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Concept and Approach of Human Signal-molecular-profiling Database: a Pilot Study on Depression Using Lab-on-chips

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Abstract— **Signal molecular profiling (SMP) in serum can reveal abundant medical information about the human body. The construction of a human signal-molecular-profiling database (HSMPD) will greatly prompt the research of medical science. However, some challenges hinder the construction of HSMPD. A promising strategy is proposed to provide a convenient way for the establishment of HSMPD. Firstly, a low-cost and high-throughput tool for measuring SMP should be developed and standardized. When the SMP-oriented tools were accepted by most hospitals worldwide, SMP information will be decoded by a cloud-based system and stored into the online database naturally. In the pilot study, an ultrasensitive Lab-on-chips (LOC) device was developed as a specific tool for SMP. Clinical serum samples from 10 women within 4 weeks of giving birth, including 2 patients with postpartum depression were studied by the LOC devices, since accumulating evidence has indicated that hormones and cytokines in patients with mood disorders are abnormal. HSMPD may be applied to diagnose depression in the future. Here, five kinds of signal molecules were quantified on the devices, namely, tumor necrosis factor-alpha (TNF-α), thyroid-stimulating hormone (TSH), interleukin (IL)-2, IL-6 and IL-8. The preliminary results showed that the concentrations of IL-2 and IL-8 in the depression group may be higher than those in the control group, whereas the other kinds of signal molecules did not change significantly. Although the correlations are not enough to induct any diagnostic criterion, the SMP-oriented tool was verified. The results also indicated that the strategy to establish HSMPD is conceivable.**

I. Introduction

Signal molecules, e.g. hormones and cytokines etc. are biochemical molecules released by a single cell or gland in one part of the body that deliver messages to affect cells in other parts. Although the concentrations of signal molecules are usually extremely low, they are essential to the normal functions of the human body, such as metabolism and immunological function, among others. Signal molecules deliver important messages between systems inside the body. They often exist in the blood, and their concentrations reveal the status of one's health.[1] When their concentrations change dramatically, it is implied that some great changes must be occurring in the body. Some cases are normal physiological processes, such as adolescence and pregnancy, among others; however, in most cases, the dramatic change indicates the advent of disease (e.g., cardiovascular disease, cancer, etc.).[2]

Signal molecular profiling (SMP) could evidently be employed as a composite health index of human body. It provides a holistic image of an entire system inside the body. In fact, great efforts have been made for years to discover diagnostic relationships between the composition of blood and respective diseases, but progress is limited due to the complexity of blood and the absence of Human Signal-molecular-profiling Database (HSMPD). Only a small proportion of such relationships have been uncovered, and most mapping functions remain unknown. [3] If a public database of human serological information could be established, similar research on disease diagnosis would be accelerated greatly. Life scientists and biometricians could study millions or billions of medical records in the public database through some specific search engines or biostatistics programs on a national network platform, instead of investigating hundreds of patients one by one. From the viewpoint of scientists, the building of HSMPD will greatly prompt the research in the field of medical science, especially disease diagnosis and chronic disease management.

On the other hand, the construction of HSMPD will result in some relevant ethical issues, which should be resolved properly. Anonymous records should be defined for the infrastructure of HSMPD, which means the database will never contain the fields of non-essential personal information, such as name, home address, birthday etc. Only physiological and medical information will be stored and shared in the public database. Everyone in the national health care system could be assigned a record number in this database, which cannot be retrieved by someone else. Thus, everyone here plays the bilateral role as an information donor to HSMPD and a beneficiary of the health IT services derived from HSMPD. The abuse of HSMPD could be prevented under the supervision of laws. Thus, ethical problems in the HSMPD construction will be minimized, but the cost of building such a useful database is still the bottleneck, because different hospitals often use inconsistent detection platforms with variable detection items, and even incompatible standards. All these challenges hinder the construction of HSMPD.

A sound strategy may provide a convenient solution for the establishment of the database. First, a low-cost and high-

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throughput tool for measuring as many serological biomarkers as possible could be developed and standardized, for instance, the SMP-oriented LOC device in this study. This tool should be cheap enough to be widely accepted by most hospitals worldwide. When patients go to hospitals or health centres, the routine blood tests could be performed by the tool due to its low cost and high efficiency. Thus, the SMP information in the tests will be decoded by cloud-based processing servers before relevant analysis reports are returned. By the way, that health information will be stored into the online database simultaneously (Fig. 1). Finally, serological investigations could be performed through the valuable database. The health IT services could generate a stable income stream to support the sustainable development of HSMPD. Once such a health IT system is installed, the serological criterion of SMP will become a multipurpose diagnostic tool for a variety of human diseases, not only for acute ones, but also for chronic disease management, even some mental diseases, such as depression etc.

In this study, a prototype of 1196-chamber LOC device was developed into a promising tool for building HSMPD. The microfluidic device can standardize the detection of SMP in blood samples, which application was primarily tested using blood samples from patients with postpartum depression, a subclass of mood disorders. Some studies have shown that mood disorders are related to the change of SMP in blood.[4] Steiner et al. proposed a biological susceptibility hypothesis to account for gender differences in the prevalence of mood disorders based on the idea that there is a disturbance in the interaction between the hypothalamic–pituitary–gonadal axis and other neuromodulators in women.[5] According to this hypothesis, postpartum depression may be related to SMP changes in blood. This pilot study was performed just to evaluate the new quantitative tool and the feasibility of the HSMPD strategy mentioned above, rather than a thorough study on the serological diagnosis of depression.

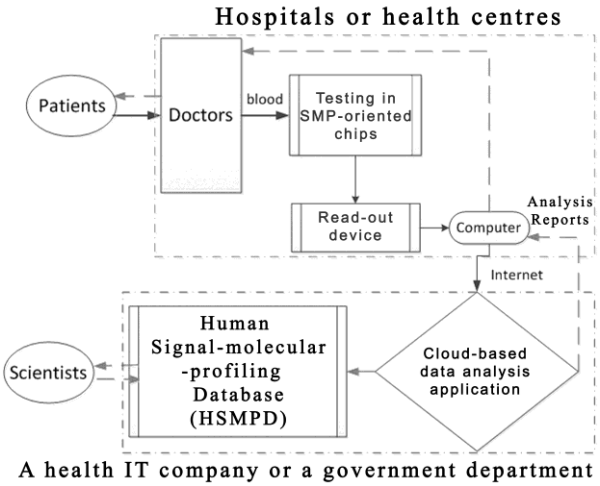

Figure 1 Conceptual framework of HSMPD system.

II. Material and Methods

A. Chip design and fabrication

The 6-channel immuno-NASBA LOC has been previously described, which depends on an isothermal amplification assay regarded as a combination of sandwich immunoassay and NASBA assay.[6] Its sensitivity provides an executable solution to quantify signal molecules in an extremely low concentration. The previous immuno-NASBA chip was designed according to a microtiter plate in order to make it readable in a microplate reader, which is widely used in biological laboratories. Since the cloud-based detection module for HSMPD has not been invented, an 1196-chamber microfluidic chip was made in reference to a standard 1536-well microtiter plate in like manner. (Fig 2) The dimensions of the total device exactly match those of a 1536-well microtiter plate.

The 1196 chambers in a LOC device are enough to quantify a few hundred of signal molecules. The details structure of the 1196-chamber LOC has been reported before. [7] The simulation of fluid field was performed using

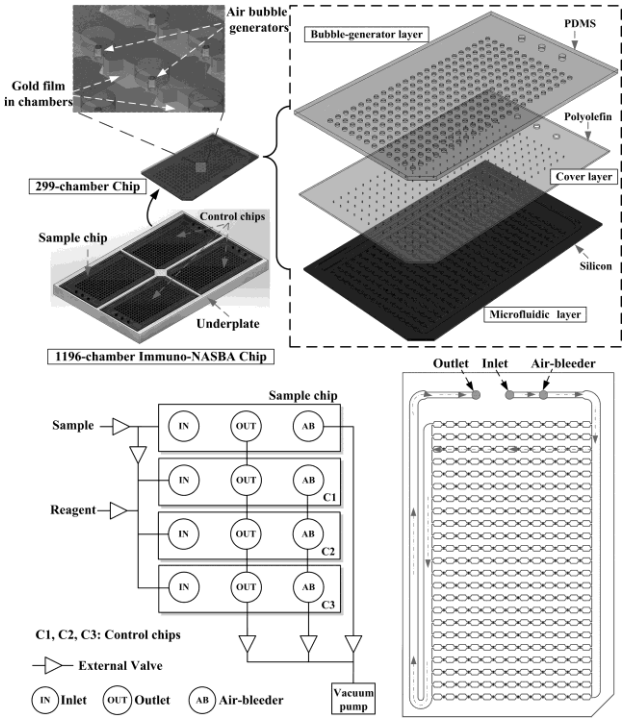

Figure 2 Layout of the 1196-chamber LOC. The chip consists of four pieces of 299-chamber chips on a specific underplate with a symmetric structure. All the dimensions exactly match those of a 1536-well microtiter plate. Each 299-chamber chip was built using a silicon-based microfluidic layer, a transparent lamina cover and a PDMS-based bubble generator layer, which introduces air bubbles into the chip deliberately to isolate neighboring reaction chambers. The 299-chamber chip has an inlet, an outlet and an air bleeder. A typical fluid pathway inside the chips is shown in the dashed lines. Except for the channels inside chips, external pipes and valves connected all inlet/outlet/air bleeder ports in different chips. The four inner chips can be operated as one microfluidic device. Some chips can be used for calibration assays, whereas some work for the testing sample.[7]

COMSOL© 4.2 as described. [8-11] The chip was fabricated using MEMS fabrication technologies. [12, 13] The bottom 'microfluidic layer' was made of a silicon wafer and using

standard bulk silicon processes. [14, 15] A 350-nm Au film was subsequently coated on the chambers by magnetron sputtering. [16-18] Before the cover layer was sealed, the chambers in the microfluidic layer were treated with biological reagents as described previously.[6] After that, 1st antibodies were immobilized on the gold films inside the chamber.

B. On-chip Immuno-NASBA assay

Five kinds of signal molecules were selected as targets, which monoclonal antibodies and related reagents were as follows: TNF-α, IL-2 (human) and their respective monoclonal antibodies (mouse) (Yapei© Biotech. Ltd., Shanghai, China), IL-8 (human) and its monoclonal antibody (mouse) (BaiWo© Biotech. Ltd., Shanghai, China), TSH (human) and its monoclonal antibody (mouse) (KeXing Biological technology Co. Ltd., Shanghai, China), IL-6 (human) and its monoclonal antibody (mouse) (Wuhan AmyJet© Scientific Inc., Wuhan, China). Streptavidin was purchased from ProSpec-Tany© Technogene Ltd. (East Brunswick, NJ); the polyclonal antisera were obtained from immune mouse via traditional methods using the above target proteins as mixed antigens. The other reagents used in the NASBA assay were obtained from PreTect™ HPV-Proofer Kit (Norchip©, Norway) as before.[12, 19] The microfluidic device was operated as described previously. [7] TNF-α was employed as the model to test the calibration tolerance of on-chip immuno-NASBA assay. Calibration solutions of TNF-α were diluted in PBS and then tested on the prototype chip. All NASBA assays were incubated at 314K in a microplate reader (Biotek® Synergy® 2). The data were recorded every minute and analysed later using Microsoft Excel® and OriginPro®.

C. Immuno-NASBA assay using clinical samples

Moreover, clinical blood samples from 10 Chinese women within 4 weeks of giving birth, including 2 patients with postpartum depression, were provided by our medical partners from China. Two groups of patients were defined here. The depression group was composed of 2 patients with postpartum depression, whereas the control group includes the 8 healthy women. For every clinical sample, five kinds of signal molecules (TNF-α, TSH, IL-2, IL-6 and IL-8) were simultaneously quantified. The clinical samples were also tested by the suppliers using common ELISA methods.

III. RESULTS AND DISCUSSION

Typical calibration curves of on-chip immuno-NASBA assays on microfluidic device were shown in Fig. 3, Inset A. When the concentration of TNF-α is between 5×10^{-15} and 1×10^{-12} mol·L^{-1}, NASBA assay can present a standard amplification curve. A linear curve about C_t and the logarithm of the concentrations of standard solutions were obtained as described before.(Fig. 3, Inset B) [12]

Polynomial regression analysis (order, 3) of the same data was performed, and the results were shown in Fig. 3, Inset C, which show better correlation coefficients. Generally speaking, the concentration range of calibration solutions ought to cover the physiological concentrations of analytes. In this case, the polynomial regression equation (order, 3) is preferred. However, the linear regression

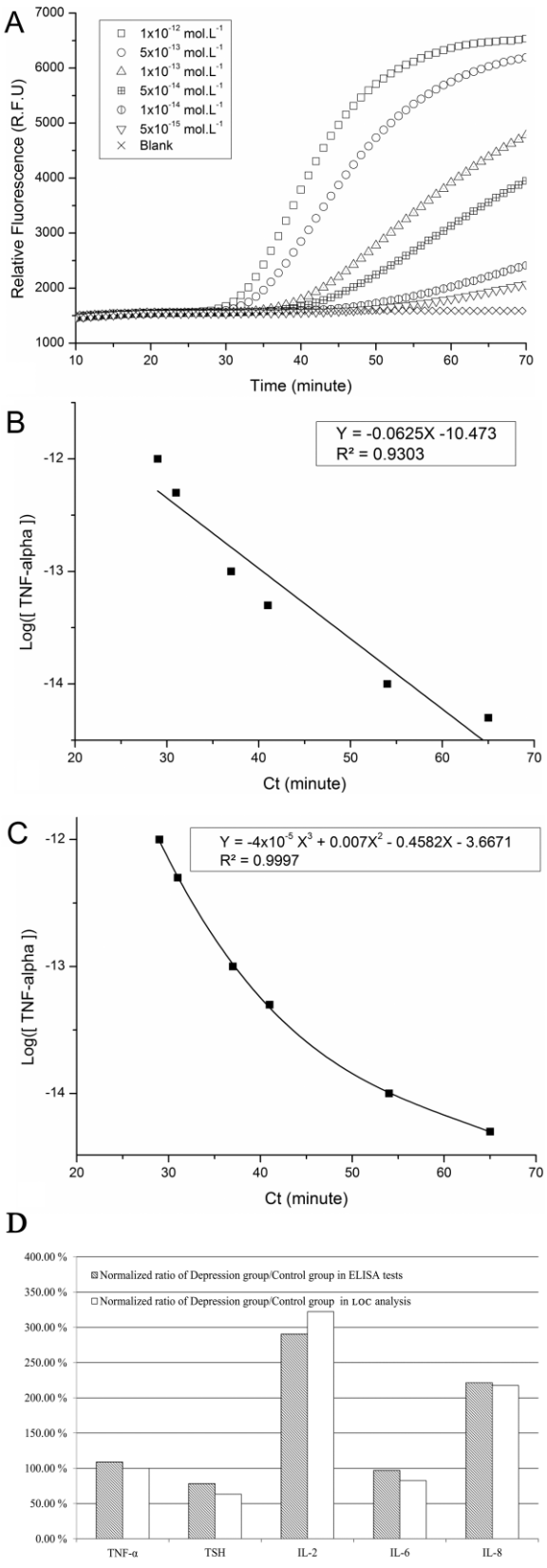

Figure 3 Determination of quantitative detection assays on the microfluidic device and test results of clinical samples.

equation shows more advantages when the sample concentration is outside the range of calibration curve.

The clinical samples were tested in the microfluidic device that described above. IL-2 and IL-8 in the plasma of patients with postpartum depression were higher than those of the control group (Fig. 3, Inset D). However, no significant difference was observed in the levels of TNF-α, TSH and IL-6 between Depression group and Control group. Due to the different calibration system, the rough values from common ELISA tests were incompatible to those analyzed by microfluidic devices. Despite this, the ratio of the average value in the depression group to that in the control group is defined as a normalized factor, which is comparable beyond different measuring systems. Normalized results in different measuring systems are shown in Fig. 3, Inset D. We found that all five groups of normalized data from the LOC device were in accord with those from common ELISA tests, which illustrates that the quantitative analysis on the LOC device is able to work as reliable as ELISA.

Using the serological criterion to assist the diagnosis of psychological disorders might be a revolutionary achievement. In fact, such efforts have been studied in many fields for a long time. However, diagnosis databases develop at an unsatisfied speed due to the bottleneck of data acquisition. For example, even the preliminary results described in this pilot study show that the changes of IL-2 and IL-8 may be related to postpartum depression. However, reliable conclusions cannot be drawn because the number of samples in this study is too limited. Without HSMPD, increasing the sample capacity of investigation requires greater cost and higher ethical risk. This investigation can be smoothly executed after HSMPD is established.

Here, we consider that the bottleneck of HSMPD development might be chalked up to the lack of cheap and suitable tools for medical data acquisition, instead of the problems of IT or biological methods. For example, microarray is a high-throughput tool for biological data acquisition, but a single piece of microarray chip usually costs hundreds of US dollars, which determines that such kind of tools are only limited at a research level. The development of health informatics requires huge databases, and consequently calls for a cheap and standardized biochip for ordinary blood testing in hospitals. The low-cost biochip maybe looks shabby but it is suitable for health information acquisition, instead of mere biological researches. Further development on HSMPD requires the contributions of knowledge in applied statistics and medical science.

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