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A novel bio-microcircuit for bio-assays

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Abstract: In this study, a novel micro-system made of a fluid circuit board and various functional components, inspired by the integrated microcircuit technique was developed. The circuit board was fabricated on a thermoplastic sheet using two-dimensional (2D) cutting technology. The functional components, including mixers, dilutors, reactors, pumps, retreaters, and detectors, were fabricated via paper (or membrane) cutting/folding and integrated into this system, similar to the way that electronic components were integrated into a micro-integrated circuit board. This system validated by the rapid detection of human immunodeficiency virus (HIV) and starch catabolism process would potentially be another microfluidic system for the analytical science.

Keywords: Paper microfluidics, Human immunodeficiency virus (HIV), Detection

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Microcircuits are a type of micro-integrated electronic system that combines transistors, diodes, resistors, capacitors and inductors, in which, the driving force from the voltage is applied to the electrodes were transported into the circuit to activate the microelectronic components, performing series of electrical functions. This microcircuit system could rationally be extended into the biological sciences.^{1,2} In this study, a novel concept of micro-system is developed, which transports, manipulates and processes bio-molecules (amino acids, sugars, fatty acids, nucleotide acids, inorganic salts, water and even bio-electrodes) through vessels, micro-channels, and certain types of fiber substrates, and then perform various biochemical reactions and complete fully integrated bio-functions.³ We called this kind of micro-system bio-microcircuit (or another type of microfluidics).

There are some other techniques for the fabrication of micro-channels, for example, photolithography,^{4,5} plotting,⁶ plasma treatment,^{7,8} ink jet etching/printing^{9,10} and wax printing^{11,12} et al., while in this work, we made use of the 2-D cutting technique to construct the micro-channels and transport the liquid by the capillary force. Most traditional microfluidics, based on the typical fabrication methods realize their functions via special planar and homogenized micro-channel structures. However, our proposed system (Bio-microcircuit) could be much more flexible and extendable.

This type of system is already found in nature in the blood circulatory system (where blood cells and other substances are transported through the blood vessels by the force of the heart's movement to maintain life movements),¹³ nerve conduction system (where bio-electrodes are transported along nerve axons by electrical stimulation to complete series of neural responses),¹⁴ and in the plant transportation system (where water and nutrients are transported through the plant vessels by the pulling force of transpiration).^{15,16}

In this project, we imitate the microelectronics and life biology, describe a novel micro-system and its construction using cutting/folding technology and demonstrate its application in the rapid detection of the human immunodeficiency virus (HIV) and starch catabolism .

RESULTS AND DISCUSSION

Schematics of this novel micro-system

This micro-system composed with a circuit board and various types of functional components. The circuit board was a flat substrate on which either vessels or channels (instead of the electrical wires in microcircuit system) were constructed and used to transport bio-substances. The functional components were fabricated and assembled in a certain way on the circuit board to realize a particular biochemical function.

A two-dimensional (2D) cutting technology was applied to construct the circuit board (Figure 1).^{17, 18} This technology utilized a cutting machine that incorporated a shape blade in place of the traditional printing/plotting pen in desktop printers/plotters. The blade rotated freely on a turret, enabling precise cutting of various patterns on a flat substrate using the computerized X-Y knife cutter software (Circuit Expression® 2, Provo Craft & Novelty, Inc., USA).¹⁹ This flat substrate could be thermoplastic plates, paper, polymers, membrane or other porous materials in which different channel/vessel depths could be constructed and patterned by adjusting the blade angle and downward force. In this study, a thermoplastic sheet was used. The depth and width of the micro channels were approximately 300 μm and 250 μm , respectively. These micro-channels generated were embedded in the thermoplastic plates, and could be sealed by tape if necessary, to transport the liquid.

The ink of red color was applied to demonstrate that the fluid could be transported in this micro-system. This pattern of micro-channels on the thermoplastic sheet could also be sealed with plastic tapes and then the fluid could be driven by a pumping force.

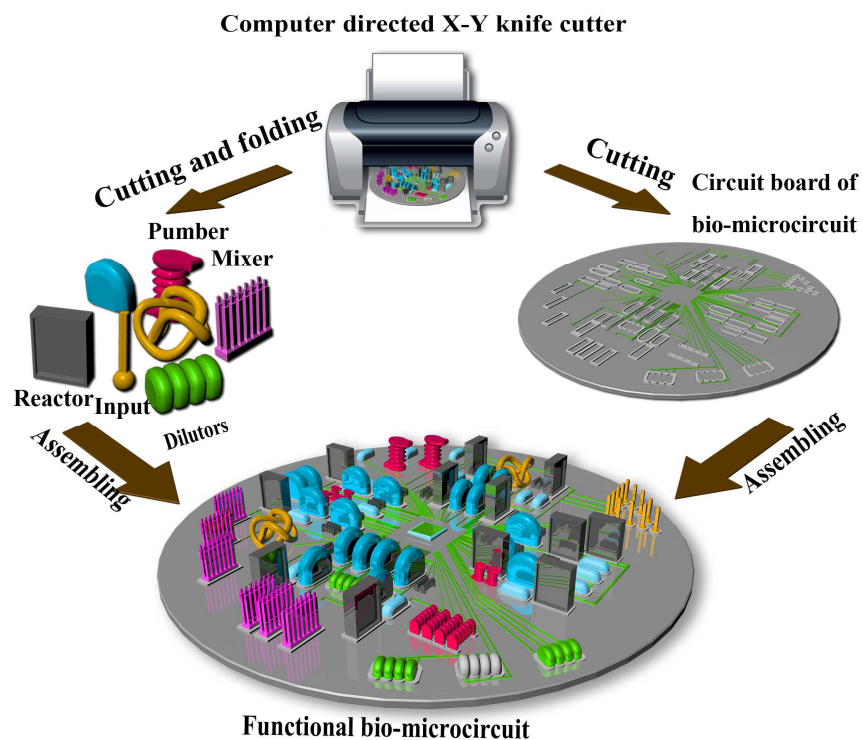


Figure1. Schematic illustration of the fabrication of this novel system. Computer-directed X-Y knife cutting machine is used to construct the circuit board and various types of functional components.

Fabrication of functional components

In addition to the circuit board, various types of functional biological components were constructed by cutting and folding technology, including sample inlet, sample pre-treater, mixer, separator, dilutor, reactor and detector. These analogues to electrical components (resistors, capacitors and transistors) can be used to carry out various functions in this novel micro-system (**Figure 2**). The sample inlet was constructed directly by cutting a spiral-like micro-channel in a thermoplastic sheet, of which the sample loading depended primarily on the dimensions of the spiral. As illustrated in **Figure 2a**, different sample volumes of 2 μL (red ink), 4 μL (yellow ink), 6 μL (green ink) and 10 μL (blue ink) were loaded at 2 mm, 4 mm, 6 mm, and 10 mm of the spiral, respectively, and were transported into the micro-system by the capillary force. The sample was pretreated with a square piece of cellulose paper coated with a pretreatment reagent. As shown in **Figure 2b**, a sample of green ink was transported from the input and became dark within 15 s, after processing by the pretreatment. Other pretreatments, such as a pH/ionic strength adjustment, impurity removal and interference shielding, could all easily be performed in a similar way using this component. Various mixers were designed in this study, including ellipsoid, sugar-coated haws-like and three/four-member ring-like mixers. The four-member ring-like mixer performed the best out of all of the designs. **Figure 2c** illustrated that two streams of green and red ink were simultaneously driven into the mixer, and the dark ink flowed out of the pretreater within 10 s, indicating that the red and green inks had mixed together successfully. 3D mixers were also constructed by paper-cutting/folding, which appeared to be much more flexible than the 2D format (**Figure 2c**). The separator and dilutor components were used to separate and converge the fluids at a certain ratio. **Figure 2d** shows that the green ink could be diluted into series concentrations. This component was useful for diluting the original sample. Reactors were constructed to carry out biochemical reactions. A large surface-to-volume ratio and a high capacity for water retention are important for the performance of the reactor (**Figure 2e**). Detectors were constructed from a porous membrane and used to determine the unknown target in a sample. Various types of

detection techniques could be combined with this system, including naked eye detection, electro-chemical detection and raman spectroscopy based on the real applications.

The pumps in this system were used as the transporting force to accelerate the fluid circulating through the system (**Figure 2e1**). This pump was constructed by stacking numerous pieces of circular cellulose paper, where the top-pad was the input port and the bottom-pad was the pump output port. The basic principle of this pump was based on the fluid microgravity. When the paper was pressed, the fluid underneath it squeezed and pumped out of the bottom-pad. When the force was released, the fluid was absorbed and flowed from the top-pad. By applying this technique, the fluid could be continuously pumped via a press and release cycling. Although manual operation was still required, other forces, such as electro-power or electrical-magnetism, could also be adapted instead of the manual operation. Apart from these, other relevant components could also be developed and integrated into this system, including continuous-flow mixers, continuous-flow micro-reactors, separations and electrophoresis. These paper-based functional components could perform various types of bio-functions and analysis, which would be potentially applied in the analytical sciences.

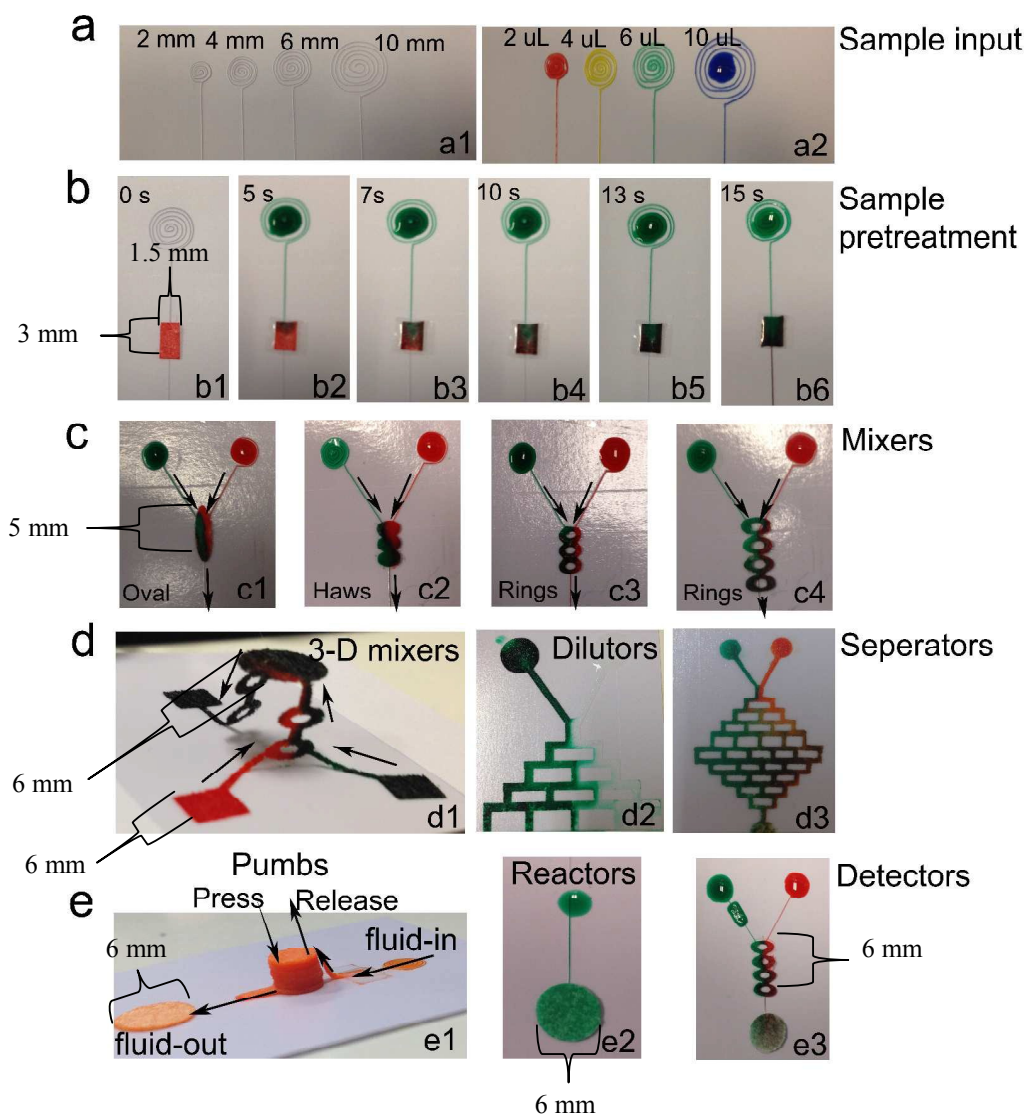


Figure 2. Demonstration of the various functional components

- (a) Sample inlet created by cutting a spiral-like microchannel on thermoplastic sheet.
- (b) The sample pre-treater was fabricated as a rectangular piece of cellulose paper.
- (c) 2D mixers of different shapes, including 1) oval, 2) sausage and 3) circular.
- (d) 1) 3D mixers, 2) dilutors and 3) separators.
- (e) 1) Pumps were made by stacking circular paper, 2) reactors and 3) detectors.

System validated for the rapid detection of the HIV

In this study, we developed a type of assay for the rapid detection of human immunodeficiency virus (HIV) based on this novel system and integrated with the gold nano-particle immunoassay (Double antigen sandwich method). As shown in the **Figure 3 (a)**. When the standard antibody of HIV (1+2) (Provided in the commercial kit of “Diagnostic kit for antibody to Human Immunodeficiency Virus (ELISA)” , Beijing Kinghawk Co. Ltd.) from the inlet A and the functional gold-nanoparticles (Bio-functionized with the HIV gp41 antigen, Beijing Kinghawk Co. Ltd.) from the inlet B meet together at the mixer micro-zone, the bio-complexes of HIV antibody (1+2)-gp41-gold nano-particles generated and were transported into the following circular detection zone. This circular detection zone composed with two layers (up layer was coated with the specific antigen of HIV gp36 (Beijing Kinghawk Co. Ltd.), while the bottom layer was used to transport the fluid via the capillary force). The HIV antibody (1+2)-gp41-gold nanoparticles-gp36 forms at the detection zone and a naked eye red dot could be detected when the positive HIV antibody exits in the sample, while negative signal displays none on the detection zone.

We evaluated the detection limit and specificity of this novel assay in this study. As shown in the **Figure 3b**, the detection limit of this assay could be 500 ng/mL with the naked eye detection during 5 min (could be compared with typical typical lateral flow technique) and could easily realize the point-of-care detection, while the specificity mainly depended on the specific antigen (this antigen gp36 was commercial on the market with high specificity) coated on the circular detection zone. Although lower detection limit of this assay when compared with other sophisticated assays, the ability of the simple, flexible and low-cost made our assay much more attractive; In addition, when compared with the traditional methods of ELISA assay (Detection Kit for Human Immunodeficiency Virus Antibody, Beijing Kinghawk Co. Ltd.), the most advantages are the extensibility, flexibility and good consistency obtained in this study.

These tests demonstrated the potential usefulness of this paper-based novel micro-system in the analytical sciences.

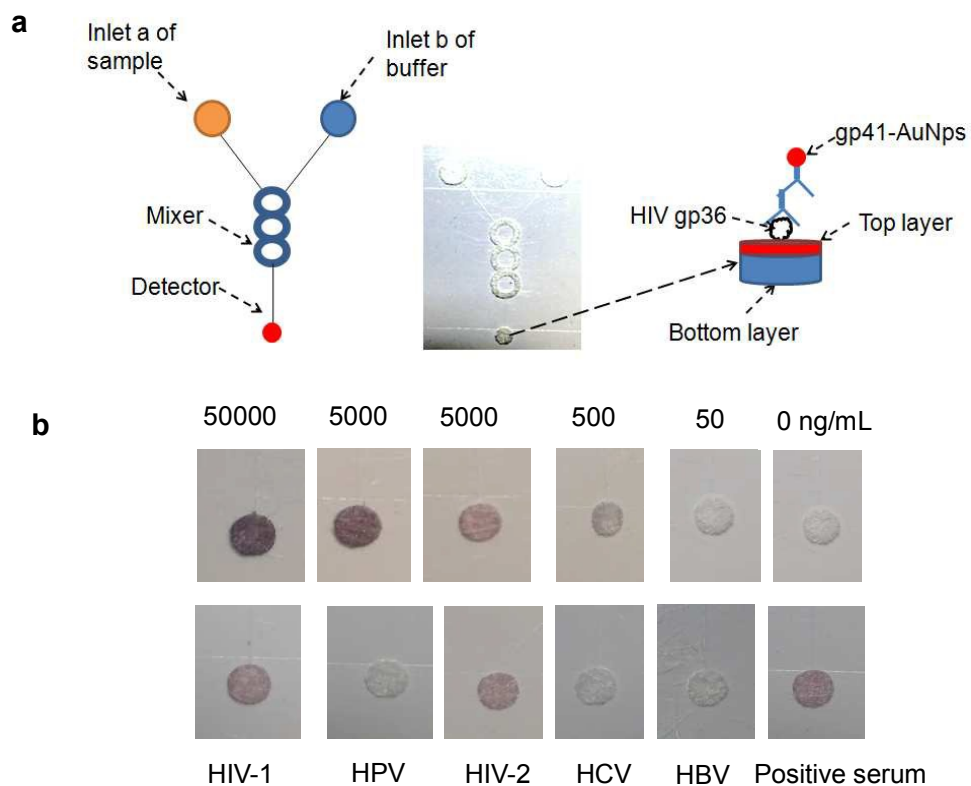
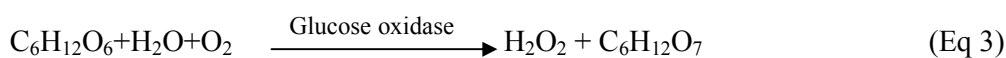
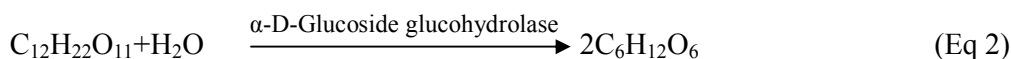
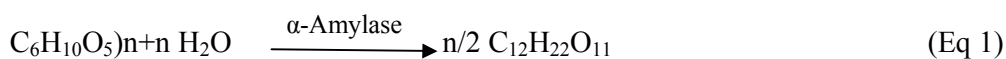


Figure 3 the novel micro-system for the rapid detection of HIV

- (a) Schematics of the micro-system for the rapid detection of the HIV.
- (b) Detection limit (50000, 5000, 500, 50, 5 ng/mL, respectively from left to right) and specificity (The standard antibody of HIV-1, HPV, HIV-2, HCV, HBV and positive serum, respectively from left to right) of the micro-system for the rapid detection of HIV.

To more describe the extensibility and real application of this novel system, include much more various components, we perform a series of biochemistry functions for starch catabolism, including stage I of starch decomposition to maltose (Eq. 1), stage II of maltose decomposition to glucose (Eq. 2), and stage III of glucose oxidation (Eq. 3) (**Figure 3**).



This system of starch catabolism included the sample/reaction buffer inlet, pretreater, mixer, decomposition reactors, pumps, and dilutors, which were connected by the bio-microcircuit channels. In this functional bio-microcircuit system, the starch sample was introduced into sample inlet a1, whereas the reaction buffer was transported from inlet a2. These two fluids were mixed in mixer b and then derived into intestine-like reactor I to perform the starch decomposition, followed by decomposition into liver-like reactor II. Pump e was able to drive the fluid to circulate around the components c and d, and finally to circular reactor III for glucose oxidation and detection. Reactors I, II, and III were soaked in α -amylase, α -D-glucoside glucohydrolase, and glucose oxidase, respectively, for approximately 12 h. Reactor III was used to detect the catabolism of glucose. We could also replace the spherical reactors with dilutors for semi-qualitative analysis of the decomposition product.

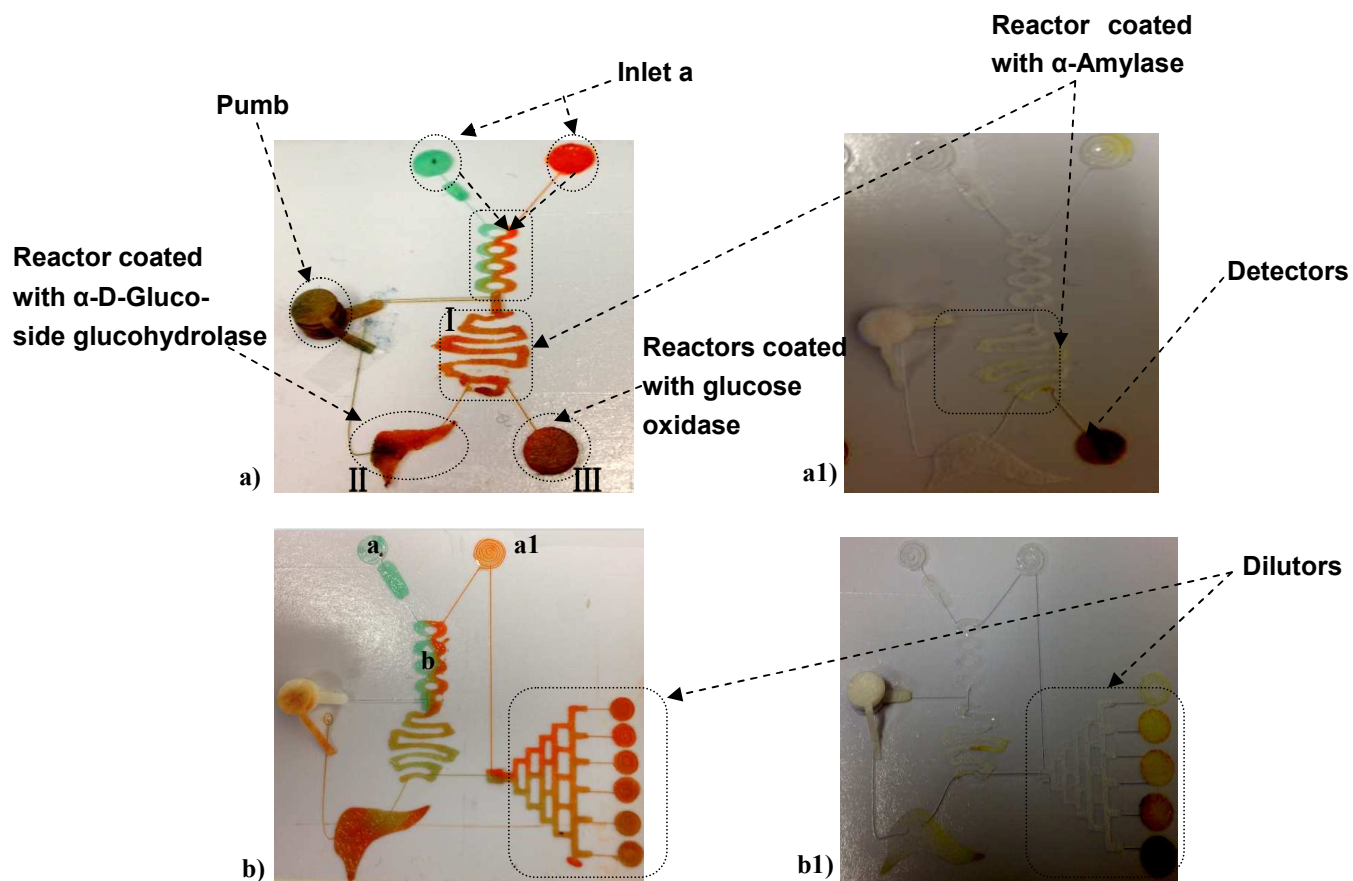


Figure 4. Bio-microcircuit system for starch catabolism

(a, a1) Assembly of the bio-microcircuit for starch catabolism with red/green ink and a real starch sample, respectively. **(b, b1)** Replacement of the single detector of glucose with dilutors with red/green ink and the actual starch sample, respectively.

Apart from the application displayed above, this micro-system was capable of integrating various other kinds of functional components in the circuit board in a flexible manner, which we think is much more attractive when compared with the planar and homogenized system of common microfluidics.²⁰⁻²⁹ Most of the traditional microfluidic systems realize their functions via special planar and homogenized micro-channel structures. However, our proposed system, with the nature of flexible 3D structure, composed with two parts, one is the fluid circuit board, acting as the platform to realize the transportation and controlling of the fluid, while another part is the functional components, used to perform all detail bio-functions. We are looking forward to establish a standard circuit board and various kinds of functional components with which we can design a functional micro-system by ourselves for special applications, such as bio-molecule purification, genetic analysis,³⁰ heavy metal detection,³¹ sperm cell analysis,³² biochemical reaction optimization and so forth. We think that this novel micro-system could attract a lot of attentions and lead to the development of microfluidics.

CONCLUSION

In this study, a novel micro-system has been introduced. This system, inspired by the microcircuit system with the nature of flexible 3D structure, composed with fluid circuit board and various functional components, was super powerful than typical planar and homogenized microfluidics. This novel system was validated in the rapid detection of human immunodeficiency virus (HIV) and starch catabolism. We believed that this novel system will open up new areas for the applications in the analytical science.

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ACKNOWLEDGMENTS

We are grateful for the kind help from the colleagues in our groups. We acknowledge the Introduce talents of Fudan university research funding (IDH1615001), the National Natural Science Foundation of China (21505024, 21427806, 21175029, 21335002, 81472032) and the Shanghai Leading Academic Discipline Project (B109), Shanghai Pujiang Program (14PJ1431400). Medicine Disciplines leaders Project of Pudong District of Shanghai (PWRd2012-09) and Key Specialty Construction Project of Pudong Health and Family Planning Commission of Shanghai (Grant No.PWZz2013-03). In addition, we thank Prof. Xinyu Jiang to help us review this article and polish the English language.