Development of Immunoassay Microfluidic Chip Using Serial-flow Method

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ABSTRACT

A serial-type microfluidic chip toward immunoassay-on-chip is presented. The proposed chip contains no active microvalves and micropumps, thus low cost and disposability can be achieved. Complexity of fluids control scheme as well as the cost per unit test can significantly be reduced due to the serial flowing of fluids. Some side-effects for this type of chips, including inactive valve after valve wetting, branch flow and undesired interaction of different fluids, are eliminated by the design of away-valves and spontaneous air-plug, respectively. Experimental testing shows that multi fluids can be controlled and transported serially with volume loss less than 6%, demonstrating the feasibility of the proposed design. The proposed chip design can provide a practical tool for immunoassay testing, and contribute to the point-of-care diagnostics and basic researches.

Keywords: Serial flow, Microfluidic, Immunoassay, LIGA-like process

1 INTRODUCTION

A serial-flow type microfluidic chip toward immunoassay-on-chip is reported. Enzyme-Linked Immunosorbent Assay, or ELISA, is a useful and powerful method in estimating very low amount of materials in the solution. Applications of ELISA are range from basic researches to diagnostics of clinical diseases. In general, a large number of repetitive steps are involved in an immunoassay, and are often carried out with labor-intensive techniques. As such, the advantages in automation and reaction rates offered by microfluidics are particularly well suited to this application [1-3]. For a process automation to reduce the high time and labor costs of ELISA, most existing immunoassay chips exploit multiple active microvalves or micropumps to control the fluids [4-8]. In addition, many researchers have reported on the integrated ELISA chip [9-11], in which micro pumps for driving fluids are embedded. In order to reach this object, the fabrication processes inevitably become more complex. In addition, due to many different liquid reagents to be processed with definite sequence and time, many pumps and valves within the chips must be controlled simultaneously in a proper manner. It may result in complex control schemes and raised chip cost. Accordingly, the cost per test often rose as a result.

In this paper, a microfluidic chip utilizing serial-flow method, which can perform immunoassay with a minimum control effort, is demonstrated. The method is basing on a microfluidic chip design with specific fluid network and an off-chip pumping method. The microfluidic chip contains no active microvalves and micropumps, thus low cost and disposability can be achieved. Only one reusable micro membrane-pump is required, so that the system can be operated with more economic benefits. Several functions required for an ELISA process, such as performing repetitive steps involved in an immunoassay, fixed-volume transformation, and control of flow sequence, are tested in this work to demonstrate the feasibility of the proposed design.

2 DESIGN OF FLUID SYSTEM

2.1 Valving of fluids

To control the flowing of fluids, micro valves with on/off controllability is required. In this work, surface tension force is exploited to automatically draw fluids from an injection channel and stop them at a desired position. In order not to increase the system complexity, the passive geometric-valves, or capillary burst valves [12-14], are exploited in this work. These stop valves can simply be constructed by expanding the cross section of channels abruptly, as shown in Fig.1. The fluids driven by surface tension force will stop flowing when their flow-front encounters a stop valve, where a capillary pressure barrier is generated at the air-liquid interface. Such passive valves provide a simple way to precisely position the location of fluids in microfluidic device.

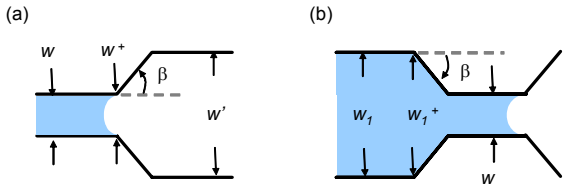

Figure 1: Top view of a geometric-valve. (a) expending type (b) shrinking type (channel depth is *h)*

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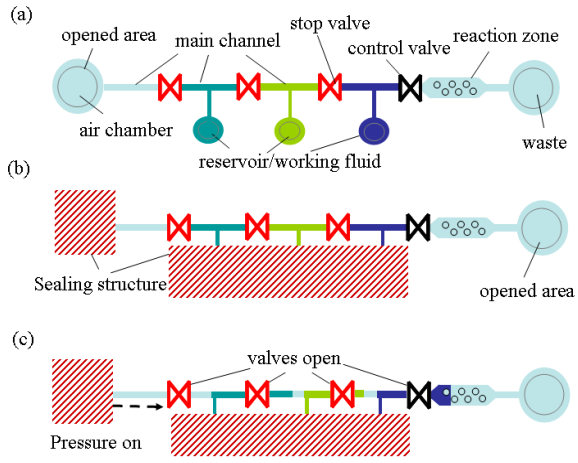

Figure 2: Illustration of the serial-flow method (a) fluids filling (b) sealing (c) pumping.

To generate geometric valves with different pressure barrier, it is feasible by changing the geometry of the valves. Refer to Fig.1, the pressure barrier of a valve can be approximated as

$$P_{\text{barrier}} = \frac{2\gamma_{la}\left[w\cdot\cos(\theta_c)+h\cdot\cos(\theta_c+\beta)\right]}{w\cdot h} \quad (1)$$

where γ_{la} and θ_c are surface tension and contact angle of the working fluid, respectively. To obtain a high pressure barrier, a simpler way is to shrink the neck-width w or increase the valve angle β.

According to eqn.(1), the hydrophilicity between working fluids and chip material must be clearly known before designing a stop valve. We chose PMMA as chip material and tested the contact angle of different fluids involved in an ELISA test. It reveals that the stop valves must be able to stop various reagents with contact angle less than 62° from flowing. In serial flow operation, however, the later fluid flow through valves which are already wetted by the former fluid is inevitable. The hydrophilicity of fluids would increase dramatically after wetting. As a result, the pressure barrier of a wetted stop valve becomes very small even for the valve with very large valve angle β. Increase pressure barrier by significantly shrinking the neck-width w is also unworkable

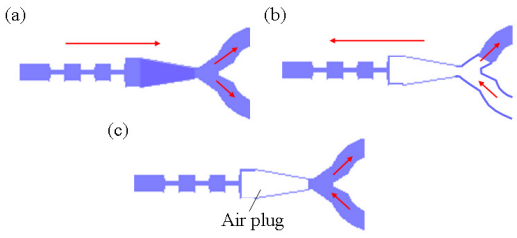

Figure 3: Formation of self-generated air-plug (a) fluid filling (b) air forced into the injection channel (c) air-plug formation.

since side effects such as generation of bubbles and corner flow of fluids would occur. Through investigations for the flow phenomenon in the vicinity of stop valves, it is confirmed that fluids tend to wet the stop valves either when entering or leaving them. In other words, the flow-front of fluids tends stay at the wetted stop valves when they are pushed away. Accordingly, we propose a design for control-valve with sufficient pressure barrier whether the valves is wetted or not. Instead of the conventional valves to keep fluids from entering them, the control valves are designed as the "away-valves" to keep fluids from leaving them. Since the valve property will not change significantly after channel wetting, neither the valve angle nor the neck-width of valves can be designed with reasonable parameters without side effects. On the contrary, for the stop valves only used for positioning multi fluids when they are first loaded into the main channel, it is undesired to keep the fluids from subsequent transporting. For this purpose, stop valves with slit shape are exploited to render themselves one-time-use valvs, thus undesired flow stopping can be eliminated.

2.2 Serial-flow type microfluidic chip

To minimize the pumping source and control efforts, a serial-flow method to control the flow sequence is proposed and illustrated in Fig.2. The most significant part of the chip is a main channel, which is divided into many segments by geometric stop valves. The working fluids are loaded to the reservoirs and drawn into a corresponding segment by surface tension force, as shown in Fig.2(a). The working fluids are pre-arranged in a desired order. A pump chip is then pushed toward the microfluidic chip to temporally seal the openings such as air chamber, and reservoirs [15], as shown in Fig.2(b). By applying a sufficient driving pressure to the sealed air chamber, working fluids would break through stop valves and be transported one by one to the reaction zone, as shown in Fig. 2(c). The mentioned control-valves are exploited to decide whether the fluids should be transported further or stand still for incubation. As a result, a single pump with minimal control effort is required to perform this operation. Assays with different flow sequence can be performed by pre-arranging working fluids in a desired order.

One important issue to perform the serial-flow is to keep the fluids transport with fixed-volume. However, it is found that a partial amount of fluids might flow back through the injection channels connected with the main channel. It is due to the finite reservoir-sealing effect. This "branch-flow" will result in serious volume loss as well as pre-interaction between different fluids. To fix the branch-flow problem, a special entry valve at the injection channel is proposed. Referring to Fig. 3(a-c), an air plug, i.e. the air trapped in-between the injection channels, would be spontaneously generated during fluids transportation. The liquid-air interfaces at both ends would stably confine the air-plug at that region. With the aid of air-plug separation,

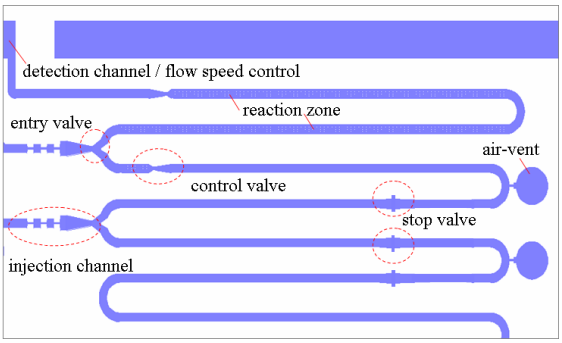

Figure 4: Proposed serial-flow type fluid-network.

the branch-flow as well as volume loss can effectively be suppressed. Undesired interaction can also be minimized. Fig. 4 shows the design of the proposed serial-flow type fluid-network.

3 FABRICATION OF MICROFLUID CHIP

Since no active components are needed for the microfluidic chip, plastic material such as PMMA can be adopted and structured by hot-embossing method. A LIGA-like process with mass production capability is developed to replicate plastic chips. SU-8, NiCo alloy, and PMMA are exploited as the material of mold template, metal mold, and plastic chip, respectively. Typical fabrication results of metal mold and PMMA chip are shown in Fig. 5. SU-8 residue left in the metal mold is easily removed since the mold template is immersed into a surfactant before being electroplated. Parallel demolding must be guaranteed during the demolding step to acquire patterns with fewer defeats. Some feathers such as the channel, the stop valve, and the air-vent are visible in the figure. Channels with high wall verticality are obtained by this method. It enables the feasibility of in-plane optical absorbance detection. Micro pillars with small dimension are successfully realized within the micro channel, as also shown in the figure. The increased surface area is helpful in enhancing the reaction

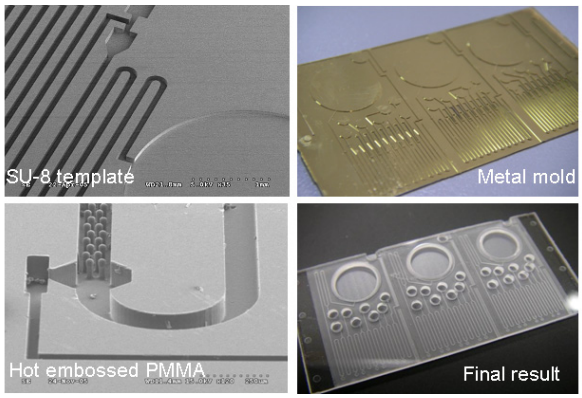

Figure 5: Results of LIGA-like process for batch fabrication of plastic chips

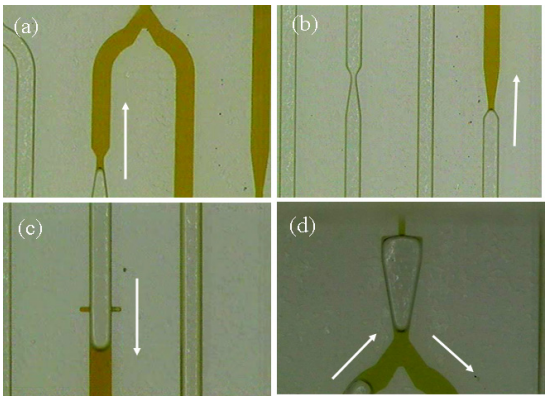

Figure 6: (a)(b)Fluid passing through and stopped by the control valve (c) Fluid passing through the one-time-use stop valve without any staying (d) Fluid passing through the air-plug, without interacting with the fluid within the injection channel.

such as protein binding rate due to increased surface-to-volume ratio.

4 FUNCTION TESTING

In this work, some experiments were conducted to demonstrate functions of the proposed design. Tincture of iodine, with its contact angle close to that of serum, was used as the working fluid. Figure 6 shows fluids passing through the stop valve for one-time-use, the control valve for fluid-leaving control, and the entry valve with an air-

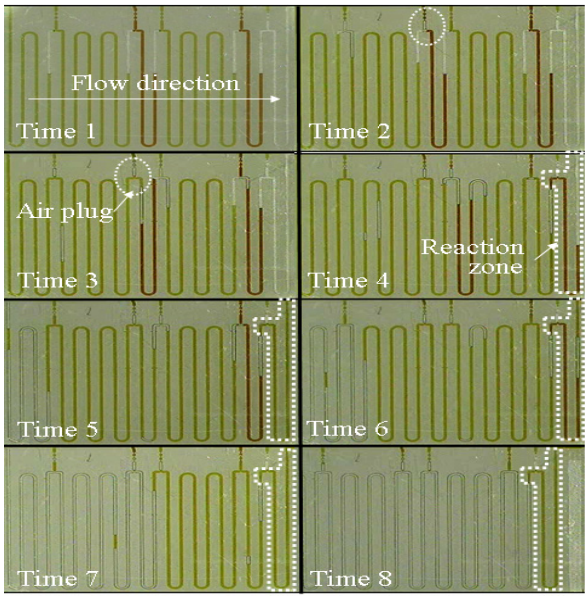

Figure 7: Progress of serially transporting of multi-fluids. Time1 to Time3: procedure of air-plug forming. Time4 to Time8: multi-fluids transporting and stay at the reaction zone, respectively.

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plug trapped in the injection channel, respectively. Both qualitative and quantitative testing results have revealed the effectiveness of the proposed designs. The serially transporting of multi-fluids are snapshot from a video and shown in Fig.7. With the design of air-vents, multiple working fluids could fill into their corresponding segments in the main channel. The channel length each fluid occupied is proportional to its volume. Since the order of these fluids could be arranged specifically, they could be transported with desired flow sequence certainly. Multi-fluids successfully flow serially in the main channel without being intervened by the injection channels. Each fluid can be stopped by the control valve with a desired holding time. According to the length change during the fluid transportation, the volume loss can be less than 6%. The fluid operations facilitate the chip to perform the procedure of on-chip immunoassay. The detection of HPV16 E7 protein for cancer diagnosis using this chip is undergoing.

5 CONCLUSION

ELISA chip can automatically perform a large number of repetitive steps involved in an immunoassay, and save the costs for laboring and processing time. In this work, a serial-type microfluidic chip for immunoassay is reported. In contrast to many other reported ELISA chip, the proposed microfluidic chips could be fabricated by hot embossing or injection-molding, manifesting their low cost and disposability. The complexity of control as well as the cost per unit test can significantly be reduced. Some side-effects for this type of chips, including inactive valve after valve wetting, branch flow and undesired interaction of different fluids, are eliminated by the design of away-valves and spontaneous air-plug, respectively. Experimental testing shows that multi fluids can be controlled and transported serially with volume loss less than 5%, demonstrating the feasibility of the proposed design. In sum, the proposed chip is capable of saving more control effort and fabrication cost. It is believed that the demonstrated chip can provide a practical tool for immunoassay testing, and contribute to the point-of-care diagnostics and basic researches.

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