer alignments of the F2F assembly using stair-like or arrow-shaped plug-in structures in comparison with controls without alignment structures.

profile produced by laser micromachining.³¹ Moreover, although the microfluidic modular layers can form a tight fit with the diagonally positioned alignment blocks through careful adjustments, the internal stress or the high elasticity can cause further deformation on the thin layer structures, leading to additional misalignment. In brief, alignment capacities are found similarly in interlocking structures with different geometrical shapes but potentially scale with the structural dimensions accordingly. Further reduction in the dimensions of pluggable structures improves alignment precision in principle; however, it also introduces multiple complications to the manual assembly process, e.g., difficulty in plugging individual layers into the positioned alignment module, reliability of miniature alignment structures (wear and tear), as well as potential structural deformation. In addition, interlocking designs (the arrow-shapes) provide additional stabilization mechanisms to lock the layers into position prior to vacuum seal, which could be crucial in a multilayer layout. Overall, multilayer alignment precision can be further improved if the sidewalls of each modular block are perfectly straight, which can be accomplished by using high-aspect ratio photolithographic molds (e.g., SU-8).³² Previous investigation has shown that a maximum misalignment of $40\text{ }\mu\text{m}$ has been achieved in a 2D planar assembly system.²⁶

B. Leakage performance

As previously described, the performance of microfluidic assembly packaging has been evaluated through a series of blister tests in which an increase of internal microchannel pressure is achieved by slow infusion of a dye solution. Figure 4 shows the characterization of the maximal leakage pressure in single-layer or multilayer microfluidic networks at various vacuum pressures ranging from 30 to 70 kPa, in comparison with a vacuum-free control, of which the interlayer adhesion solely relies on interfacial molecular interactions (e.g., hydrogen interactions or Van de Waals forces).³³ As expected, a linear correlation is observed experimentally between the external vacuum source and the sealing performance. Qualitatively, a more negative pressure applied through the vacuum networks leads to a stronger interlayer adhesion surrounding the fluidic passages, and as a consequence, results in a higher leakage pressure. Furthermore, the maximum average leakage pressure of $39.46 \pm 1.41\text{ kPa}$ is obtained in a single-layer device, whereas the multilayer assembly of microfluidic networks has a similar maximal average leakage pressure of $38.47 \pm 2.37\text{ kPa}$. Our results suggest that the assembly of multiple PDMS microfluidic layers can be reversibly sealed by vacuum suction with comparable performance to traditional single-layer microfluidic devices operating under identical sealing principles.³⁴ Fluidic leakage usually occurs when the hydrostatic pressure built up within the microchannel exceeds the vacuum holding force, which can be caused by high flow rate and/or channel constriction from multilayer misalignment. In particular, the leakage can originate from any point along the flow passage but has higher occurrence in the upstream region around the inlet where a higher pressure gradient presents across the layer (in this case, the leakage starts

FIG. 4. Characterization of the maximal leakage pressure vs. vacuum pressure in both single-layer and multilayer configurations.

FIG. 5. Leakage behavior within microfluidic channels in a multilayer F2F assembly (scale bar: 1 mm). As expected, the leakage starts in the bottom channel, where the internal hydrostatic pressure is higher.

from the bottom channel), as can be seen in Figure 5. Once the fluid escapes to the vacuum network, it spreads throughout the network until the built-up hydrostatic pressure is relieved to reestablish vacuum seal. In addition, the through-holes in the middle layer are designed with extended dimensions to compensate potential layer-to-layer misalignments and to eliminate possible fluidic blockage. This ensured similar packaging performance between single-layer and multilayer configurations since pressure drop is considerably higher in the planar channels than that in the perpendicular through-layer vias. Another potential solution could be using PDMS modular layer with higher adhesiveness and/or higher conformability, which can be achieved through altering the processing time of PDMS crosslinking (adjusting curing ratio, temperature, time, etc.).^{35,36}

C. Reconfigurable microfluidics

The reversibility of three-dimensional packaging offers an appealing feature that would allow microfluidic systems to be reconfigurable or re-routable. Figure 6 shows transition from a

FIG. 6. Reconfiguration of a F2F assembly device from (a) a single zip-zag fluidic pattern to (b) two or (c) three inter-tangled flow streams (scale bar: 2 mm).

single zip-zag fluidic pattern to two or three inter-tangled flow streams on the F2F assembled microfluidic platform, of which the bottom microfluidic base layer is shared among the designs, but the top layers are interchangeable to switch the flow pattern. As shown in Fig. 1(b), the surrounding vacuum microchannel network provides an adequate adhesion strength (suction force) to keep hold of the positively pressurized fluid flow. As demonstrated, the modular packaging concept of the F2F assembly approach not only provides versatility to the three-dimensional microfluidic device platform but also enable individual part replacement as well as further expansion with multifunctionality. Besides its potential in standardization of 3D microfluidic packaging, the F2F assembly can also be extended to academic education of microfluidics in a regular wet laboratory setting, where the entire chip can be basically assembled in amateurs' hands with sufficient care, similar to the assembly of renowned Lego building bricks.

IV. CONCLUSIONS

In this paper, we have extended the plug-and-play interconnection concept, known as F2F, from a world-to-chip microfluidic interface to a versatile packaging scheme for reversible and reconfigurable assembly of three-dimensional microfluidic systems. The F2F assembly, consisting of a world-to-chip interfacial layer, two diagonally located alignment modules, and monolithically fabricated modules of microfluidic channels, proves advantageous for rapid prototyping and modular integration in a standardized, mass production environment. In particular, the multilayer F2F microfluidic packaging with easy yet accuracy assembly allows complex and multiplexed three-dimensional fluid operations as well as large-scale fluidic integration. In addition, the packaging scheme is realized by using a highly adaptive modular architecture with simple pluggable planar components, which provides sufficient consistency in its repeatability and performance. The modality of the F2F technology has been demonstrated through re-routing parts of the microchannels to form reconfigurable fluidic patterns in a 3D fashion. The presented F2F microfluidic assembly can be extended to high-throughput biological analysis and large-scale biochemical synthesis as well as serves as an effective technological education toolkit of microfluidics and nanomedicine to the general public and high school students.

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