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Review

Development of micro chemical, biological and thermal systems in China: A review

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ABSTRACT

The interest in micro technologies for application in chemical, biological and thermal systems has increased considerably in China because microreactors or microfluidics in general demonstrate some remarkably advantages such as inherent miniaturization and portability, intensification of heat and mass transfer, quick analysis results, high-throughput and low consumption of reagents. This review focuses on the recent advances in the micro technologies in China including microreactor-based systems for chemical processing and analysis, biological microfluidics for DNA analysis, cell handling and analysis, separation based detection, protein based applications and immunoassays, micro heat transfer and micro power systems, as well as design and manufacturing. A comparison of the research output of China with different countries is made in terms of publications. The weaknesses and bottle necks in these fields of research and development are discussed. It is anticipated that microreactors and microfluidic chips are on the eve of large-scale industrial applications in China. More international collaborations are expected to prompt the progress in both the research and commercialization.

1. Introduction

The development of micro and nanotechnologies has evoked much interest from the industrial and scientific community. Silicon chips had been an ideal subject for miniaturization, which led to the increase of capacity and functionality of microelectronics and micro electro-mechanical systems (MEMS). The same approach has been implemented in chemical, biological and thermal engineering. Owing to the small scale of microreactors or microfluidics, the flow and process conditions in the micro space greatly enhance the heat- and mass transfer and conversion efficiency, which constitutes a new frontier for the disciplines involved.

In the last decade (2000–2009), there has been an increasing awareness of the importance of micro chemical, biological and thermal systems. The rapid development of the field can be evidenced by a search of literatures in Web of Science (Thomson Reuters) using microreactor and microfluidics related keywords, as shown in Table 1. A total of 13,942 records were found. Main contributions have been from chemistry (analytical chemistry, multidisciplinary chemistry, physical chemistry, electrochemistry, and applied chemistry), bioscience and biotechnology (biochemical research methods, biotechnology and applied microbiology, biophysics, and biochemistry and molecular biology), nanoscience and nanotechnology, and instruments and instrumentation. Some major subject fields are enlisted in Table 2.

Many countries have contributed to the rapid development of this field. Table 3 illustrates the TOP ten countries or regions that contributed most of the papers. China produced about 1292 papers which share 9.27% the total publications (Taiwan not included). However, the United States produced 4.4 times that by China. Following China, among the list are Japan, Germany, United Kingdom, France, Canada, Republic of Korea, Taiwan China and Switzerland. The trends of development of different countries are illustrated in Fig. 1. In comparison with the world average shares of different subject areas, as shown in Table 2, one could find that China has been doing relatively well in analytical chemistry, physical chemistry, biochemical research, chemical engineering and applied chemistry. However, in the areas of nanoscience & nanotechnology, instruments & instrumentation, electrical & electronic engineering, mechanical engineering, and biomedical engineering, the performance of Chinese researchers has been relatively weak.

The major progresses of in China includes microreactor-based systems for chemical processing and analysis, biological microfluidics for DNA analysis, cell handling and analysis, separation based detection, protein based applications and immunoassay, micro heat transfer and micro power systems, and design and manufacturing.

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Table 1
Literature growing in last decade (2000–2009).

Year	Records[a]	% of 13,942	Histogram
2009	2633	18.89	
2008	2434	17.46	
2007	2157	15.47	
2006	1830	13.13	
2005	1521	10.91	
2004	1188	8.52	
2003	836	5.60	
2002	614	4.40	
2001	407	2.92	
2000	250	1.79	

[a] A search of SCI Expanded by using ("micro react*") OR microfluid* OR ("microstructured reactor*") OR ("micro chemical") OR ("micro thermal") OR ("micro heat*") OR (micro pump*) OR (micro valve*).

2. Microreactor-based systems for chemical processing

As important tools for intensification of heat and mass transfer, microreactors have been used in varieties of reaction recently, and show much more excellent performance than conventional batch reactors [1]. Most of the reactions are multiphase ones catalyzed homogeneously or heterogeneously.

2.1. Liquid phase reactions

Typically, in order to run a liquid–liquid reaction to achieve high yield and selectivity, both mass and heat transfer must be carefully controlled, especially for immiscible liquid–liquid reaction mixtures. Conventionally, for a two immiscible liquids reaction mixture, long times for completion of the reaction are always required depending on the interfacial area generated.

Microreactors should provide more possibilities for fast and efficient mixing [2]. But the Reynolds numbers calculated on typical liquid flow in microchannels are usually low and far from the turbulence regime, indicating a long mixing time which highly depends on the molecular diffusion. Thus, such kind of mixing will result in the inhomogeneity of both chemical distribution and temperature gradient. Sun et al. have used micro-capillary reactor to synthesize biodiesel and reach the yield of 99.4% at the residence time of 5.89 min [3]. In order to further enhance the mass transfer of the reaction by forming smaller drops, Wen et al. [4] developed zigzag microchannel reactors to synthesize biodiesel (see Fig. 2). The biodiesel yield of 99.5% can be achieved with the residence time of 28 s. This is the fastest residence time reported at mild reaction conditions so far in comparison with the time around 1 h for the conventional stirred reactors. Besides, the unit energy consumption for per gram of biodiesel is much less than that of conventional stirred reactors. Chen et al. [5] compared various continuous reactors for biodiesel synthesis (see Table 4). They concluded that the zigzag microchannel reactor by Wen et al. achieves the shortest residence time for high biodiesel conversion.

Table 2
Literature distribution among different subjects.

Subject	World records[a]	% of 13,942	China records	% of 1292
Analytical chemistry	3149	22.52%	432	33.43%
Nanoscience & nanotechnology	3078	22.08%	186	14.4%
Multidisciplinary chemistry	2562	18.38%	233	18.0%
Biochemical research methods	2382	17.09%	235	18.2%
Material science, multidisciplinay	1797	12.89%	146	11.3%
Instruments & instrumentation	1539	11.04%	92	7.12%
Electrical & electronic engineering,	1459	10.46%	76	5.88%
Applied physics	1429	10.25%	91	7.04%
Physical chemistry	1124	8.06%	118	9.13%
Mechanics	853	6.12%	61	4.72%
Electrochemistry	676	4.85%	42	3.25%
Mechanical engineering	588	4.22%	35	2.71%
Chemical engineering	584	4.19%	73	5.65%
Fluid & Plasmas physics	553	3.97%	27	2.09%
Optics	474	3.40%	28	2.17%

[a] A search of SCI Expanded by using ("micro react*") OR microfluid* OR ("microstructured reactor*") OR ("micro chemical") OR ("micro thermal") OR ("micro heat*") OR (micro pump*) OR (micro valve*).

Table 3
Top ten countries or regions that published most papers in past decade in micro chemical, thermal and biological systems.

Country/region	Records[a]	% of 9442	Histogram
USA	5655	40.60%	
P.R. China	1292	9.27%	
Japan	1051	7.54%	
Germany	1033	7.41%	
France	777	5.57%	
England	754	5.41%	
South Korea	740	5.31%	
Canada	634	4.55%	
Taiwan China	516	3.70%	
Switzerland	379	2.72%	

[a] A search of SCI Expanded by using ("micro react*") OR microfluid* OR ("microstructured reactor*") OR ("micro chemical") OR ("micro thermal") OR ("micro heat*") OR (micro pump*) OR (micro valve*).

2.2. Gas-phase reactions

Compared with liquid–liquid phase systems, there are more examples for using microreactors for gas-phase reactions. Traditional fixed-bed microreactors packed with catalyst particles normally suffer from poor intra-particle mass/heat transfer, low contacting efficiency, high pressure drop, mechanical attribution, and catalyst clumping in a way that leads to fluid bypassing. To solve these issues, Liu et al. [6] used micro fibrous entrapped Ni/Al_2O_3 to produce H_2 from NH_3 and got a high ammonia conversion of 99.5% at 650 °C.

Ethylene is a crucial material for the petrochemical industry and therefore, catalytic dehydration of ethanol to ethylene has become a more competitive and promising route. The reactors used in industry are usually tube-array fixed-bed reactors with low liquid hourly space velocity (LHSV: 0.3–0.6 h^{-1}), low ethylene yield, and relatively high reaction temperature (350–450 °C), resulting in high energy consumption and low utilization of equipment capacity. In order to fulfill the process intensification of ethanol catalytic

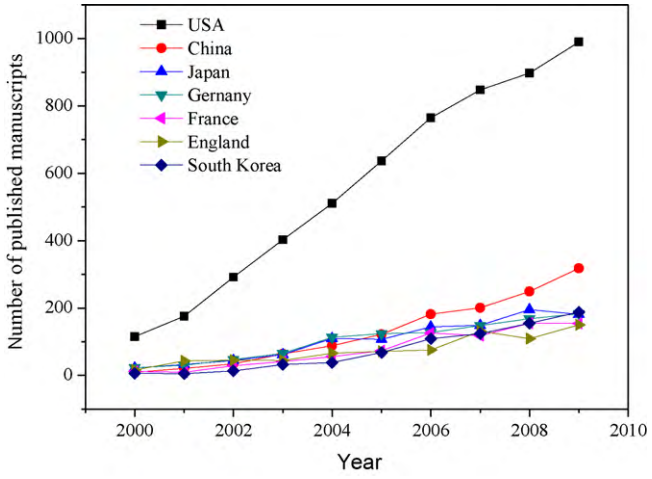

Fig. 1. The trends of development of different countries.

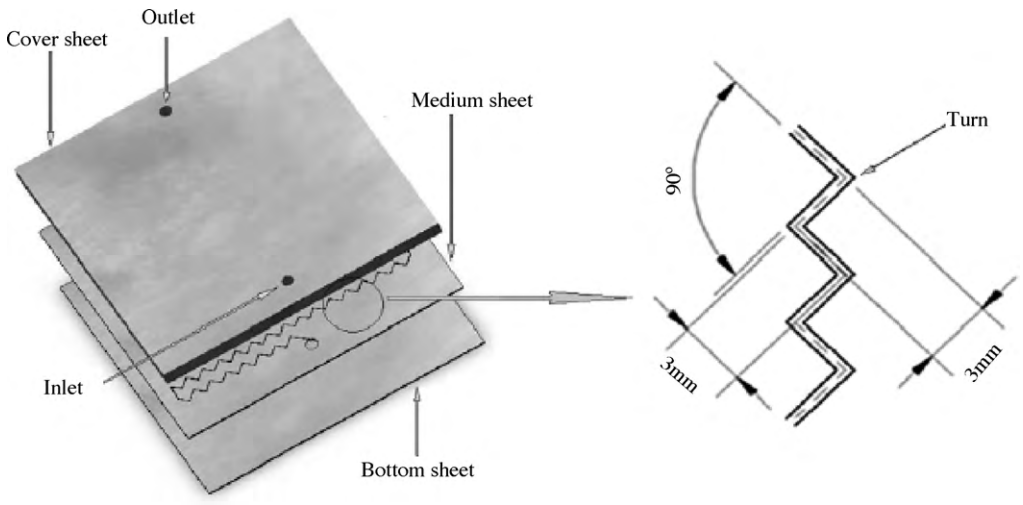

Fig. 2. Reprehensive configuration of a zigzag microchannel reactor for biodiesel synthesis [4].

dehydration to ethylene, Chen et al. [7] used a combination of a microreactor with 40–60 mesh catalyst particles to perform this reaction. High ethanol conversion of 99.96% and ethylene selectivity of 99.4% as well as an ethylene yield of 26 g(g cat h)$^{-1}$ was achieved, which demonstrates the feasibility of microreactors for ethylene production.

Gas-phase partial oxidation of toluene has many competitive advantages over existing liquid phase reactions to obtain benzaldehyde and benzoic acid. Up to the present, with all kinds of improved catalysts built, the selectivity and yields of benzaldehyde and benzoic acid are still rather low. In some cases relative high selectivity (approx. 70%) was obtained, but inevitably large amount of inert gas or steam were introduced and the space-time yield was fairly poor. To solve the problem, Ge et al. [8] packed a catalyst into microchannels (Fig. 3) and also investigated various promoting elements of V/Ti catalysts. The best catalyst performance with the maximum space-time yield (STY) of 4.82 kg h^{-1} L^{-1} was obtained for a catalyst doped with Ag.

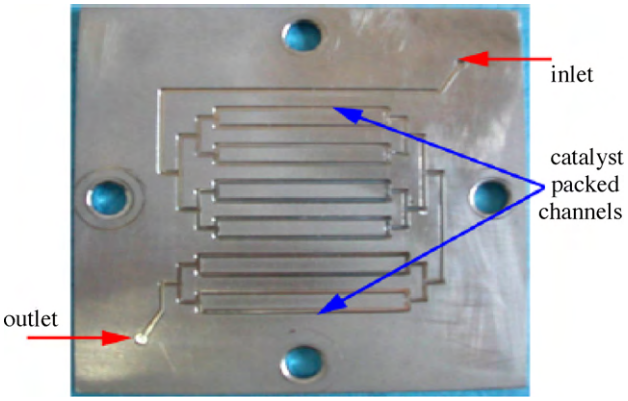

Fig. 3. Microchannel reactor for gas-phase partial oxidation of toluene [8].

2.3. Nanoscale particles synthesis

The preparation of monodispersed nanocrystals is a very significant and challenging work for the development of nano-industry. The preparation of nearly monodisperse nanocrystals is the prerequisite to permit investigations that distinguish truly novel properties intrinsic in nanoscale structures from those associated with structural heterogeneities or polydispersity. In classic colloid chemistry, the particle formation will go through three stages in the liquid phase, i.e., nucleation, growth companying with Ostwald ripening, and aggregation. The nucleation processes are usually rapid compared with the rate of reactants mixing. The nonuniform distribution of the crystal nucleus and different residence time of growth and aggregation may occur due to the insufficient mixing in batch reactors, thus broadening the crystal size distribution and debasing the nanomaterial's quality. As microreactors exhibit the better heat/mass transfer, applying of microreactors are expected for nano-synthesis. Ying et al. [9] employed T-type

Table 4
The reaction conditions, biodiesel yield and their residence time obtained from various reactors compared with those obtained using zigzag microchannel reactors.

Authors	Reactor	Temperature (K)	Biodiesel yield (%)	Residence time (min)
Darnoko et al.[a]	Stirred-tank reactor	333	58.8–97.3	40–70
Barnard et al.[b]	Microwave heating reactor	323	94.4–95.3	0.56–2
Chen et al.[c]	Rotating packed bed reactor	307–340	5.5–97.3	0.43–1.67
Sun et al.[d]	Capillary microreactor	303–343	45.0–99.4	3–20
Stavarache et al.[e]	Ultrasonication reactor	311–313	50.0–96.0	10–30
He et al.[f]	Reactive distillation reactor	340	61.9–94.4	2
Wen et al.[g]	Zigzag micro-channel reactor	313–350	81.5–99.5	0.3–0.47

[a] D. Darnoko, M. Cheryan, Continuous production of palm methyl esters. J. Am. Oil Chem. Soc. 77 (2000) 1269–1272.
[b] T.M. Barnard, E.L. Nicholas, B.B. Matthew, M.S. Lauren, A.W. Benjamin, Continuous-flow preparation of biodiesel using microwave heating. Energy Fuel 21 (2007) 1777–1781
[c] Y.H. Chen, Y.H. Huang, R.H. Hsien, N.C. Shang, A continuous-flow biodiesel production process using a rotating packed bed. Bioresour. Technol. 101 (2010) 668–673.
[d] J. Sun, J. Ju, L. Ji, L. Zhang, N. Xu, Synthesis of biodiesel in capillary microreactors. Ind. Eng. Chem. Res. 47 (2008) 1398–1403.
[e] C. Stavarache, M. Vinatoru, Y. Maeda, H. Bandow, Ultrasonically driven continuous process for vegetable oil transesterification. Ultrason. Sonochem. 14 (2007) 413–417.
[f] B.B. He, A.P. Singh, J.C. Thompson, A novel continuous-flow reactor using reactive distillation for biodiesel production. Trans. ASAE 49 (2006) 107–112.
[g] Z. Wen, X. Yu, S.-T. Tu, J. Yan, E. Dahlquist, Intensification of biodiesel synthesis using zigzag microchannel reactors, Bioresour. Technol. 100 (2009) 3054–3060.

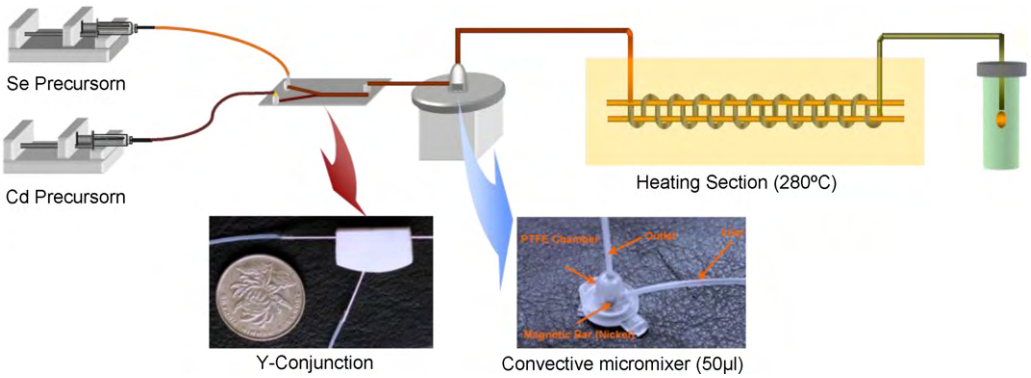

Fig. 4. Schematic drawing of the microfluidic capillary reactor [15].

microchannel reactors to prepare successfully nanocrystals with high-throughput. $BaSO_4$ nanocrystals with various sizes could be obtained by simply changing the flow rate, that is, the performance of micromixing. Monodispersed $BaSO_4$ nanocrystals of 18 nm were prepared. Chang et al. [10] firstly used microfluidic assisted methods for *in situ* synthesizing poly-DL-lactide-co-glycolide (PLGA) microgel matrices for entrapping CdSe/ZnS quantum dots. They also proposed that this microfluidic system would be useful for the encapsulation of many other materials such as biomaterials, enzymes, drugs, catalysts, and nanoparticles into PLGA microcapsules. Ju et al. [11] used microchannel reactors to synthesize NaA zeolites successfully. It was found that the mean particle size and the particle size distribution of zeolite NaA synthesized in the microchannel reactor were, respectively, smaller and narrower than in the batch system.

Quantum dots (QDs) represent the typical nanomaterials that are expensive, and imposing significant requirement on the size, and size distribution. The synthesis of QDs in batch reactions is usually restricted by the inefficient heat and mass transfer, poor reproducibility and low reaction yield. The synthetic technique can be improved by passing the precursors and ligand through a microfluidic reactor integrated with a micromixer and a microscale reaction channel, while reaction temperature is controlled with high precision. In this way, the entire process can be performed continuously with better reproducibility. Microreactors also provide close systems to perform reactions that sensitive to air. Luan et al. [12] developed a PTFE capillary microreaction system based on the classic reaction process for quantum dots, shown in Fig. 4. The magnetic stirring inside a miniature chamber was powerful for achieving the efficient mixing for precursors under flow conditions with low Reynolds number. Based on this reactor, monodisperse CdSe QDs ranging from 2.5 to 4.3 nm was successfully synthesized directly open to air. Furtherly, the optimization for the microreaction system were conducted and the large-up synthesis of CdSe QDs was achieved via a microfluidic reactor with parallel operation of four channels, resulting in high reproducibility, superior stability, low cost and also with a high-throughput of 72.66 mL/h [13]. An ideal kinetic for QDs synthesis must ensure that nucleation of seed particles occurs on a timescale that is short enough to separate from the characteristic growth phase. Moreover, the growth should occur in a gentle environment that allows the slow and controlled growth of the formed QDs. Yang et al. [14] has addressed this concern via a microfluidic method. In their study, symmetrically distributed temperature zones in a small tube furnace chamber were utilized to achieve discrete temperatures in the heating section of a capillary microreactor. In this way, the well controlled temperature provided a powerful route to separate the nucleation and growth during the synthesis of CdSe nanocrystals, resulting in good size uniformity of the formed products. For single-phase, laminar flow in a straight microchannel, the shear force imparted by the channel walls will induce a parabolic velocity profile over the cross section, which yields a residence time distribution for the liquid flowing through the channel. For this concern, a three-dimensional serpentine microchannel was applied in the reaction part of a microreactor for the synthesis of CdSe nanocrystals. The local fluctuation of velocity in the turns created by the continuous variation of channel geometry was demonstrated to be effective to maintain a uniform residence time and monomer concentration for the constrained fluid under fast flow rates. Therefore, an enhanced growth rate for nanocrystals within a narrow size distribution (8%) was accomplished with a short residence time (8–10 s) [15]. Recently, a two-step microreaction system for the totally continuous synthesis of full color emitting core/shell QDs was developed based on temperature-gradient microreaction system, containing a 3-D serpentine microfluidics [16].

Concerning the synthesis of QDs, the comparison between batch reaction and miroreaction is presented in Table 5. From the pure research point of view, microreaction will undoubtedly impact the field of kinetic studies for nanoparticles synthesis. The ultra fast mixing and high control precision realized in these cases allowed the study of nucleation, growth and shape evolution of nanocrystals with precisely defined time, and the integrated sensors facilitate the on line or *in situ* monitoring the properties for the samples.

Although many progresses have been made concerning microreactor-based systems for chemical processing, there is still a long way to go for the commercialization of these technologies. The bottle neck is that the capacity per microreactor unit is very small due to its structural features. That is, the feed flow rate is usually in the range of mL min^{-1}, suggesting that it is hard or impossible to meet the volume requirements of most of the industrial reactions. It is reasonable to apply the technology of microreactors in some highly dangerous reactions or the reactions with the products that are very expensive and difficult to be obtained by conventional methods. In these cases, high cost and low capacity is tolerable. On the other hand, it will be much better instead of focusing on a microchannel with more investigations in the microstructured reactors that provide the structurization of

Table 5
Comparison of microreactions and batch reactions in the synthesis of QDs.

Effectivity	Batch reaction	Microreaction
Mass transfer	Fast	Slow
Heat transfer	Fast	Slow
Reaction time	Second scale	Minute scale
Reaction yield	Gram per hour	Gram per batch
Products quality	Good	Good
Reproducibility	Good	Bad
Applicability	Limited (low viscosity system)	Wide

a fluid stream in the micro-scale. A lot of efforts are needed to focus on the essential, to overcome mass- and heat transfer restrictions by pre-structurization and pre-heating of the fluids into micro parts before combining them for reaction. Some pilot-scale reactors for the production of fine chemicals are in use, especially in Japan and Germany. They were micro-scaled parts indispensable to perform difficult chemical reactions. It is a great pity that most of the papers are published without a strict approval of the industrial potential with the microreactor technology. Therefore, more detailed investigations are needed to evaluate the feasibility of the microreactor technology for industrial or commercial applications.

3. Biological microfluidics

The microfluidic technology associated with micro-total analysis systems (μTAS), or Lab-on-a-Chip (LoC), has been developing rapidly and is set to revolutionize the chemical, pharmaceutical, healthcare and food industries. The global market for microfluidic technology is growing at a great pace and is estimated to be worth US $6.2 billion by 2011. Microfluidic devices can offer many advantages including quick analysis results, high-throughput and low consumption of reagents. Microfluidics has been the motivation for various biochemical application advancements in point-of-care diagnostics, bioterrorism detection, and drug discovery. Potential applications include biotechnology, pharmaceuticals, life science, defense, public health, and agricultures, each of which has its own needs. During the past 5 years, advances in microfluidics in the biological field have been developed very rapidly, yet, much more research is required due to the drastic research in length scale accompanying miniaturization, the increased role of electro kinetic effect, and most importantly, the inherent difficulties in interfacing the macroworld to the micro-scale. The progresses by researchers in China in the field of biological microfluidics mainly cover the polymerase chain reaction (PCR), capillary electrophoresis (CE), cellular analysis, protein analysis, and immunoassays.

3.1. DNA analysis

Driven largely by huge potential markets and thanks in big part to the Human Genome Project, of all the areas into which microfluidics has been introduced, the general field of DNA analysis has produced the most highly integrated chips. For DNA analysis, amplification is indispensable. As one of the methods of amplification, PCR has been most popular due to its simplicity. Application-oriented research endeavors have been expanded gradually in the last decade in China.

Lien et al. [17] presented a new miniature reverse-transcription polymerase chain reaction (RT-PCR) system integrating a sample pretreatment device for fast nucleic-acid amplification and diagnosis of viruses and bacteria. A two-way serpentine-shape (s-shape) pneumatic micropump and a magnetic bioseparator were developed for separation and enrichment of viruses and bacteria. It was also used as microheating chambers to perform RT-PCR. Consequently, the target virus bacteria was successfully separated and enriched by the high specificity and selectivity of anti body-conjugated magnetic beads, and the subsequent amplification of RNA/DNA was automatically completed by utilizing the on-chip microheaters and the micro temperature sensor. Yu et al. [18] presented a flow-through PCR device with integrated chromium resistive heaters. The PCR device was composed of a polydimethylsiloxane channel chip fabricated using soft lithography and a glass heating chip produced by standard photolithography. PDMS bonding layers were used to assemble the device instead of oxygen plasma treatment. The formation of air bubbles within the device was suppressed by treating the flow channel with 20% (v/v) Tween 20. DNA fragments with different lengths (219, 298, and 842 bp) were successfully amplified with the device a clear product band was observed after gel electrophoresis. Also, the device showed no cross contamination after appropriate washing. In addition, Liu et al. [19] presented an automatic ultramicro-volume DNA ligation process using a coplanar electrode type of electrowetting-on-dielectric (EWOD) microfluidic system. And it can be improved as an efficient parallel DNA-cloning system. Zhang and Xing [20] developed a temperature-gradient microfluidic system for DNA amplification. The microfluidic system mainly consists of modular thermally conductive copper flake which was attached onto a finned aluminum heat sink with a small fan. By making full use of the hot (90–97 °C) and cold (60–70 °C) regions on this temperature-gradient device, the MG-PCR from three parallel reactions of 112-bp *Escherichia coli* DNA fragment was performed in a continuous-flow format, in which the flow of the PCR reagent in the closed loop was induced by the buoyancy-driven nature convection. It was emphasized that the presented MG-PCR thermocycler can be further scaled-down, and the temperature-gradient technology can be applied onto other analytical purposes such as parallel and combination measurements, and fluorescent melting curve analysis. But the single integrated PCR system cannot realize a complete DNA analysis. One of the main challenges in the miniaturization of DNA analysis devices is the integration of various functional components to completely perform several operations without the need of external macro apparatus or manual operation. Several research groups in China concentrate on the integrated DNA analyzers. Liu et al. [21] demonstrated a highly integrated microfluidic chip with giant electrorheological-fluid actuated micromixer, micropump and microheater array. All internal functional components are based on polydimethylsiloxane (PDMS) and silver/carbon black-PDMS composites and formed using soft lithography. This integration approach shows promise for a broad range of applications in chemical synthesis and biological sensing/analysis because different components can be combined to target desired functionalities with flexible designs of different microchips through soft lithography.

Although the partially integrated DNA analysis chips have been successfully developed, the complete LoC still requires further development. Since the source of raw template samples is varied and the sample preparation methods are diverse, the miniaturization of conventional sample preparation and functionalities on a chip remains a challenge. In addition, the bulky optical detection systems such as charge-coupled device (CCD) and laser-induced fluorescence (LIF) are difficult to miniaturize onto a single chip. Recently, along with the development of MEMS technology, the optical-MEMS apparatus such as optical fibers, light-emitting diodes (LED) and photo-multiplier tubes (PMT) can be applied onto the PCR chip to realize a portable DNA analysis device. To obtain a portable DNA analysis device, Chen et al. [22] have made progress with the fabrication of a microfluidic chip with the size of 2 cm × 1.2 cm consisting of a silicon substrate within an etched coiled channel (see Fig. 5). Porous silicon layer developed on the surface of the internal wall of the coiled channel is designed to absorb DNA. The rapid cell lysis and PCR-amplifiable genomic DNA purification can be achieved within 20 min. The potential of this microfluidic biochip is illustrated by treating a whole blood sample, which shows the possibility of integration of sample preparation, PCR, and separation on a single device to work as portable point-of-care medical diagnostic system.

3.2. Devices for separation based detection

To work on biological systems, the first issue is to know the total elements that are included in the target system, then evaluating connections of these elements and their dynamics over

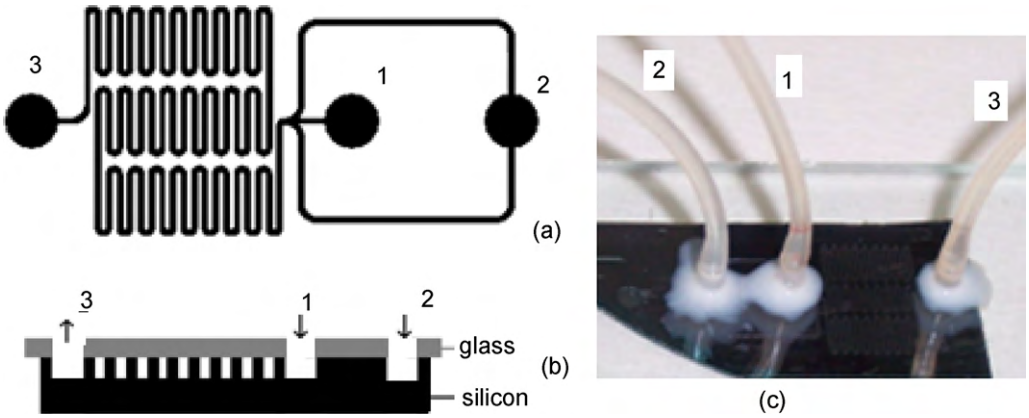

Fig. 5. Schematic top view (a), cross view (b), and photograph (c) of the microfluidic biochip. (1) Cell inlet, (2) buffer inlet, and (3) outlet [22].

time become possible in practice. From this point of view, separation method acts as a fundamental role in the systems of biology. Capillary electrophoresis is one of the major separation tools for bio-analysis, based on difference in mobility of analytes in electric field. Since its introduction in earlier 1980s, CE has undergone rapid progress due to its high performance, high speed, and low sample requirement and reagent consumption. Many useful separation modes have been originally developed by researchers in China for improving separation selectivity and efficiency [23,24].

Shen et al. [25] developed a capillary array electrophoresis (MCAE) chip for parallel chip electrophoresis of biomolecules. The microfluidic array layout consists of two common reservoirs coupled to four separation channels connected to sample injection channel on the soda-lime glass substrate. Under the computerized control of program, the sample injection and separation in multichannel can be achieved through six high-voltage modules' output. A CCD camera was used to monitor electrophoretic separations simultaneously in four channels with LIF detection. Liu et al. [26] reported a microfluidic chip that couples isotachophoresis (ITP) preconcentration and zone electrophoresis (ZE) separation. A hepatitis B virus (HBV) genotyping method with only one amplification round was developed by the application of the ITP-ZE chip. All the analysis steps of the ITP-ZE separation including sample injection, stacking and separation were performed continuously, controlled by sequential high-voltage switching. The results showed that this ITP-ZE chip analysis provides HBV genotyping information in reduced PCR amplification time with higher detection rate when compared with conventional method. Long et al. [27] presented an integrated microfluidic device with the online coupling of solid-phase extraction to microchip electrophoresis (chip SPE-CE). With a nanoporous membrane sandwiched between two PDMS substrates, the SPE preconcentration and electrophoretic separation were carried out in upper and lower fluidic layers, separately and sequentially. SPE was performed on a 2.5 mm long microcolumn, with two weirs on both sides to retain the C-18-coated silica beads. High separation efficiency and thousand-fold signal enhancement were achieved. Cong et al. [28] established a microchip of two-dimensional capillary electrophoresis platform, combining isoelectric focusing (IEF) and capillary zone electrophoresis (CZE). During the separation, peptides were focused by IEF in the first dimensional channel, and then directly driven into the perpendicular channel by controlling the applied voltages, and separated by CZE. The digests of bovine serum albumin (BSA) and proteins extracted from *E. coli* were separated, and the results suggested the promise of multidimensional separation on a microchip for the high-throughput and high-resolution analysis of complex samples. Zhang et al. [29] developed an approach to introduce picoliter-scale sample for capillary electrophoresis. The concept of the translational spontaneous injection was based on the droplet splitting phenomenon at the capillary inlet end at the beginning of the injection. It can reduce sample injection volumes to sub-100 pL range. On the basis of the injection approach, a versatile high-speed capillary electrophoresis (HSCE) system was built with separation performance comparable to or even better than those reported in microfluidic chip-based CE systems.

There are more interests in combing the CE and the detection technologies such as fluorescence detection, electrochemiluminescence (ECL), amperometric detection, and bioluminescence (BL) method. Among these methods, the fluorescence detection is the dominant optical detection technique used in microfluidics due to its advantages such as high sensitivity and easy incorporation into microfluidic devices. Several researchers concentrated on the combination of the fluorescence detection and microfluidics. Fang's group presented several microfluidic systems combining capillary electrophoretic separation and laser-induced fluorescence [30,31]. They developed a functional integration of cell sampling, single cell loading, docking, lysis and CE separation with laser-induced fluorescence detection in a single glass chip with a crossed-channel design to determine reactive oxygen species (ROS) and reduced glutathione (GSH) in single erythrocyte cell. Also, they developed a flow-through sampling reservoir with a crossed-channel design for CE separation and continuously monitored the sampling system with LIF detection. Liu et al. firstly and successfully used a microfluidic chip-affinity CE method based on indirect LIF detection to study protein–drug interactions [32]. The binding constant was in good agreement with that reported in the literature. As conventional optical devices need to be miniaturized to make a portable lab-on-a-chip system, large amount of current research is directed towards integrating detection mechanisms. For example, Li et al. [33] constructed an integrated LIF detector for microchip electrophoresis. Their simple detector for fluorescence detection was proved suitable by baseline separation of fluorescein isothiocyanate (FITC)-labeled arginine, phenylalanine, and glycine and FITC within 30 s at separation length of 3.8 cm and electrical field strength of 600 V cm^{-1}. Qiu et al. [34] described an indium tin oxide (ITO) electrode-based Ru(bPY)(3)(2+) ECL detector for CE. The microchip consisted of a PDMS layer containing separation and injection channels and an electrode plate with an ITO electrode fabricated by a photolithographic method. They demonstrated that the high separation electric field had no significant influence on the ECL detector, and decouplers for isolating the separation electric field were not needed in this microchip CE-ECL system. The ITO electrodes employed in the experiments displayed good durability and stability in the analytical procedures.

Amperometric detection exploits the use of a potential applied between a reference and a working electrode, causing the oxidation or reduction of an electroactive species. Wang et al. [35] constructed a polycarbonate (PC) CE microchip with end-channel electrochemical detection. The on-chip integrated three-electrode system consisted of a gold working electrode, an Ag/AgCl reference electrode and a platinum counter electrode, which was fabricated by photo-directed electroless plating combined with electroplating. The first one was positioned against the separation channel exit to reduce post-channel band broadening. The second one was positioned in close proximity to the working electrode to diminish the electrophoresis high-voltage interference. Xu et al. [36] fabricated the carbon nanotube/polystyrene (CNT/PS) composite electrodes as sensitive amperometric detectors of microchip CE. The composite electrode was manufactured on the basis of the *in situ* polymerization of a mixture of CNT and styrene in the microchannel of a piece of fused-silica capillary under heat. The new CNT-based CE detector offered significantly lower detection potentials, yielded substantially enhanced signal-to-noise characteristics, and exhibited resistance to surface fouling and hence enhanced stability. Yao et al. [37] from the same group, presented a carbon nanotube/poly(methyl methacrylate) (CNT/PMMA) composite electrode. It also used as an amperometric detector, developed in the similar method with the former one and showed good performances.

Bioluminescence is the generation of light due to the release of living organisms or from chemical systems derived from them. Liu et al. [38] developed a microfluidic platform which integrated CE and BL in order to monitor cellular adenosine triphosphate (ATP) of *E. coli* coupled with on-chip cell lysis and sample extraction. BL detection of ATP and ATP-conjugated metabolites was realized using a firefly luciferin–luciferase BL system. Under optimized conditions, ATP analysis was realized within 30 s, with a detection limit of down to 0.20 μM and a dynamic linear range of at least two orders of magnitude. No interference from adenosine diphosphate (ADP), adenosine monophosphate (AMP) and the cell lysis detergent Triton X-100 was found within the standard deviation of the analysis.

3.3. Devices for cell handling, sorting and general analysis

In addition to on-chip DNA analysis and capillary electrophoresis, there has been a large amount of research in China directed towards the integration of microfluidic technologies with different aspects of cellular analysis. And several excellent reviews authored by Chinese researchers have been also published [39,40]. Here, we summarize some very recent advances in China on cell culture, cell sorting and capture.

Compared with traditional static cell culture in homogeneous conditions across the substrate, microfluidic perfusion culture can affect a defined artificial microenvironment by continuously controlling the supply and removal of soluble factors. Moreover, microfabrication techniques offer the advantages of increased fluid control, ability to address the cellular length scale, approximating the physiological culture environment, and improved culture efficiency. Ye et al. [41] developed an integrated microfluidic device consisting of continuous concentration gradient generators of stimulator and parallel cell culture chambers. In the chambers, the processes of liquid dilution and diffusion, micro-scale cell culture, cell stimulation and cell labeling could be integrated into a single device. They used this device for high-content screening and rapidly extracted the maximum of information from tumor cells in response to several drugs varying in concentration, with minimal sample and less time. And also they used it to characterize doxorubicin-induced apoptosis in human hepatocellular carcinoma (HepG2) cells and investigated multiple parameters relating stimulation to apoptosis [42]. Liu et al. [43] fabricated a microfluidic chip featuring parallel gradient-generating networks etched on glass plate. The microchip contains five gradient generators and 30 cell chambers where the resulted concentration gradients of drugs are delivered to stimulate the on-chip cultured cells. The intracellular glutathione (GSH) level and cell viability were assessed by fluorescence image analysis. The results indicated that high intracellular GSH level has a negative effect on chemotherapy sensitivity, while depletion of cellular GSH may serve as an effective way to improve chemotherapy sensitivity. The integrated microfluidic chip is able to perform multiparametric pharmacological profiling with easy operation, thus holding great potential for extrapolation to the high-content drug screening. Wu et al. [44] reported a new perfusion-based, micro three-dimensional (3-D) cell culture platform for high-throughput cell culture using enabling microfluidic technologies. The micro 3-D cell culture platform was fabricated based on SU-8 lithography and polydimethylsiloxane replication processes. The 3-D culture of oral cancer cell was successfully performed, showing that the cell viability remained as high as 95–98% during the 48 h cell culture. Huang and Lee [45] presented a new chip capable of automating the cell culture process by using microfluidic technology. This microfluidic cell culture system comprising microheaters, a micro temperature sensor, micropumps, microvalves, microchannels, a cell culture area and several reservoirs was fabricated using micro-electro-mechanical-systems' fabrication processes. A typical cell culturing process for human lung cancer cells (A549) was successfully performed to demonstrate the capability of the developed microfluidic system.

Cell sorting includes many techniques that are used to collect a group of cells with similar identities. Liu et al. [46] fabricated a smart microfluidic device integrating nickel micropillars, microvalves and microchannels for the specific capturing and sorting of cells. As a result, A549 cancer cells were effectively captured and sorted on the microfluidic device with the capture efficiency ranging from 62 to 74%. Jang and Wang [47] presented a microfluidic device including a glass substrate with electrodes and a PDMS channel with micro pillars to capture physically single cells within microstructures. Besides, the impedance of a single HeLa cell was measured using an impedance spectroscopy.

In recent years, there has been an increasing interest in optical manipulation of biological species on microfluidic device due to its non-contact and contamination-free manipulation process. As Luo et al. [48] reported, a microfuidic device combined with microwell array and optical tweezers was set up for cell manipulation, localization and cultivation. Yeast cells were successfully manipulated by a 1064 nm laser and transferred to microwell array as a demonstration. And they also proved a trapping laser power of 0.30 W is harmless for yeast cell cultivation.

3.4. Devices for protein based applications

In general the development of integrated microfluidic devices that are specifically designed for protein analysis, beyond traditional CE chips, is less mature than some of the applications already listed.

One of the most attractive contemporary endeavors is the mapping of proteins and establishing their linkages to normal and pathological conditions. Protein digestion is an essential procedure prior to identification. Subsequently, the sequencing of proteins can be accomplished by matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) in conjunction with database searching. A lot of efforts have been taken to speed up the digestion of protein in China. An on-chip enzymatic reactor providing rapid protein digestion is presented by Liu et al. [49]. Trypsin-embedding stationary phase within the microchan-

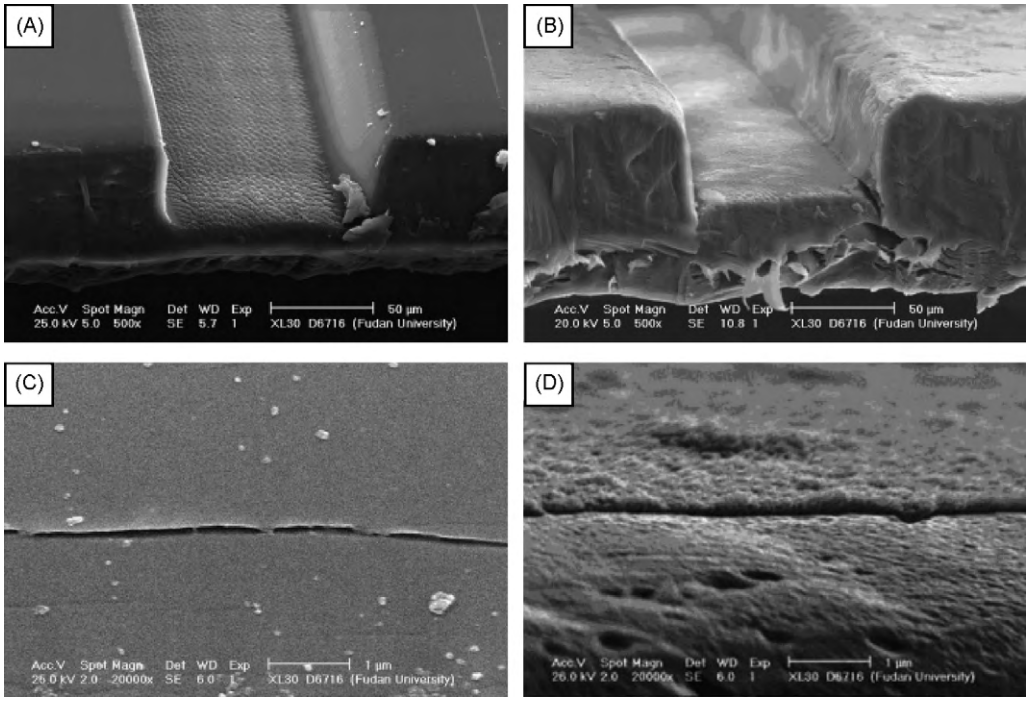

Fig. 6. SEM images of the microchannel (A and B) and its side face (C and D). A and C are the unmodified microchip. B and D are the microchannels with (PDDA/nano-zeolite)n (n = 3) assembled layers [55].

nel has been prepared by a sol–gel method. Such a microfluidic reactor has been used for low-level protein digestion at 16 fmol per analysis. The analytical potential of the microreactor combined with the strong cation exchange and reversed-phase liquid chromatography-electrospray ionization tandem mass spectrometry (RPLC ESI-MS/MS) for the identification of real samples from the cytoplasma of the human liver tissue has been demonstrated. Feng et al. [50] reported a nanoliter trypsin-based monolithic microreactor coupled with micro RPLC–MS/MS for shotgun proteome analysis. The proteins were rapidly digested by the microreactor, and the resulting protein digests were directly loaded onto a micro RPLC column for separation followed with detection of the eluted peptides by tandem mass spectrometer. A novel kind of immobilized trypsin reactor based on organic–inorganic hybrid silica monoliths has been developed by Ma et al. [51]. By monitoring the reaction of a decapeptide, C-myc (EQKLISEEDL), the enzymatic activity of the immobilized trypsin was calculated, and the results showed that the digestion speed was about 6600 times faster than that performed in free solution. A nanoreactor based on mesoporous silicates is described by Qiao et al. [52] for an efficient tryptic digestion of proteins within the mesochannels. This nanoreactor combines the advantages of short digestion time with retention of enzymatic activity, providing a promising way to advance the development of proteomics.

The fabrication and performance of a fiber-packed channel bioreactor in microchip along with its application in protein analysis were reported by Fan and Chen [53]. The feasibility and performance of the unique microfluidic bioreactor were demonstrated by the tryptic digestion of myoglobin (MYO) and BSA. The on-chip digestion was carried out at a flow rate of 2.0 mL min^{-1} and the digestion time was significantly reduced to less than 5 s. The digests were identified by MALDI-TOF MS with sequence coverages of 66% (MYO) and 40% (BSA) that were comparable to those obtained by the conventional in-solution tryptic digestion. The present fiber-based microchip bioreactor provides a promising platform for the high-throughput protein identification. A core-changeable needle enzymatic reactor was developed by Wang et al. for highly efficient proteolysis [54]. The feasibility and performance of the unique bioreactor were demonstrated by the tryptic digestion of BSA and lysozyme and the digestion time was significantly reduced to less than 5 s.

Modification of solid substrates and the subsequent immobilization of biomolecules are relevant to many areas in research applications. Surface-bound molecules are applied in biosensors, DNA microarrays, protein chips, cell culturing, biomolecule interaction investigations, and so forth. Liu et al. developed an on-chip microreactor towards the acceleration of protein digestion through the construction of a nano-zeolite-assembled network. The nano-zeolite microstructure was assembled using a layer-by-layer technique based on poly(diallyldimethylammonium chloride) and zeolite nanocrystals (see Fig. 6) [55,56]. The maximum proteolytic velocity of the adsorbed trypsin was about 600 mM min^{-1} μg^{-1}, thousands of times faster than that in solution. BSA, myoglobin, and cytochrome c were used as model substrates for the tryptic digestion. The standard proteins were identified at a low femtomole per analysis at a concentration of 0.5 ng μL^{-1} with the digestion time <5 s. This simple technique may offer a potential solution for low-level protein analysis. Qing et al. [57] reported that bioactive surfaces with appropriate hydrophilicity for protein immobilization can be achieved by hydrophobin II (HFBI) self-assembly on mica and polydimethylsiloxane surfaces. The results suggest that HFBI assembly, one kind of hydrophobin from Trichoderma reesei, may be a versatile and convenient method for the immobilization of biomolecules on diverse substrates, which may have potential applications in biosensors, immunoassays, and microfluidic networks. A biocompatible interface was constructed by Liu et al. [58] on a microchip using the layer-by-layer (LBL) assembly of charged polysaccharides incorporating proteases for highly efficient proteolysis. The efficient on-chip proteolysis was obtained within a few seconds, and the identification of biological samples was feasible. Various silica-based microreactors have been designed by Shui et al. and used enzyme immobilization to address technical concerns in proteolysis including inefficient and incomplete protein digestion [59]. Smart surface created in a microfluidic chip by Mu et

al. has shown the capability of adsorbing and releasing proteins under electrical control [60]. The inner surface of the chip channel was first coated by a thin layer of Au through sputtering and was subsequently modified with loosely packed self-assembled monolayers (SAMs) of thiols with terminal carboxylic or amino groups. A hydrophobic or hydrophilic channel surface was established and could be reversibly switched electrochemically. Accordingly, the microchips prepared in this way can reversibly and selectively adsorb and release differently charged proteins under electrical control.

In the past years, enzymatic microreactors have been developed to facilitate routine work in biochemical analysis and in biocatalysts. A monolithic enzymatic microreactor was prepared by Duan et al. in a fused-silica capillary by *in situ* polymerization of acrylamide, glycidyl methacrylate (GMA) and ethylene dimethacrylate (EDMA) in the presence of a binary porogenic mixture of dodecanol and cyclohexanol, followed by ammonia solution treatment, glutaraldehyde activation and trypsin modification [61,62].

An easily replaceable microchip enzymatic microreactor has been fabricated by Liu et al. [63] based on the glass microchip with trypsin-immobilized superparamagnetic nanoparticles. Magnetic nanoparticles with small size (50 nm in diameter) and strong magnetism were synthesized. This microreactor was successfully applied to the analysis of an RPLC fraction of the rat liver extract. After a database search, six proteins were identified. This opens a route for its further application in bottom-up proteomic analysis. A regenerative protease microreactor with metal-ion chelated adsorption of enzyme has been fabricated by Li et al. on a chip [64]. The capability of the proteolytic microreactor was demonstrated by cytochrome c and bovine serum albumin as model proteins. After a database search, 23 unique peptides corresponding to 7 proteins were identified when one RPLC fraction of rat liver extract was digested by the microreactor. This represents the future route for top-down proteomic analysis.

In China, one of the focuses in the fields of microchip application is to develop new protein analysis techniques. Yao et al. [65] presented a simply fabricated microfluidic device using a green organic light-emitting diode (OLED) and thin film interference filter as integrated excitation source and applied it to fluorescence detection of proteins. This system has been used for fluorescence detection of Rhodamine 6G, Alexa 532 and BSA conjugates in 4% linear polyacrylamide (LPA) buffer (in 1 6 TBE, pH 8.3) and 1.4 fmol and 35 fmol mass detection limits at 0.7 nL injection volume for Alexa and Rhodamine dye have been obtained, respectively. A facile and disposable microfluidic device for rapid protein concentration was fabricated by Yu et al. using a direct-printing process [66]. Using this device, about 103–105-fold protein concentration was achieved within 10 min. Based on the dimer–monomer equilibrium movement of the fluorescent dye Pyronin Y (PY), Xu et al. [67] developed a rapid, simple, highly sensitive, label-free method for protein detection by microchip electrophoresis with LIF detection. Hu et al. [68] presented a microfluidic protein chip for an ultrasensitive and multiplexed assay of cancer biomarkers by combining the high-throughput capabilities of a microfluidic network with the high sensitivity and multicolor imaging ability offered by highly fluorescent quantum dots.

3.5. Devices for immunoassay

Microchip-based immunoassays, which are immunoassays performed on a microchip, has recently been used in various fields owing to its advantages, such as reduction in sample and reagent consumption, short analysis time and simple operation. Different types of immunoassay have been applied to the miniaturization on a microchip.

Tang et al. [69] synthesized magnet core/shell $NiFe_2O_4/SiO_2$ nanoparticles and fabricated an electrochemical magnetic controlled microfluidic device for the detection of four tumor markers. The sensor detection limit was $<0.5\,\mu g\,L^{-1}$ (or <0.5 kunits L^{-1}) for most analytes. Intra- and interassay imprecisions were <4.5% and 8.7% for analyte concentrations $>5\,\mu g\,L^{-1}$ (or >5 kunits L^{-1}), respectively. Luo et al. [70] developed a simple but highly specific immunoassay system for goat anti-human IgG using gold nanoparticles and microfluidic techniques. By controlling the reaction time and flow velocity, a dynamic range of 3 orders of magnitude and a detection sensitivity of $10\,ng\,mL^{-1}$ of coat anti-human IgG were achieved. Wu et al. [71] introduced a disposable multianalyte electrochemical immunosensor assay for automated simultaneous determination of tumor markers with the detection limits of $1.1\,ng\,mL^{-1}$, $1.7\,ng\,mL^{-1}$, 1.7 kilounits L^{-1}, and 1.2 units L^{-1} for carcinoembryonic antigen (CEA), α-AFP, CA 125, and β-hCG using the horseradish peroxidase (HRP)-labeled antibodies. A bead-based microfluidic device was developed and demonstrated by Liu et al. to achieve rapid and sensitive enzyme-linked immunosorbent assay (ELISA) with quantum dots as the labeling fluorophore for virus detection [72]. Tang et al. [69] reported the fabrication of a novel bead-bed immunoassay system on a microchip for multiplex measurement of 4 tumor markers, AFP, CEA, CA 125, and CA 15-3, with the switching and controlling of electrochemical signals by means of an external magnet.

The electrocatalytic properties of nanoparticle labels have been used for signal amplification in clinical immunoassays [73]. One major merit of using nanoparticles is that the nanoparticles can provide unique chemical and physical properties to enable new and advanced functions, such as a high surface-to-volume ratio and surface free energy in comparison with bulk materials [74]. Wang et al. [75] and Fan et al. [76] used gold nanoparticles as labels for the IgG detection in human with low detection limits. Luo et al. [70] showed a simple technique of protein immunoassay based on the deposition of gold nanoparticles coated with protein antigens in the presence of their corresponding antibodies to a microfluidic channels' surface.

While great progresses have already been made for microchip applications in the bio-field, the size of analytical apparatuses remains more or less unchanged. The volumes of the analytical apparatus such as MS, ILF and Raman are generally several orders than that of the microchips. The advantages of portability are hence weakened. As these apparatus can be very important in the healthcare, industries and research applications, the miniaturization is thus very essential for further applications. Nevertheless, it is rather challenging to develop such a miniaturized device because the sensitivity is usually degraded with the remarkable volume decrease. More international cooperation are expected from both theoretical and technological sides.

4. Micro power systems and micro heat transfer

4.1. Fuel processing devices and micro fuel cells

In recent years, the micro fuel cells have become a research focus because they are suitable for the portable electronics, such as notebook, personal digital assistants (PDAs), camcorders, and mobile phones. The polymer electrolyte membrane or proton exchange membrane (PEM) fuel cell, or direct methanol fuel cell (DMFC) when methanol is used as fuel, is favorable for portable equipment due to its low operating temperature and high electric conversion efficiency.

Many attempts in China have been made to reduce the total size of the fuel cells, such as Yu et al. [77] and Lee and Chuang [78]. Pan et al. [79] developed the micrometer-sized fuel cell using

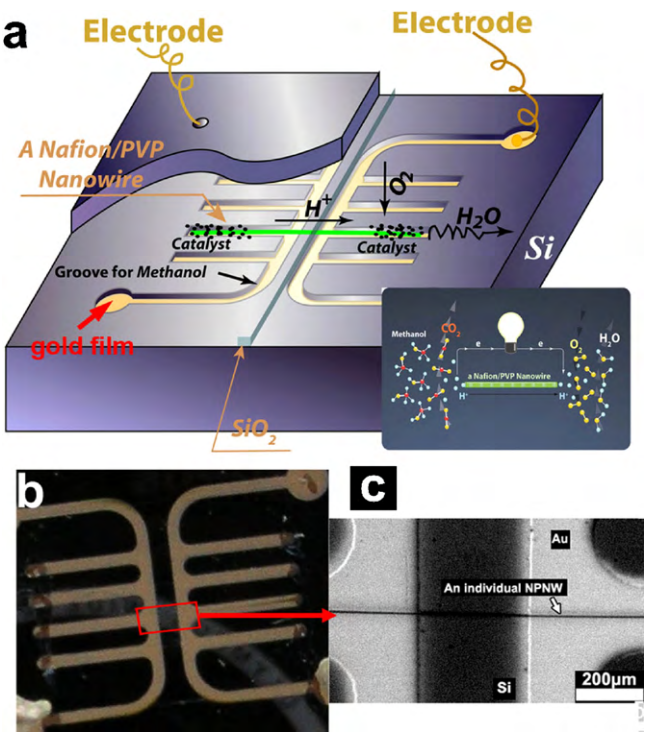

Fig. 7. (a) Schematic 3D representation of the structure of the micro fuel cell. A groove in the Si substrate (with a 50 nm Au film deposited on the groove) serves simultaneously as a "microchannel" fuel container and an electrode. The NPNWs were kept in place on the Si substrate by mounting a silicon oxide "dam" (of 100 mm width and several mm thickness) on top in the middle of the silicon substrate. The inset shows the working principle of the micro-fuel cell. (b) A top-view (overhead view) of the micro fuel cell. (c) A magnified image of the area marked by the red rectangle in (b) [79].

Nafion/poly (vinyl pyrrolidone) (PVP) nanowires (NPNWs) as the electrolyte. This micro fuel cell was monolithically integrated on a silicon substrate and consisted of NPNWs, PtRu/C and Pt/C catalysts, two electrodes, methanol as fuel, and air as oxidant (see Fig. 7). The performance of the micro fuel cell was several orders of magnitude higher than that of traditional fuel cell power sources. NPNW-based micro fuel cells may provide a good future for integrated, self-powered nanodevices.

Silicon-based fuel cell micro power system using a microfabrication technique of comparable power density to the conventional counterparts has been widely concerned [80]. Zhang et al. [81] reported a silicon-based μDMFC integrated with a temperature control system, consisting of a heater and a temperature sensor. This work would make it possible for a μDMFC to enhance the performance by adjusting to an optimal temperature. μDMFCs can be employed in extreme environments, such as severe winter, polar region, outer space, desert and deep sea area. The fabrication and performance evaluation of an MEMS-based planar 6-cell PEMFC stack combined with a small hydrogen storage canister were reported by Zhang et al. [82]. Operating on dry H2 at a 40 mL min^{-1} flow rate and air-breathing conditions at room temperature and atmospheric pressure, the linear polarization experiment gave a measured peak power of 0.9 W at 250 mA cm^{-2} for the stack and average power density of 104 mW cm^{-2} for each cell. Suo et al. [83] presented two kinds of μDMFC stack in planar array. The silicon-based DMFC stack is designed in a flip-flop configuration using MEMS technology, and the stainless steel stack is fabricated using stamping technology. Experimental results show that these two kinds of stacks have similar output 6.75 mW and 6.77 mW at same operation conditions. The performance of the MEMS-based DMFC stack is rather good for application in portable electronic products. Wei et al. [84] reported a novel star-shaped micro solid oxide fuel cell, which can power an USB fan with good performance with diluted methane–oxygen mixture. The addition of carbon nanotubes into anodic micro-porous layer of the membrane electrode assembly significantly improves the performance of the passive μDMFCs [85]. Liu et al. [86] presented a modification method of the commercial proton exchange membrane Nafion® 117 to produce an improved polymer electrolyte membrane for μDMFCs. The method involves γ-ray radiation and electroless palladium deposition. The single μDMFC produced power density performance as high as 4.9 mW cm^{-2} under 2 M methanol solution at room temperature. Membraneless fuel cell using laminar flow in a Y-shaped microchannel was also reported by Chang et al. [87] and Sun et al. [88]. To reduce the membrane transport resistance, Kwan et al. successfully employed microfabricated micromembranes from zeolites as a PEM in a micro fuel cell [89,90]. A rubber pad forming process was used to fabricate the metallic bipolar plate for a proton exchange membrane fuel cell, which has multi-array micro-scale flow channels on its surface. This rubber pad forming process has a lot of advantages such as high surface quality and dimensional accuracy of the formed parts, low cost of the die and high efficiency [91].

4.2. Micro heat-exchangers and microheat sinks

The importance of the convective heat transfer with and without phase change in microchannel structures has increased significantly due to practical applications in thermal control of electronic, power, and laser devices as well as other applications where lightweight, small heat-exchangers are required. In contrast to the conventional heat-exchangers, the main advantage of the micro-heat-exchangers is their extremely high heat transfer area per unit volume.

Convective flow with phase change can achieve very high heat-removal rate for a constant flow rate, while maintaining a relatively constant surface temperature determined by the saturation properties of the cooling fluid. Recently, great attention has been paid to phase-change flow and heat transfer in the micro-scale, using silicon or copper microchannel heat sinks, due to its effective heat transfer performance as compared with single-phase liquid flow in microchannels. A set of microchannel heat sinks, integrated with temperature sensors, varying in channel height from 5 to 510 μm, have been fabricated for the study of forced convection boiling in microchannel flow by Lee et al. [92].

Xu et al. [93] performed the boiling two-phase flow experiment in ten parallel triangular microchannels at high heat fluxes. Wu et al. [94] described and explained the two-phase flow instabilities occurring in the silicon-based micro heat sinks. Cheng et al. [95] described the evolution of phase-change heat transfer, including flow boiling and flow condensation in microchannels (with application to microchannel heat sinks and microheat-exchangers) as well as bubble growth and collapse on microheaters under pulse heating (with applications to micropumps and thermal inkjet printerheads). Qi et al. [96] developed microchannel tube with heat transfer enhancement characteristics in mobile air conditioning (MAC) industry to improve system performance and reduce the effect of MAC on environment. In their work, two retrofitted compact and high efficient microchannel evaporator and condenser were proposed to replace the currently used MAC heat-exchangers. The enhanced system could supply more cooling capacity to car compartment under all test conditions. Cooling capacity and coefficient of performance of the enhanced system was increased by about 5% and 8% under high vehicle speed condition.

Capillary-assisted evaporation is gaining more and more attention in recent years for its high heat transfer coefficient benefited from the extremely thin evaporating liquid film. Xia et al. [97]

introduced such a micro-scale heat transfer method into normal-scale applications. A series of enhanced heat transfer tubes with circumferential rectangular micro-grooves on the outside surfaces have been experimentally investigated. The experimental results have indicated that there is a positive correlation between the evaporation heat transfer coefficient and evaporation pressure, and negative for the superheating and immersion depth.

Up to now, very little information is available on condensation in microchannels with a hydraulic diameter smaller than 100 μm. A recent study of condensation in microchannels was reported by Wu and Cheng [98] for channels with a hydraulic diameter of 82.8 μm where various flow patterns, such as fully droplet flow, droplet/annular/injection/slug-bubbly flow, annular/injection/slug-droplet flow, and fully slug-bubbly flow, were observed. They found that pressure and temperature oscillations correlated with these flow patterns. Zhang et al. [99] reported condensation flow in a single silicon microchannel with a depth of 30 μm, a width of 800 μm and a length of 5.0 mm, covered with a Pyrex glass to allow for visualization of the bubble formation process. This study suggested that a method for controlling the size and generation frequency of microbubbles could be so developed, which may be of the interest for microfluidic applications. The breakup of the elongated bubble was caused by the large Weber number at the tip of the elongated bubble induced by the maximum vapor velocity at the centerline of the microchannel inside the elongated bubble and the smaller surface tension force of water at the tip of the elongated bubble. In addition, the flow and heat transfer performance of a microchannel heat-exchanger and a micro-porous heat-exchanger were also theoretically and experimentally investigated and evaluated by Jiang et al. [100]. The results showed that the deep microchannel design offers a better overall performance than either the porous media or shallow microchannel alternatives.

Although the great advances are achieved in the fields of micro power systems and micro heat transfer, there is still a lack of fundamental understanding of the heat and mass transfer in the systems. The systematic and detailed investigations on the heat and mass transfer phenomena at the micro-scale are needed. However, it is rather challenging to measure the distributions of some data such as temperature, pressure, and concentration at the micro-scale because the uncertainty is increased with the dimension decrease and the effects of interference during the measurement become more obvious. Therefore, we suggest that the future research should focus on developing accurate and sensitive measurement approaches for the high-efficiency micro power systems and micro heat-exchangers.

5. Design and manufacture

5.1. Modeling and simulation

Before any rules or criteria are made available for design, computer simulation is primarily considered to be a design tool though it can also be used to support the interpretation of experimental data. Computational fluid dynamics (CFD) simulation can provide valuable insight into various micro processes, such as mixing, fluid transport, cell sorting and separation of bimolecular.

Guan et al. [101] used a VOF (Volume Of Fluid) based CFD scheme to simulate the flow in impinging jet micromixers which were developed as a solution against fouling in microreactors. It showed that the angle of jets and the inject speed have important effects to the mixing effect. Design improvements of the impinging jet micromixers are thus proposed. Wu and Liu [102] numerically and experimentally studied the electroosmotic mixing of two fluid streams in a three-dimensional microchannel. The obtained images of fluid mixing in their studies are generally two dimensional and do not provide a detailed description of the fluid mixing dynamics from a cross-section perspective in these electroosmotically driven mixers. Therefore, Chang and Yang [103] investigated the secondary flow induced by these specific designs and showed how to mix the fluid streams from a cross-section perspective. In addition, the study also presented a new straight diagonal/symmetric herringbone mixer. Tsai et al. [104] performed 2-D and 3-D numerical simulations to investigate fluid flow behavior in a sudden expansion microchannel, which is commonly applied in fuel cell devices or biochips.

Zhuang et al. [105] described a numerical investigation of different electrokinetic injection techniques to deliver a sample plug within electrophoresis microchips. Droplet formation in immiscible fluids has applications in many fields including pharmaceuticals, biology, foods and cosmetics. As microdroplets can be generated in microfluidic devices, Hong and Wang [106] numerically investigated the flow rate effect on the droplet formation in a co-flow microfluidic device and demarcated four drop pattern regions which is helpful to assure the accuracy and efficiency in the droplet production.

5.2. Manufacture technologies

To produce a reliable microfluidic device, the selection of suitable materials and fabrication methods is of high importance. At present, materials for microfabrication can be silicon, glass, polydimethylsiloxane and poly methyl methacrylate (PMMA), stainless steels, etc. However, no single process flow can be used to fabricate all possible microfluidics or microreactors. The company foundations established during the last 2–3 decades for MEMs fabrication offer a great feasibility. Process flows of silicon based microfluidics, for instance, may include wet bulk etching and wafer bonding, surface micromachining, deep trench silicon micromachining, and micromoulding. The microfabrication technology in general may cover deep lithography, photolithography, etching, micromoulding, diffusion bonding, deposition, plasmatreatment, laser micromachining, mechanical micromachining, and micrometrology.

He et al. [107] presented the fabrication of glass microfluidic chips which can be achieved at room-temperature bonding without the requirement of clean-room facilities. Meantime, they also produced monolithic sampling probes on glass chips with tip diameters of a few hundred micrometers using simple tools including a glass cutter and a bench drill. Guan and co-workers [108] fabricated 1–3-stages electroosmotic pump (EOP) using a porous silica particles packed-columns, fused-silica capillaries and stainless electrodes. Zhong et al. [109] developed two methods to fabricate the two-weir structured glass chips: a “two-side etching/alignment” method and a simplified “one side/two-step etching” method. The former method required a straightforward alignment step, while the latter approach comprised a simplified wet etching process using paraffin wax as the temporary protective layer. Both methods were convenient and rapid as compared to the previous approaches. Sun and Yin [110] produced a novel multi-depth microfluidic chip on glass substrate using the conventional lithography and three-step etching technology. Qu et al. [111] demonstrated the rapid fabrication of poly methyl methacrylate microfluidic chips using polydimethylsiloxane template fabricated by soft lithography. Liu et al. [112] developed a facile and rapid one-step technique for design and fabrication of passive micromixers in microfluidic devices using a direct-printing process. Jin et al. [113] used a facile one-step laser etching method to achieve rough PDMS surface containing micro-, submicro- and nano-composite structures. Such surface shows a super-hydrophobic character. Sun et al. [114] described the fabrication of microfluidic channel struc-

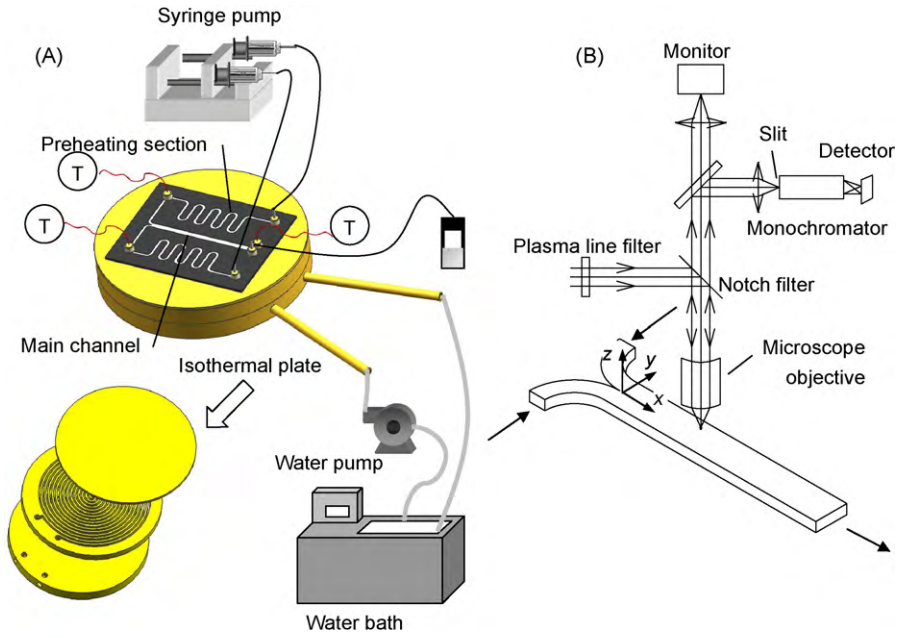

Fig. 8. (A) Setup for temperature-dependent diffusion measurements. Two liquids were separately injected into the two inlets and fully pre-heated in each pre-heating section. After converging at the Y junction, they interdiffused into the main channel and then flowed out. Three thermocouples were set to measure the temperatures at the two inlets and one outlet. Pre-heating water was circulated in the isothermal plate by the pump, and its temperature was controlled by water bath. (B) Schematic of Raman measurements in the microchannel. A Raman microscope was operated at the hydro- and thermodynamic steady states. The coordinate system is presented [117].

tures on the surface of a borosilicate glass slide by femtosecond laser direct writing for optical waveguide application. Chang and Su [115] proposed PDMS emulsification devices to serve as versatile and low-cost emulsification tools for various biological and pharmaceutical applications, thus overcoming the difficulty in integrating the surface treatment or manual assembly process with the fabrication of common micro-fluidic system.

Stainless steel owns the merit of corrosive resistance and high temperature stability, Yu et al. [116] developed a microchannel reactor concerning steam reforming of methanol using the diffusion bonding of FeCrAl alloy and coating of catalyst at the micro-scale. The diffusion bonding technique allows the fabrication of the stainless steel microreactors in a low price. Catalytic surface was prepared by coating the aluminum oxide on the surface and impregnation in an aqueous solution. The test results show that the microchannel reactor generated enough hydrogen with a power of 11 W.

A recent example of fabrication of a coated microfluidic chip is given by Lin et al. [117]. An Al-coated microfluidic chip is developed to measure the diffusion coefficient (D) using a confocal Raman microscope, schematically presented in Fig. 8. Silicon was chosen as the substrate material because of its high thermal conductivity, which is required by the isothermal conditions for D measurements. The shape of the microchannel was patterned on the silicon substrate by Deep Reactive Ion Etching (DRIE). Six access holes were drilled on a Pyrex glass wafer. Two of these were located on the arms, and one was located on the outlet, all of which were used for the insertion of three thermocouples for liquid temperature measurements. The three other holes formed two inlets and one outlet for liquid flow. The substrate with microchannel was then enclosed by the Pyrex glass wafer using anodic bonding. A 200 nm-thick Al film was sputtered onto the silicon surface after DRIE. The fabrication process is schematically shown in Fig. 9 A. The image reversal photoresist AZ5214E has unique characteristics

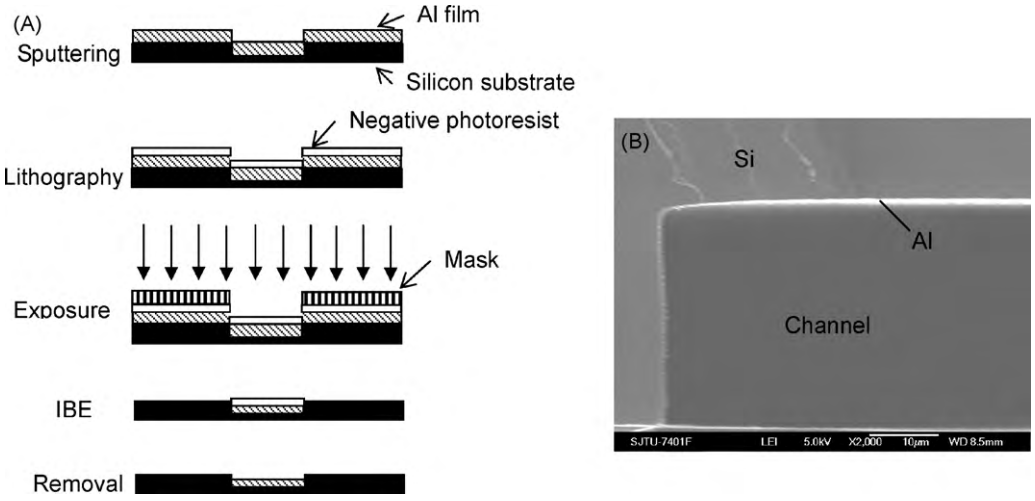

Fig. 9. (A) Fabrication process of depositing the aluminum film onto the channel bottom. (B) SEM photograph of the microchannel cross-section with the aluminum film [117].

in image reversal processing and is widely used for lift-off metallization. This photoresist was used as the negative photoresist and spin-coated onto the surface with the Al film for lithography. The Al film on the channel bottom was protected by the photoresist, while the Al film outside the microchannel was lifted off through Ion Beam Etching (IBE). After cleaning, the substrate with the Al film on the channel bottom was covered with the Pyrex glass wafer by anodic bonding. Fig. 9B presents the SEM photograph of the microchannel cross-section with the Al film. The Al film can clearly be distinguished from the silicon substrate. The Al film, serving as a reflection structure, was formed via a combination of negative resist photolithography and ion beam etching after sputtering. The Al film substantially reduced absorption of laser power, thus ensuring the measured *D* values in excellent agreement with theoretical data.

With increasing interest and investment for micro-engineering, the fabrication technologies will surely be developed much faster than before. However, the standardization of these technologies is in general lack of serious efforts, which may limit the large-scale industrial application. It is expected that more industrial partners be involved in future developments as the manufacturing technology is versatile and can be used in other cases in addition to the microsystems.

6. Concluding remarks

This paper reviewed the progresses achieved in recent years in China in the fields of chemical, thermal and biologic systems by applying micro technologies. The recent advances include microreactor-based systems for chemical processing and analysis, biological microfluidics for DNA analysis, cell handling and analysis, separation based detection, protein based applications and immunoassay, micro heat transfer and micro power systems, and design and manufacturing. It is suggested that deep investigations are required to evaluate the feasibility of the microreactor technology in the industrial or commercial aspects. Further research efforts should be taken to miniaturize analytical apparatuses in order to achieve a compatible size with current microfluidic chips. The integration of separate operation units and the evaluation on the stability and reliability of the complex integrated system require further research and development. The systematic and detailed studies on the heat and mass transfer behaviors at the micro-scale are urgent with the expected breakthrough in the accurate and sensitive measurements at the micro-scale. For the large-scale industrial application of the micro systems, more efforts should be taken in the establishment of the corresponding standards concerning the design and micro manufacturing technologies. It is believed that the microreactor and microfluidic chip is on the eve of the larger-scale industrial application. More international cooperation is expected in prompting the progress in both research and commercialization.

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