转化医学研究（电子版）2011，1（1）：46－68
Translational Medicine Research（Electronic Edition），2011，1（1）：46－68

Review



Jing Cheng, *expert in medical biophysics. Member of the Chinese Academy of Engineering. He has been engaged and made great achievements in biotechnology researches related to the basic and clinical medicine, especially in the field of biochip technology. He has established series of biochip-based molecular typing systems for disease prevention, diagnosis and prognosis; and developed a variety of biochips for the analysis of genes, proteins and cells. Some of the chips have been certified by the State Food and Drug Administration and applied in the hospital. Additionally, he has been responsible for developing and manufacturing a full line of instruments for biochips.*

Editorial note: *This article presents a variety of diagnostic kits/reagents and devices/instrument based on microarray and microfluidic chip technologies studied an developed by Translational Institute of Medicine and Engineering (TIME), Tsinghua University and collaborators according to the demands of clinical practice; many of these products have been or will be soon applied clinically. These kits and devices/instrument include "Deafness Gene Mutation Detection Array Kit", "Mycobacteria Identification Detection Array Kit", "M. tuberculosis Drug Resistance Detection Array Kit", microfluidic chip for detection of multiple respiratory pathogens, "HBV Antiviral Drug Resistant Mutation Detection Array Kit", "AntiNuclear Antibody Test Array Kit", a real-time PCR detection kit for early diagnosis of cervical cancer, a potent biomarker set that can predict the clinical outcome of node-positive breast cancer patients, devices for sperm health screening and in vitro fertilization (IVF), and biomedical instruments. Some instruments have already been exported to over 20 countries, including ten countries in Europe and U. S. A.*

Advances in translational medicine for individualized diagnosis of diseases

Lan Xie[1,2], Yong Guo[1,2,3,4], Guo-liang Huang[1,2,3,4], Wan-li Xing[1,2,3,4], Hua-fang Gao[4], Ling-xiang Zhu[4], Dong Wang[4], Guan-bin Zhang[4], Yi-min Sun[4], Zhi-wei Zhang[4], Can Wang[4], Gao-shan Jing[4], Wen-yan Qin[4], Keith Mitchelson[4], Yu-xiang Zhou[1,2,3,4], Jing Cheng[1,2,3,4]*

1. Translational Institute of Medicine and Engineering (TIME), Tsinghua University, Beijing 100084, China
2. Department of Biomedical Engineering, Tsinghua University School of Medicine, Beijing 100084, China
3. National Engineering Research Center for Beijing Biochip Technology, Beijing 102206, China
4. CapitalBio Corporation, Beijing 102206, China

Abstract Translational medicine is a newly emerging concept which emphasizes a link from "Bedside to Bench and back to Bedside". It plays an important role in disease diagnosis and prevention, individualized treatment and prognosis evaluation through the translation of basic research achievements to clinical solutions. Using the advantages of biochip technology, systems biology, chemistry, micro-electronics, precision instruments and mechanology, and collaborating closely with leading clinical hospitals, Tsinghua University has established a new transformative model to link "Clinical Demands - Research in Universities or Institutions - Enterprise Transformation - Clinical Application". After ten years of practice, great progress in translational medicine has been achieved in diverse medical fields including the diagnosis of hereditary diseases, infectious diseases, autoimmune diseases, early diagnosis for cervical cancer and risk prediction of distant metastasis for breast cancer, assisted reproductive technology and biomedical instrument development.

Correspondence: Jing Cheng E－mail: jcheng@tsinghua.edu.cn

Key words translational medicine, clinical application, disease diagnosis, biochips, individualized

Recently, translational medicine has become a popular concept, in which the wealth of new biological discoveries are turned into appropriate drugs or used to create medical devices and then introduced in a new form of medical practice that aims for improved and personalized treatment of patients. Translational medicine emphasizes the link of "Bedside to Bench and back to Bedside", where medical issues are brought to the research bench for new solutions that can be returned to the clinic. Looking at previous practices, basic biomedical research was isolated from clinical practice for a number of reasons. Basic research such as drug development is usually carried out independently in the laboratory and lacked direct communications with clinics. On the other hand, clinical work itself still follows the traditional medical model closely, and has not yet benefited fully from the rapid developments that have occurred in basic science. A new medical model, termed "translational medicine" has emerged, intending to shorten the distance between basic research findings and clinical medical practice, proposing a "clinic - basic research - clinic" progression model to improve the efficiency of the translation of medical research. It maintains patient-centered principles and yet emphasizes that basic scientific discoveries should be linked with the arena of clinical investigations, while the results of clinical trials should be translated into changes in clinical practice, particularly in providing more immediate personalized care, utilizing genetic information from pathogens and from the patients themselves to realize better care. Translational medicine is now playing increasingly important roles in the drug discovery process, in the discovery of new biomarkers, in the early diagnosis of diseases, disease prevention and particularly in realizing more individualized treatment.

Tsinghua University has established a new model of "Clinical Demands-University Research-Enterprise Transformation-Clinical Application" by employing the latest discoveries and methods in biochips, chemistry, microelectronics, precision instruments and mechanology, systems biology approach and device development, and by working in close collaboration with clinical hospitals to realize the desired new medical tools and devices. Specifically, we have engaged in intimate discussion on the urgent needs of clinical practice and found solutions through interactive development involving the clinics, university and industry. These scientific discoveries have been transformed into fully developed products through close interactions with biotechnology/ biomedical companies, and finally the outcomes are being brought into widespread clinical practice. After ten years of development, significant progress in translational medicine is being accomplished in different medical fields, including the diagnosis of hereditary diseases, infectious diseases and autoimmune diseases. Additional progress is also achieved in early diagnosis for cervical cancer and risk prediction of distant metastasis for breast cancer, assisted reproductive technology, and clinically directed biomedical instrument development.

1 Diagnosis and prevention of hereditary hearing loss conditions

1.1 Clinical demand

The Chinese second national disabled sample survey held in 2006 found that congenital hearing loss affects 27.8 million people, accounting for 33.5% of the disabled and ranking as the first of all the disabilities in China. Currently, the number of new hearing-impairment cases diagnosed is about 60 000 per year (including infant hearing loss, late-onset hearing loss and drug-induced deafness), constituting a great social and economic burden[1]. Most of the deafness conditions have a strong genetic component and at least 60% of the cases are inherited[2]. Sequence variants of many genes can contribute to hereditary hearing loss. The Chinese People's Liberation Army (PLA) General Hospital suggested that a rapid and effective genetic diagnostic method was urgently needed to prevent birth defects and for early diagnosis of hereditary deafness.

1.2 University research

Because of its laboratory utility, a research team at Tsinghua University applied microarray technology for detection of hereditary hearing loss mutations. Molecular epidemiological surveys in China found that mutations in the GJB2, SLC26A4 and mtDNA MTRNR1 genes are the major genetic causes of hereditary hearing loss[3-5]. Li et al. at Tsinghua University developed a universal array platform termed multiplex allele-specific PCR-based universal array (ASPUA) to detect mutations at these major hereditary hearing loss loci. This technology allowed successful detection of the most common mutations occurring in four genes (GJB2, GJB3, SLC26A4, and mtDNA MTRNR1) in less than 5 hrs. Detection of these gene loci facilitates the diagnosis of drug-induced deafness, congenital profound sensorineural hearing loss, enlarged vestibular aqueduct syndrome and acquired high-frequency sensorineural hearing loss, and other conditions. Initial chip analysis of 141 patient samples generated results that were fully concordant with direct sequencing[6].

1.3 Enterprise translation

Clinical support provided by the Chinese PLA General Hospital aided this initial research to be developed by CapitalBio Corporation into the "Deafness Gene Mutation Detection Array Kit" which can evaluate nine mutations simultaneously (35 del G, 176 del 16, 235 del C and 299 del AT for GJB2, 538 C>T for GJB3, 2168 A>G and IVS 7 −2 A>G for SLC26A4, 1555 A>G and 1494 C>T for mtDNA MTRNR1) (Fig. 1) accounting for major hereditary hearing loss loci. When the developed kit was used to analyze over 1000 patient samples from three hospitals, the array results were again fully concordant with direct sequencing[7-8]. In September 2009, the "Deafness Gene Mutation Detection Array Kit" received a certificate for *in vitro* diagnostic (IVD) use from the China State Food and Drug Administration (SFDA) and can now be used for molecular diagnosis of clinical deafness. Compared with routine sequencing, the kit allows rapid, accurate and high throughput screening cost-effectively. The protocols have been standardized and are easy to implement in clinical practice, and it is expected to soon become a common clinical diagnosis in hospitals for pre-conception, prenatal and neonatal screening. Knowledge of the mutations can help parents to identify potential hearing impairment risk for their babies. Furthermore, it can also provide a warning against exposure to aminoglycoside which is known to cause deafness in children carrying certain gene mutations, and allows the child to avoid suffering this impairment throughout life.

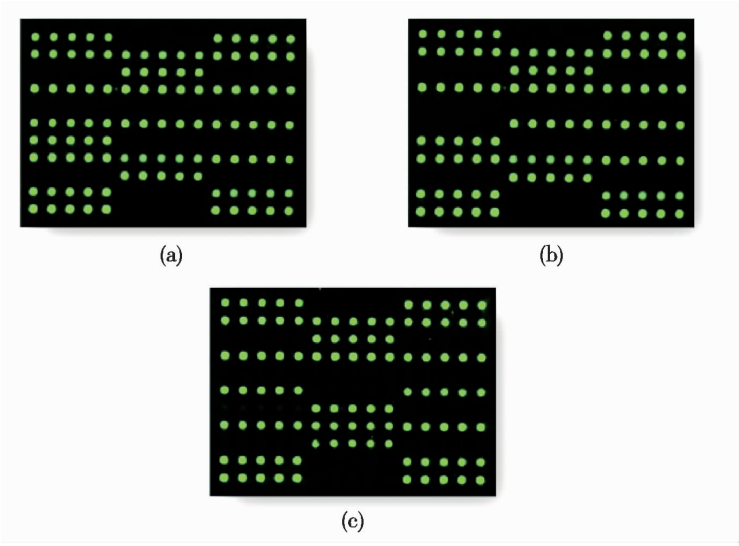


Fig. 1 Detection results of the CapitalBio Deafness Gene Mutation Detection Array Kit
(a) Heterozygote Mutation of GJB2, related to congenital profound sensorineural hearing loss; (b) Homozygous mutation of GJB2, related to congenital profound sensorineural hearing loss; (c) mtDNA 1555A>G mutation, related to drug-induced deafness

2 Diagnosis of infectious diseases

With the development of modern microbiology and pharmacology, many infectious diseases have been cured or gradually controlled to acceptable levels. However, some newly emerging infectious pathogens such as SARS virus and H1N1 influenza virus, and the re-occurrence of ancient infectious diseases such as tuberculosis, cholera and malaria again severely threaten human health because of their wide influence, high infectivity and mortality. The rapid and accurate detection and identification of such dangerous pathogens are therefore urgently required, and should also provide improved guidance for efficient treatment. Tsinghua University not only developed biochips for pathogen identification and drug resistance detection but tested them in clinical laboratory settings, finding greatly improved early diagnosis and treatment guidance for the target infectious diseases.

2.1 Identification of Mycobacterial species

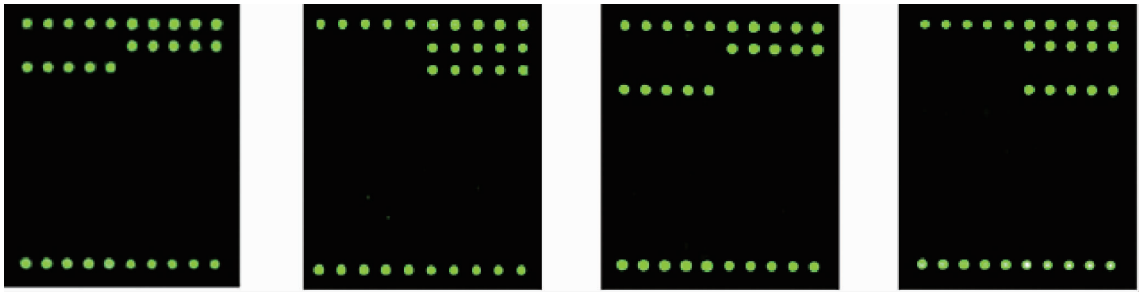
2.1.1 Clinical demand

Mycobacterium tuberculosis (MTB) is the causative agent of most cases of tuberculosis (TB). Tuberculosis-induced lung disease occurred frequently in parts of rural China, and in some regions was as infectious as classical smallpox or the plague. With the use of anti-tuberculosis drugs in 1950s, the TB epidemic appeared to have been effectively and successful controlled, and was even declared absent in some areas. However, from the 1990s the resurgence of tuberculosis has become a serious worldwide public health problem, thought to be the consequence of several factors. A rapid rise of the world population and changes in the mobility of populations, as well

as the neglect of TB in many countries have each contributed to this resurgence. Currently, over 2 million people die of tuberculosis each year worldwide, and 8 million new TB cases have emerged[9]. Non-tuberculosis mycobacteria (NTM) infection refers to disease caused by other members of the genus Mycobacterium which do not cause tuberculosis or leprosy. A nation-wide random survey in year 2000 in China on the epidemiology of tuberculosis revealed that 11.1% of the 441 bacterial strains isolated from patients were NTM[10]. The laboratory tests currently used for tuberculosis detection include microbiological smears and cultures. The main problem with these two methods is the difficulty in culturing this slow-growing organism in the laboratory and the low sensitivity[11]. As a consequence, the National Tuberculosis Reference Laboratory at China Center for Disease Control and Prevention (CDC) proposed that rapid molecular methods for accurate identification of mycobacterial species would be beneficial for improved diagnosis and disease control.

2.1.2 University research

Zhu et al from Tsinghua University developed a DNA microarray which can simultaneously identify 17 mycobacterial species (*M. tuberculosis*, *M. intracellulare*, *M. avium*, *M. gordonae*, *M. kansassii*, *M. fortuitum*, *M. scrofulaceum*, *M. gilvum*, *M. terrae*, *M. chelonae*/*M. abscessus*, *M. phlei*, *M. nonchromogenicum*, *M. marinum*/*M. ulcerans*, *M. aurum*, *M. szulgai*/*M. malmoense*, *M. xenopi* and *M. smegmatis*) that are frequently isolated from clinical samples. This set of species covers more than 95% of the common mycobacterial species. The array system takes less than 6 hours from sample preparation to result report, which saves much time compared to the conventional clinical examination methods. The concordance between the DNA array and DNA sequencing results with standard clinical isolates was 100%[12] (Fig. 2).



(a) (b) (c) (d)

Fig. 2 Detection results of CapitalBio Mycobacteria Identification Array Kit

(a) *M. tuberculosis* complex; (b) *M. intracellulare*; (c) *M. avium*; (d) *M. gordonae* clade[12]

2.1.3 Enterprise translation

The CapitalBio "Mycobacteria Identification Detection Array Kit" product was subsequently developed from the aforementioned initial lab work from Tsinghua University and the China CDC, which provides a fully controlled method to identify mycobacterial species. The kit was approved and granted with Registration Certificate for Medical Device by the SFDA in 2010 and a CE-IVD mark afterwards and is now in use at dozens of topnotch hospitals across China.

2.2 *Mycobacterium tuberculosis* drug resistance detection

2.2.1 Clinical demand

With the successful development of anti-tuberculosis drugs such as rifampicin and isoniazid in 1950s, the spread of tuberculosis was gradually under control. However, after several decades, drug resistance of *M. tuberculosis* is presently growing more and more serious. Multidrug-resistant tuberculosis (MDR-TB), *i. e.*, strains resistant to at least rifampicin (RMP) and isoniazid (INH) are now associated with high fatality[13]. Conventional drug susceptibility testing (DST) is time-consuming and usually takes 4 to 12 weeks to complete. Automated systems such as the BACTEC/BacTAlert (Becton Dickinson, Sparks, MD) are currently used widely, yet complete resistance testing still takes about 14 days from the time the culture is obtained[14]. The National Tuberculosis Reference Laboratory at China Center for Disease Control and Prevention strongly suggested that the rapid identification of resistant strains will facilitate early administration of appropriate treatment and is crucial to reducing the spread of MDR-TB through better understanding of infection epidemiology.

2.2.2 University research

The mechanisms of resistance to RMP and INH have been recently delineated. The vast majority of RMP resistance is caused by mutations located in an 81 base-pairs (bp) mutation hotspot region of the rpoB gene[15]. Investigations of strains with high level INH resistance found that between 50% and 95% of the strains contain

mutations in codon 315 of the katG gene, while between 20% and 35% of resistant strains possess mutations in the inhA regulatory region[16]. Knowledge of these resistance mutations allows the direct testing of patient specimens by fast molecular detection methods. Guo et al at Tsinghua University developed a DNA chip which can detect 14 of the most frequently detected site mutations in three genes which are associated with resistance to rifampicin and isoniazid[17]. The DNA chip-based genotype assay uses a simple semi-automatic protocol which can be completed within 6 hours. Tested on more than 1000 clinical cases, the DNA chip assay and DNA sequencing were 100% concordant. The concordance rates between the DNA chip results and the conventional drug susceptibility testing using culture isolates were 93% for RMP resistance and 83% for INH resistance, respectively.

2.2.3 Enterprise translation

By collaborating with the above two organizations, CapitalBio Corporation developed the "*M. tuberculosis* Drug Resistance Detection Array Kit". The kit was granted a Registration Certificate for a Medical Device by the China SFDA in 2010 and a CE-IVD certificate soon afterwards and has been employed in dozens of the first tier Chinese hospitals to help clinicians make effective individualized treatment plans.

2.3 Detection of different respiratory pathogens

2.3.1 Clinical demand

RTI (Respiratory Tract Infection) is a commonly occurring condition which could be induced by a variety of bacteria. However, conventional methods such as isolated culture for RTI bacteria identification have long detection period or low sensitivity problems. The People's Hospital of Peking University proposed that more rapid and accurate high throughput method for RTI bacteria detection is urgently needed in clinical practice.

2.3.2 University research

Xing et al from Tsinghua University developed the "Respiratory Pathogen Detection Chip" (Fig. 3) which integrates microfluidic technologies with isothermal nucleic acid amplification and real-time confocal fluorescence detection. It can achieve rapid, parallel detection of 12 common respiratory pathogens (*Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia*, *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Mycobacterium tuberculosis*) directly from patient sputum without need for cell culture. The platform has several advantages: detection is very fast taking less than one hour; the throughput is high as 12 pathogens can be detected simultaneously; the specificity is high and no false positive signals from non-target nucleic acid (human nucleic acid, 26 other common bacteria or other microorganisms) are observed; the detection process is fully automated and thus convenient for the clinical laboratory.

2.3.3 Enterprise translation

The main design and structure of the microfluidic chip has now been established at CapitalBio Corporation and its associated detection instrumentation and software have also been developed. After completion of clinical trials and relevant registration and certification they can be manufactured cost effectively in large numbers in the future.



Fig. 3 Microfluidic chip for respiratory pathogen detection

2.4 Detection of the anti-HBV drug resistance mutations

2.4.1 Clinical demand

Hepatitis B infection is an illness caused by the hepatitis B virus (HBV) which is associated with liver inflammation and chronic liver damage. More than 2 billion people worldwide are infected with HBV, including 350 million chronic carriers of the virus. HBV is endemic in China, with a carrier rate for HBV surface antigens of about 10%. HBV strains can vary genetically under the pressure of drug exposure, resulting in new drug resistance. Drug abuse and excessive antibiotic consumption are also factors that may exacerbate emergence of drug resistant strains, leading to increased prevalence in the community, and to individual consequences such as requiring prolonged treatment or experiencing disease recurrence. So far, resistance mutations have been reported associated with lamivudine and adfovir, two first-line anti-HBV drugs approved by the SFDA. Direct detection of the mutations known to provide resistance to HBV antiviral drugs can be useful for clinical HBV treatment monitoring. Peking University People's Hospital clinicians suggested that rapid and accurate detection of the drug resistance would allow the early administration of appropriate treatment and prevent

the further spread of drug-resistant virus.

2.4.2 University research

Guo et al. from Tsinghua University presented an array system specific for detecting HBV mutations that confer antiviral resistance to lamivudine and adfovir. The wild-type amino acids in the HBV reverse transcriptase gene include rtL180, rtM204, rtA181 and rtN236. The Lamivudine resistant mutations detected are rtL180M, rtM204I and rtM204V, while the adfovir resistant mutations rtA181T, rtA181Vand rtN236T are also detected, covering the most common mutations found in the Chinese population[18] (Fig. 4).

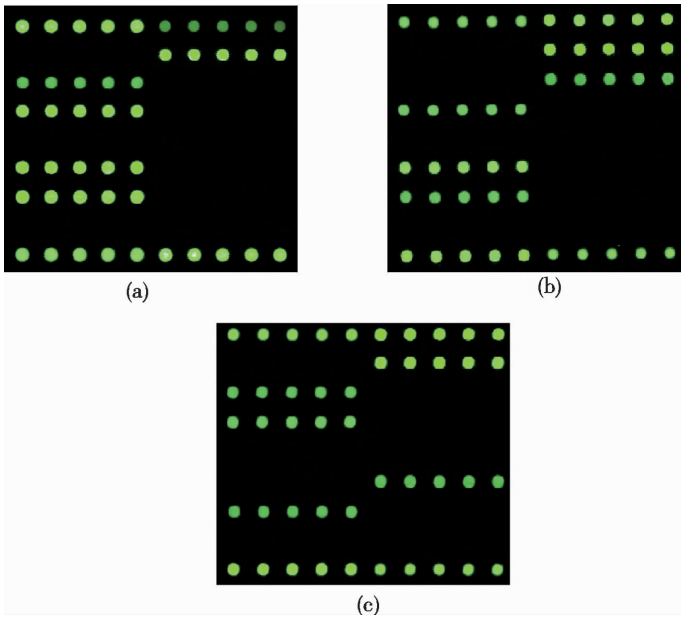


Fig. 4 Results of HBV antiviral drug resistant mutation detection kit

(a) wildtype; (b) Lamivudine resistant mutation rtL180M; (c) Adfovir resistant mutation rtN236T

2.4.3 Enterprise translation

Based on these research results, CapitalBio Corporation developed the "HBV Antiviral Drug Resistant Mutation Detection Array Kit". The detection chip is easy to use, rapid and accurate, automatic and has a high throughput. It has been evaluated in three pilot hospitals and has great potential of further use in HBV treatment monitoring.

3 Diagnosis of autoimmune diseases

3.1 Clinical demand

Autoimmune diseases refer to a category of diseases that may involve multiple organs and systems, characterized by the presence with a variety of autoantibodies. Detection of autoantibodies in the serum or other body fluid of patients with suspected autoimmune disease is an important part of the diagnostic process. A fast, specific and sensitive detection method can benefit for differential diagnosis, treatment and prognosis evaluation and help to improve the life quality of the patients and decrease the mortality. The routine detection methods for autoantibodies used at Peking Union Medical College Hospital are complicated (screening and confirmation assay are both involved) and labor-intensive. Based on the clinical feedback, the throughput is not high enough for the clinical load encountered.

3.2 University research

Peking Union Medical College Hospital and Tsinghua University collaborated to establish a protein array chip to determine the autoantibody profile. The detection process is based on an indirect immunofluorescent reaction and can be as rapid as 2 hours with sample consumption as low as 10 μL serum. Eight antibodies (anti-SSA 52, anti-SSA 60, anti-SSB, anti-Sm, anti-Scl 70, anti-CENP B, anti-RNP 68 and anti-Jo 1) related with systematic autoimmune diseases such as systemic lupus erythematosus, scleroderma, and Sjogren's syndrome can be detected simultaneously with high sensitivity and specificity[19].

3.3 Enterprise translation

In collaboration with the above two institutes, CapitalBio Corporation developed the "AntiNuclear Antibody Test Array Kit". This kit is time-saving and easy to use, with high throughput, high sensitivity and specificity (Fig. 5). In 2009, the kit was approved and granted with Registration Certificate for Medical Device by SFDA. It is now recommended for clinical detection of autoantibodies and has great significance for diagnosis, monitoring and prognosis evaluation of autoimmune diseases.

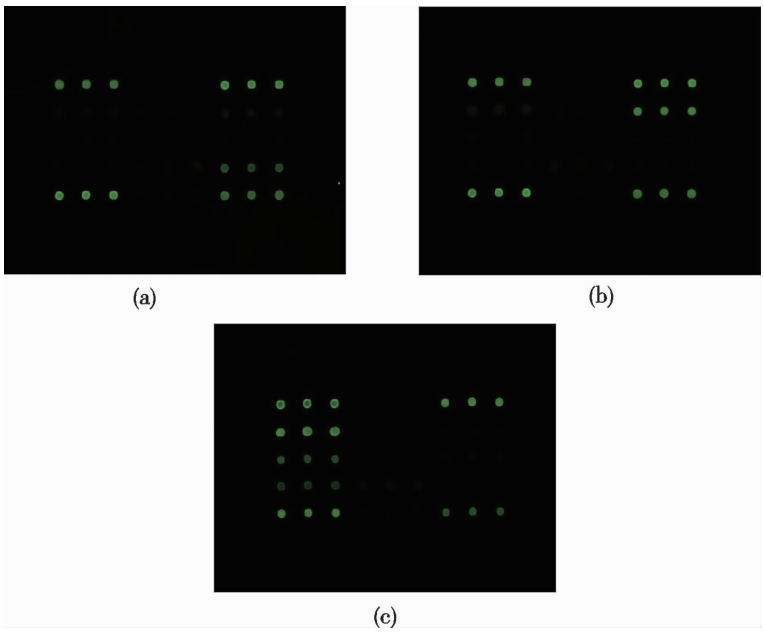


Fig. 5 Detection result of CapitalBio autoantibody detection kit
(a) systemic lupus erythematosus; (b) scleroderma; (c) Sjogren's syndrome (picture source: www. capitalbio. com)

4 Early diagnosis for cervical cancers and risk prediction of distant metastasis for breast cancer

4.1 Early diagnosis for cervical cancer

4.1.1 Clinical demand

Uterine cervical cancer is the second most common cancer in women worldwide with an estimated 493 000 new cases and 274 000 deaths in the year 2002, and has a high incidence in young women[20]. According to data from the Chinese Centre for Disease Control and Prevention, there are 131 000 new cases of cervical cancer in China each year, accounting for 26% of the world's burden; and 53 000 deaths, accounting for 20% of the world's burden. Thus, the situation for prevention and control of cervical cancer is particularly serious in China. In general terms, cervical cancer is much more common in developing countries than in the developed world, where 83% of cases occur[20]. The low incidence rates in developed countries are attributed to the introduction of cervical cancer screening programs. Current screening tests include the cytological examinations (Pap smear and the improved Thinprep Cytology Test) and the HPV DNA test, but each of them has both advantages and disadvantages and not all cases are detected. HPV testing together with routine cytology screening increases the cost of screening and is difficult to popularize in low income areas. Thus, physicians at the First Teaching Hospital of Xinjiang Medical University suggested that discovery and validation of new biomarkers exhibiting high sensitivity and specificity for the disease have great significance for the prevention and treatment of cervical cancer.

4.1.2 University research

HPV is the only completely identified cancer-causing virus in human cancers, and although more than 100 subtypes of HPV are known, only several high risk subtypes are associated with cervical cancer. HPV DNA is detected in virtually 99.8% of cervical cancers. Yet, although HPV is capable of initiating pre-cancerous development by the disruption of multiple host tumor-suppressor pathways and is the principal factor for predisposition to cancer[21], additional host cell factors are required for development of the fully transformed malignant phenotype[22]. MicroRNAs (miRNAs) are small non-coding RNAs that help to regulate every aspect of cell life, including cell growth, differentiation, cell movement and migration, and apoptosis[23]. Altered miRNA expression levels have been correlated with various cancers and their phenotypes[24]. Qin and co-workers at Tsinghua University collaborated with the First Teaching Hospital of Xinjiang Medical University and profiled the deregulation of miRNA expression in cervical cancers by miRNA microarray. The study revealed that the expressed levels of 20 miRNAs were deregulated in cervical carcinomas. Amongst them, miRNA133b expression was confirmed to increase during the progression of cervical carcinoma[25]. Functional studies indicated that miRNA133b can promote tumorigenesis and metastasis in cervical cancer cells[25]. MiRNA133b could be a potent biomarker for the early onset of cervical carcinoma and a real-time PCR

detection kit for miRNA133b is under development by CapitalBio Corporation.

4.2 Risk prediction of distant metastasis for breast cancer

4.2.1 Clinical demand

Tumor invasion and metastasis are the major causes of treatment failure and death in breast cancer patients. Although many patients apparently have the same pathological status, their prognoses can be very different. Approximately 20% ~30% of node-positive patients remain free of distant metastases over 15 ~30 years, and regional treatment has been shown to be adequate for node-positive patients who do not develop distant metastases[26]. As pointed out by Tianjin Medical University Cancer Institute and Hospital, markers for effective prognosis are lacking, and as a result, patients are often put on treatment regimes without knowing whether the regime will be actually beneficial. It is therefore important to develop molecular markers that are able to accurately predict the risk of distant metastasis, and also to design patient-tailored therapeutic strategies according to the marker results.

4.2.2 University research

Auxiliary lymph node metastasis is considered an important event in the metastatic progression of breast cancer and the lymph node metastases may represent the most metastatic cell fraction of the primary cancers. Feng et al. from Tianjin Medical University Cancer Institute and Hospital collaborated with colleagues at Tsinghua University to compare the gene expression profiles between the primary tumors and their paired lymph node metastases in tissue samples collected from 26 patients. A set of 79 differentially expressed genes were determined that can correctly separate most of the primary cancers from their lymph node metastases[27]. This set of genes also classified 35 primary cancer patients into two groups with a different future prognosis: a "high risk group" and a "low risk group". Patients in the "high risk group" had a 4.65-fold hazard ratio to develop a distant metastasis within 43 months compared with the "low risk group"[27]. The gene signature may represent a potent biomarker set that can predict the clinical outcome of node-positive breast cancer patients and guide patient-tailored therapy. Further investigation of the findings of the initial study is continuing.

5 Assisted reproductive technology

5.1 Sperm health screening

5.1.1 Clinical demand

Since the first "test-tube baby" was born in 1978, *in vitro* fertilization (IVF) has been used increasingly in cases of infertility. Sperm screening is an essential step in the IVF procedure. Technologies that could safely and efficiently identify and select motile sperm would be desirable. Experts at the Center of Reproduction Medicine in the Third Hospital of Peking University noted that the swim-up method and the density-gradient centrifugal method are currently used for sperm motility screening. However, centrifugation steps used in both of these methods can cause damage to sperm through a mechanism involving oxygen burst or DNA fragmentation[28,29]. Besides motility, other parameters including acrosome reaction capability, chemotaxis, and thermotaxis are also important indicators of mammalian sperm health. Ideally a system providing a more comprehensive evaluation is clinically required to select the ideal sperm for subsequent IVF manipulation.

5.1.2 University research

A microdevice to monitor both sperm motility and chemotaxis simultaneously during sperm screening was designed and constructed by Xie et al. at Tsinghua University. It comprises a straight channel connected with a bi-branch channel that mimics the mammalian female reproductive tract. Sperm with good mobility are sorted in the single straight microchannel, followed by chemotaxis selection in the bi-branch channel where cumulus cells were cultured in one branch to form a chemoattractant gradient to select sperm with good chemoattractive characteristics[30] (Fig. 6). The microdevice mimics *in vivo* conditions well and thus reduces the chance of damage to the sperm whilst providing selection on several different physiological characteristics. The device has great potential for future clinical applications as it is easy to use and rapid for analysis compared to the other discrete selective procedures.

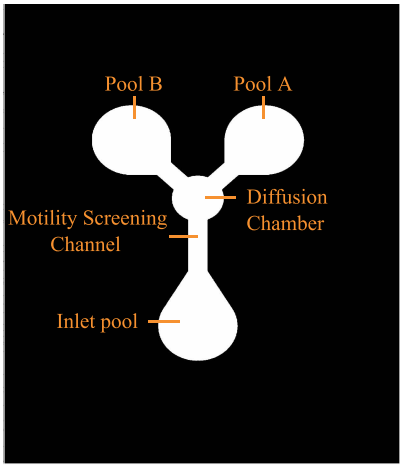


Fig. 6 Schematic diagram of sperm health screening chip[30]

5.2 *In vitro* fertilization

5.2.1 Clinical demand

Although IVF methods have improved progressively

over the past several decades, concerns exist about the significant difference from natural fertilization. The major steps of conventional IVF including gamete collection, gamete selection, oocyte fertilization, medium replacement and embryo culture are conducted in a Petri dish which is far from the physiological environment of the female genital tract. In addition, the traditional manipulations needed might cause unexpected damage to the gametes because of the frequent physical manipulation of the sperm and oocytes[28,31]. Reproductive experts at the Center of Reproduction Medicine in the Third Hospital of Peking University pointed out that a safer, more efficient and more automatic manipulation system is in high demand for use in the IVF clinic.

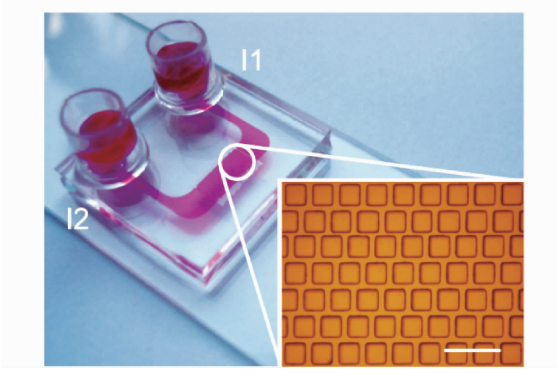
5.2.2 University research

Microfluidics could provide alternative solutions for IVF processes with the advantages of high level of integration and control, better resemblance to the gentle *in vivo* conditions and small sample-consumption. Working with colleagues from the Third Hospital of Peking University, the team led by Prof. Cheng at Tsinghua University designed and fabricated two types of PDMS-silicon microfluidic devices for integrated IVF, a microwell-structured[32] device and an octa-column-unit-structured device in which each step of IVF, including: oocyte positioning, fertilization, medium replacement and embryo culture are integrated[33] (Fig. 7). Up to now, experimental mouse IVF has been successfully conducted on these devices, with nearly identical embryo growth rates compared to the conventional IVF in a Petri dish, indicating an immediate practical significance. Further IVF work using model species is being currently undertaken to validate the effectiveness and safety of the device, before it is ultimately tested for use in the clinic.

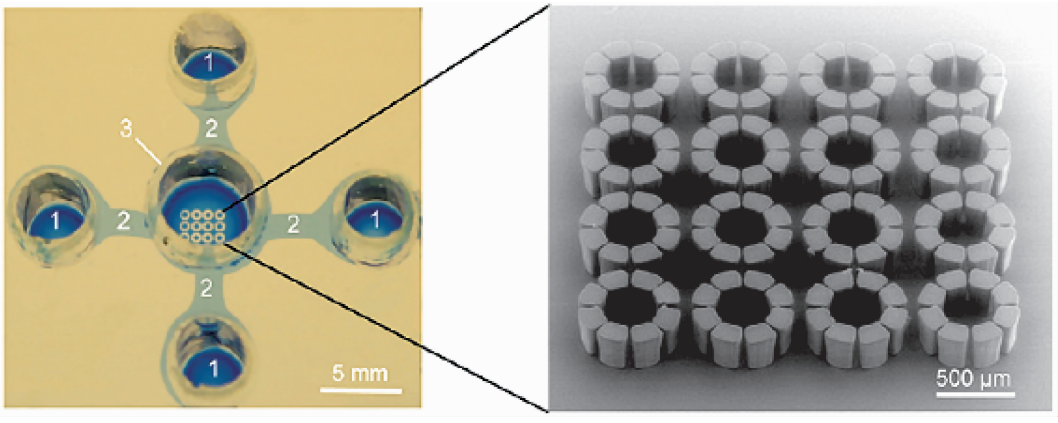
6 Biomedical instruments

Besides a variety of biochips for clinical diagnostics, a series of IVD certified biomedical instruments are now being made available by CapitalBio Corporation in collaboration with Tsinghua University, providing automated, accurate and rapid total solutions for both biomedical research and clinical practice.

The CapitalBio LuxScan™ 10K Microarray Scanner is a compact high performance scanning system for microarray



(a)



(b)

Fig. 7 Illustration of two types of IVF chip

(a) microwell based IVF chip[32]; (b) octa-column-unit based IVF chip[33]

imaging and data analysis. It integrates many advanced designs in optical, signal processing and motion control systems. The scanner is suitable for dual-color or single-color fluorescence detection of DNA, protein, cell and tissue chips with high, middle or low density. It has a superior performance with high resolution (min. 5 μm), high sensitivity and flexible configuration compared with other competitor scanners on the market. It was awarded the "national key and new product" prize by the State Department of Science and Technology, State Commerce Department, State Quality Inspection Administration and National Environmental Protection Agency. As a product with CE-IVD, TÜV and SFDA certificate, it has been exported to nearly twenties countries including the United States, the United Kingdom, Germany, France, Austria, Spain, Italy, Sweden, Switzerland and South Korea, *etc.*

The LuxScan™ Dx24 microarray scanner is a significantly upgraded product providing higher throughput (1-24 slides) and faster scanning speed. The supporting software integrates multiple functions, including chip scanning, image processing, data analysis and statistics, results reading and report generation. The instrument has received a number of domestic and international certificates (European CE-IVD, TÜV North America, TÜV Germany and SFDA). With higher Performance-to-Price Ratio than similar overseas products, the scanner has been exported to Europe.

The CapitalBio BioMixer™ II is a microarray hybridization station that aids the aqueous hybridization mixture spread evenly over the microarray surface by continuous 3-D rotation in a temperature adjustable environment. This exposes each and every sample spot uniformly with the reaction reagent solution, resulting in enhanced reaction signal intensity and signal consistency. It is easy to operate and can hold 6 or 12 microarray slides simultaneously. It was issued a variety of certificates including the CE mark, TÜV North America and TÜV Germany.

The CapitalBio SlideWasher™ 8 was developed for the clean-up of residual DNA or protein from microarray slides after a binding reaction. Slide washing and centrifuge functions for slide drying are integrated in one unit. The real-time heating system and accurate temperature control helps optimize washing conditions. It simplifies the manual operations and improves greatly the reliability, reproducibility of operations and improves the signal-to-noise ratio. It is CE and TÜV North America marked. As an important part of the microarray platform, the SlideWasher™ 8 stands out from similar alternative products and gained the approval of Roche Applied Science Inc. A version of the instrument fitting the Roche-NimbleGen microarray workflow will soon be manufactured.

The CapitalBio RT-Cycler™ Real-time PCR instrument utilizes centrifugal real-time fluorescence detection and performs thermal PCR amplification of specific target genes. It is easy to operate and has wide applications, high accuracy, small size and high Performance-to-Price Ratio. A total of 36 reactions can be completed at one time, which can fully meet the requirements of basic biomedical research and medical diagnostics. It has received the SFDA, CE certificate and TÜV North America certificate for safety compliance (Fig. 8).

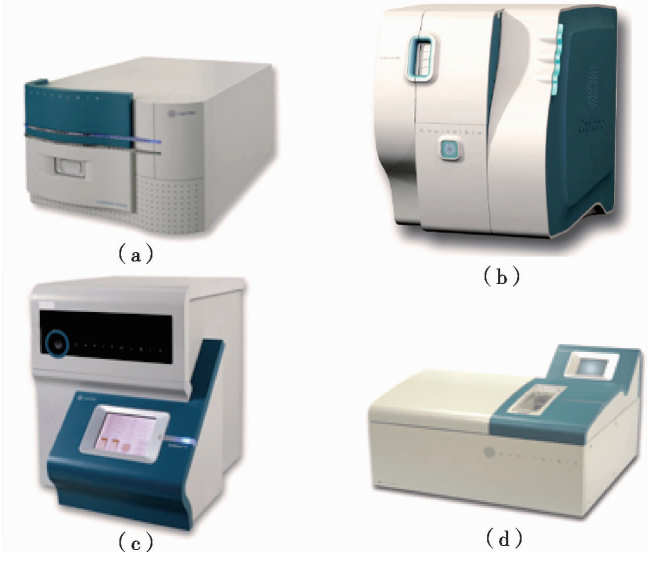


Fig. 8 Some representative instruments

(a) LuxScan™ 10K microarray scanner; (b) LuxScan™ Dx24 microarray scanner; (c) BioMixer™ Ⅱ; (d) SlideWasher™ 8 (Image source: www. capitalbio. com)

In summary, Tsinghua University has begun to realize the promise of translational medicine, conferring with leading clinicians from different branches of medicine and then taking advantage of its outstanding research capability and advanced technologies to develop devices and tools for translational medicine. Then these academic developments have, in collaboration with a biotechnological manufacturing company CapitalBio, made remarkable transformations into devices and instruments that provide new tools to the clinic for disease diagnosis, disease prevention and for better individualized therapy. With the maturation of translational medicine, Tsinghua University will play an expanding role in other important fields of clinical practice in the future.

References

[1] Chinese first summit conference for gene diagnosis and prevention of hearing loss held in Beijing. http://www.cdp.net.cn (2008-7-17) (in Chinese).

[2] Petit C. Genes responsible for human hereditary deafness: symphony of a thousand. Nat Gen, 1996, 43 (14): 385-391.

[3] Dai P, Liu X, Yu F, et al. Molecular etiology of patients with nonsyndromic hearing loss from deaf-mute schools in 18 provinces of China. Chin J Otol, 2006, 4 (1): 1-5. (in Chinese)

[4] Guan MX, Zhao LD. Mitochondrial DNA mutations associated with aminoglycoside ototoxicity. Chin J Otol, 2006, 4 (2): 98-105. (in Chinese)

[5] Liu X, Dai P, Huang DL, et al. Large-scale screening of mtDNA A1555G mutation in China and its significance in prevention of aminoglycoside antibiotic induced deafness. National Med J China, 2006, 86 (19): 1318-1322. (in Chinese)

[6] Li C, Pan Q, Guo Y, et al. Construction of a multiplex allele-specific PCR-based universal array (ASPUA) and its application to hearing loss screening. Hum Mut, 2008, 29 (2): 306-314.

[7] Zhang Y, Bian YH, Xu PF, et al. Study on the gene mutations of non-syndromic deafness using DNA microarray. Letters Biotechnol, 2010, 22 (1): 22-26. (in Chinese)

[8] Wang GJ, Dai P, Han DY, et al. Application of DNA microarray in rapid genetic diagnosis of non-syndromic hearing loss. Chin J Otol, 2008, 6 (1): 61-66. (in Chinese)

[9] Harries AD, Dye C. Tuberculosis. Ann Trop Med Parasitol, 2006, 100 (5-6): 415-431.

[10] National Technical Steering Group of the Epidemiological Sampling Survey for Tuberculosis. Report on fourth national epidemiological sampling survey of tuberculosis. Chin J Tuberculosis Respiratory Dis, 2002, 25 (1): 3-7.

[11] Department of Diseases Control. Social assessment for tuberculosis control project in China: 2004.3-2005.3. Beijing: Peking Union Medical College Press. 2006. (in Chinese)

[12] Zhu L, Jiang G, Wang S, et al. Biochip system for rapid and accurate identification of Mycobacteria species from isolates and sputum. J Clin Microbiol, 2010, 48 (10): 3654-3660.

[13] Aziz MA, Wright A, Laszlo A, et al. Epidemiology of antituberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. Lancet, 2006, 368 (9553): 2142-2154.

[14] Piersimoni C, Olivieri A, Benacchio L, et al. Current perspectives on drug susceptibility testing of Mycobacterium tuberculosis complex: the automated nonradiometric systems. J Clin Microbiol, 2006, 44 (1): 20-28.

[15] Musser JM. Antimicrobial agent resistance in mycobacteria: molecular genetic insights. Clin Microbiol Rev, 1995, 8 (4): 496-514.

[16] Zhang M, Yue J, Yang YP, et al. Detection of mutations associated with isoniazid resistance in Mycobacterium tuberculosis isolates from China. J Clin Microbiol, 2005, 43 (11): 5477-5482.

[17] Guo Y, Zhou Y, Wang C, et al. Rapid and accurate determination of MDR in M. tuberculosis isolates and clinical sputum using a biochip system. Int J Tuberc Lung Dis, 2009, 13 (7): 914-920.

[18] Yang RF, Du SC, Cong X, et al. Evaluation of the performance of a DNA microarray method for detecting HBV mutations conferring antiviral resistance to lamivudine and adfovir. Chin J Lab Med, 2010, 33 (11): 1049-1053. (in Chinese)

[19] Li YZ, Zhao ZX, Tong DW, et al. Application of ANAs protein chip in autoantibodies profile detection. Chin J Lab Med, 2006, 29 (12): 1116-1119. (in Chinese)

[20] Parkin DM, Bray F, Ferlay J, et al. Global cancer statistics, 2002. CA Cancer J Clin, 2005, 55 (2): 74-108.

[21] Elfgren K, Kalantari M, Moberger B, et al. A population-based five-year follow-up study of cervical human papillomavirus infection. Am J Obstet Gynecol 2000, 183 (3): 561-567.

[22] Burk RD. Pernicious papillomavirus infection. N Engl J Med, 1999, 341 (22): 1687-1688.

[23] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004, 116 (2): 281-297.

[24] Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med, 2009, 60 (2): 167-179.

[25] Qin WY, Dong P, Ma CL, et al. MicroRNA-133b is a key promoter of cervical carcinoma development through the activation of the ERK and AKT1 pathways. Oncogene, 2011. (in press.)

[26] Joensuu H, Pylkkanen L and Toikkanen S. Long-term survival in node-positive breast cancer treated by locore-gional therapy alone. Br J Cancer, 1998, 78 (6): 795 -799.
[27] Feng Y, Sun B, Li X, et al. Differentially expressed genes between primary cancer and paired lymph node metastases predict clinical outcome of node-positive breast cancer patients. Breast Cancer Res Treat, 2007, 103 (3): 319 -329.
[28] Younglai EV, Holt D, Brown P, et al. Sperm swim-up techniques and DNA fragmentation. Hum Reprod, 2001, 16 (9): 1950 -1953.
[29] Parrish JJ, Krogenaes A, Susko-Parrish JL. Effect of bovine sperm separation by either swim-up or Percoll method on success of in vitro fertilization and early embryonic development. Theriogenology, 1995, 44 (6): 859 -869.
[30] Xie L, Ma R, Han C, et al. Integration of sperm motility and chemotaxis screening with a microchannel-based device. Clin Chem, 2010, 56 (8): 1270 -1278.
[31] Eichenlaub-Ritter U, Shen Y, Tinneberg HR. Manipulation of the oocyte: possible damage to the spindle apparatus. Reprod Biomed Online, 2002, 5 (2): 117 -124.
[32] Han C, Zhang Q, Ma R, et al. Integration of single oocyte trapping, in vitro fertilization and embryo culture in a microwell-structured microfluidic device. Lab Chip, 2010, 10 (21): 2848 -2854.
[33] Ma R, Xie L, Han C, et al. In vitro fertilization on a single-oocyte positioning system integrated with motile sperm selection and early embryo development. Anal Chem, 2011, 83 (8): 2964 -2970.