Biotechnology and Bioprocess Engineering **2003**, 8: 213-220

Miniaturization of Polymerase Chain Reaction

Ji Youn Lee, Jae Jeong Kim, and Tai Hyun Park*

School of Chemical Engineering and Institute of Chemical Processes, Seoul National University, Seoul 151-744, Korea

Abstract Polymerase chain reaction (PCR) is one of the most widely used analytical tool and is an important module that would benefit from being miniaturized and integrated onto diagnostic or analytical chips. There are potentially two different approaches for the miniaturization of the PCR module: chamber-type and flow-type micro-PCR. These miniaturized PCRs have distinct characteristics and advantages. In this article, we review the necessity of micro-PCR, the materials for the chip fabrication, the surface modification, and characteristics of the two types of micro-PCR. The motivation underlying the development of micro-PCR, the advantages and disadvantages of the various materials used in fabrication and the surface modification methods will be discussed. And finally, the precise features of the two different types of micro-PCR will be compared.

Keywords: polymerase chain reaction (PCR), micro-PCR, miniaturization, chamber-type micro-PCR, flow-type micro-PCR

Polymerase Chain Reaction

The polymerase chain reaction (PCR) is an *in vitro* DNA amplification method that was introduced in 1985 [1]. The adoption of the thermostable Taq polymerase in 1988 greatly simplified the process, and enabled the automation of PCR [2]. Theoretically, the target DNA is doubled every cycle through the denaturation, annealing and polymerization reaction steps, and consequently the amount of DNA increases exponentially with time [3]. This process enables one to amplify and detect small amount of target DNA. Since the introduction of the traditional PCR technology, newly modified PCR methods, such as multiplex, hot start, touchdown, reverse, long and real-time PCR, have been developed, and are now being used practically. PCR technology has boosted the molecular biological research and it becomes one of the most useful and versatile processes in the biological sciences.

Motivation of Micro-PCR: Conventional PCR to Micro-PCR

For various purposes there have been many efforts to miniaturize and integrate the analytical tools onto a chip-based device, which has become possible due to the advancement of micro-electro-mechanical system (MEMS) technology and include DNA chip, protein chip and lab-on-a-chip, which can be directly utilized for commercial diagnostic testing [4,5]. A wide range of analytical reactions and procedures are currently miniaturized and integrated into small sized chips, including DNA trapping and separation [6], sizing and sorting [7], sample concentration [8], automated enzyme assay [9], immunoassay [10], SSLPs analysis [11], ligase chain reaction [12], capillary (array) electrophoresis [13], sequencing [14], mass spectrometric assay [15,16] and PCR [17,18], as well as other biotechnology/biochemical devices [19]. These chips, smaller than a credit card, are used to analyze many samples in a short time. PCR is important in clinical assays, and is an essential analytical tool in diagnostic testing. The most important factor for successful PCR is the maintenance of three distinct temperatures. The miniaturization of PCR might be beneficial as the thermal mass would be reduced and the physical limitation of thermal diffusion, caused by the large dimension of a tube, overcome.

The principal advantages with the miniaturization of analytical tools are: the small sample requirements (microliter to nanoliter), the short analyses times, the creation of relatively high surface-to-volume ratios, the cost reduction and the possibility of manufacturing portable or hand-held devices [18,20]. The surface-to-volume ratio of conventional PCR reaction tubes is about 1.5 mm^2/L, which varies depending on the geometry of the reaction chamber or capillary, and the volume of the reactor [21]. The performance changes caused by the miniaturization have been presented by Jakeway *et al.* [22]. However, the miniaturization may lead to a reduction in the scale of the reaction, and make material handling and product detection difficult. The handling and processing of materials in microstructured devices are also challenging issues [23]. In these cases, an integrated system is necessary, and there have been many approaches for the integration of

***Corresponding author**
Tel: +82-2-880-8020 Fax: +82-2-875-9348
e-mail: thpark@plaza.snu.ac.kr

different tools onto one chip, the so-called, 'micro-total analysis system' (μTAS) [22,24]. PCR has been integrated with other units, *e.g.* the integration of cell isolation and PCR onto a silicon chip [25], PCR with capillary electrophoresis [26-29], PCR and capillary gel electrophoresis [30-32], cell lysis, PCR and CE [33], multiple enzyme reactions in genetic assays [34], disruption of *Bacillus* spores and real-time PCR [35] and various DNA analysis tools [36]. In addition to conventional PCR, other modified PCR methods, such as multiplex PCR [26, 33] and real-time PCR [20,35,37,38] have been miniaturized successfully. For the real-time PCR, optical windows are employed for the real-time fluorescence monitoring of the amplified DNA product; transparent substances, such as quartz or glass, are used for the fabrication of this type of chip [39]. The fabrication methods for these chips are well developed, and are summarized in the literature [40].

The miniaturization of PCR offers three major advantages: 1) the volume reduction; which is directly related to the reduction in the thermal mass, sample and cost; 2) the time reduction; fast thermal cycling is possible due to the small thermal mass; and 3) its ease of integration with other analytical tools. The detailed features relating to these advantages will be discussed later.

Materials for Micro-PCR

For the fabrication of micro-PCR, various materials, or hybrid materials, are used. The key properties of the chosen materials include: thermal conductivity, temperature gradient and temperature uniformity, as the temperature control is the most important factor to be considered. The most popular choice is silicon, which is a traditional fabrication material. Glass, quartz and polymers, such as PDMS (polydimethylsulfoxane) and PMMA (polymethylmethacrylate), are also applied to chip fabrication. These materials have different properties, and their own advantages and disadvantages for their use in fabrication. The thermal conductivity of silicon is 1.457 W/cm-K, which is almost 200 times higher than glass, whose thermal conductivity is 0.008 W/cm-K. Therefore, the temperature patterns between a glass and a silicon-glass hybrid chip will be greatly different. Hybrid materials are also used in the fabrication of micro-PCR, such as glass-glass-bonding and silicon-glass-bonding chips.

Silicon has been widely used as a fabrication material in traditional semi-conductors and it was successfully adopted for the biological chip systems. Silicon is thermally conductive, compared with polypropylene (PP) tube used in the conventional PCR, so has the potential for rapid cycling and formation of uniform temperature zones [18,41]. The high thermal conductivity also makes the sample temperature gradient small, and precise temperature control possible. Moreover, the fabrication processes are well developed, so the precise and complex structures can be easily fabricated in silicon using current patterning methods. An example of the fabrication process is shown in Fig. 1. Although silicon has these advantages, it also has some shortcomings, one being its

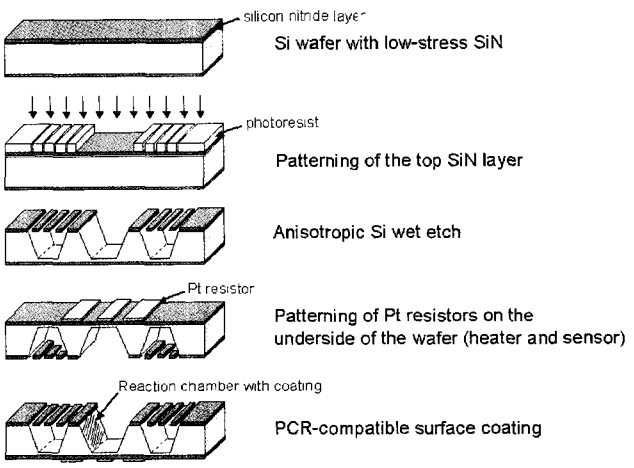

Fig. 1. Silicon PCR chip fabrication process.

opaque property. Micro-PCR is often integrated with other analytical tools, especially capillary electrophoresis, for sizing of the amplified products. In the above case, silicon is difficult to incorporate, as it is electrically conductive and optically opaque. However, this can be overcome by introducing of a low stress silicon nitride optical window for the optical readout [18].

Glass is a transparent material, so is a good candidate when optical properties are needed. In many cases, optical methods, such as fluorescence or chemiluminescence are useful for the detection and quantification of molecules, where transparency is often required. Therefore, optical methods are usually used in integrated chips for PCR and CE. The micromachining technology for glass is also well developed. Quartz has good optical properties also, and is another transparent fabrication material of choice; however, it is expensive and relatively hard to fabricate.

Microchips fabricated from polymer materials, by soft-lithography technology, are increasingly being studied for their practical applications. The materials mentioned above are hard to use, and time-consuming for chip fabrication, and consequently the chips are expensive. There are some polymer materials that can be used for chip fabrication, such as PDMS and PMMA, and have advantages over traditional materials [32,42].

PDMS is the most preferred polymer material for chip fabrication in biological applications. Various kinds of microchip, based on soft-lithography, have recently been fabricated for various purposes [43]. For example, a PDMS chip, mounted on rigid substrates, such as glass and PMMA, with holes punched through the PDMS, used as access ports for liquid, air and electrodes has been fabricated [44]. The fabrication process was relatively simple, as shown in Fig. 2. PDMS is a transparent material, so can be used when optical detection is needed. It is cheap, compared with silicon, glass or quartz, and mass production is easy. However, the silicon elastomer, PDMS, is naturally hydrophobic, so surface modification is needed to make the surface hydrophilic for practical

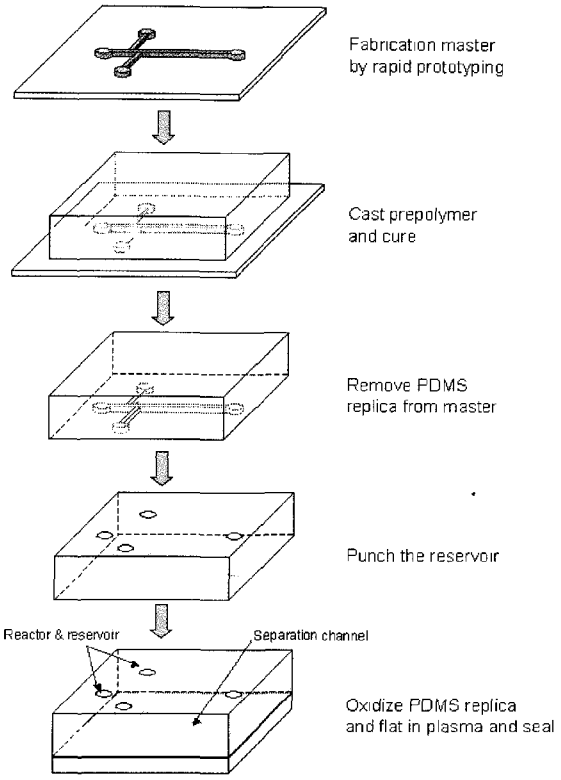

Fig. 2. PDMS PCR-CE chip fabrication process.

usage. The surface treatment methods will be discussed in detail below. Though PDMS is not suitable for complex structure fabrications, such as multi-layer chip or integrated systems containing heater or electrode, it does have merit in the fabrication of simple two-dimensionally structured chips. Developing micro-PCR systems, based on inexpensive polymer materials, has great advantages in commercial applications.

Two Types of Micro-PCR

Miniaturization of PCR can be divided into two types, depending on the structure and temperature control approaches. These are the chamber- and continuous-flow type PCRs. Since PCR is an analytical tool based on an enzyme reaction, temperature control is a key factor, as with other enzymatic reactions. Chamber-type PCR is just a miniaturized version of conventional PCR in that the temperature is controlled by a programmable instrument. The other type is continuous-flow PCR, which utilizes microfluidics, where it is divided into three different temperature zones, and the PCR reaction occurs in a channel. The recent studies on these two types of PCR will be discussed in more detail later.

Temperature Control in Micro-PCR

PCR is a cyclic process, and consists of three different steps: denaturation of double-stranded template DNA, annealing of a primer to the template, and extension of the primer by a thermostable polymerase. These three steps are carried out at three different characteristic temperatures. Since the temperature control is key to a successful PCR, there have been a number of studies on temperature analysis [45], simulation and modeling [46, 47]. Conventionally, temperature control is performed with programmable instruments, consisting of a heating element and cooling fan. The most widely used heating method relies upon Peltier effect and the achievable heating and cooling rate is about 1~3°C/s in the temperature range of 4~100°C. Conventional systems have a large thermal mass, which results in low heating and cooling rates. This decreases the efficiency of the polymerization reaction, and extends the required incubation time. In a micro-PCR system, the cycling reaction is completed in a shorter time, due to the small thermal mass. Therefore, the precise and uniform maintenance of the temperature in each step becomes more important. In many cases, the temperature uniformity is monitored with integrated thermal sensors or an IR camera [48,49].

Several heating methods have been developed for micro-PCR systems, and are categorized into two major categories, exterior and integrated. Exterior methods are divided into two further heating types. One uses a conventional heating element, Peltier [21,25,27,31] or a resistive heater [49,50], which are small devices placed under the chip, and heat the whole chip. The heat is transferred to the sample through the thermally conductive materials, as shown in Figs. 3 (a) and (d). The heating rate ranges between 5~15°C/s, with an accuracy in the range of ± 0.5°C. The other is a non-contact type, where a tungsten lamp is used as an inexpensive infrared radiation source for rapid and effective thermocycling [51,52]. In this method, the temperature heating and cooling ramping rates can be as rapid as 10°C/s and 20°C/s, respectively, in a glass microchamber. However, this infrared radiation heat source requires a lot of space, and it is not suitable for miniaturization.

These exterior heating methods are not suitable for integrated systems that require local heating and cooling. Therefore, there have been many studies on the integration of thermal sources into the chip by patterning the heating elements, such as thin film indium-tin-oxide (ITO) [39], platinum [18,48], copper, nickel-chromium [41] or polysilicon resistors [50]. ITO has some advantageous features as a thermal source, since it has a low resistivity (about 10^{-4} Ω cm), strong adhesion to glass substrates and good transmittance in visible regions (> 80%). The ITO thin film is deposited on the backside of the etched substrate using a lift-off technique. Platinum or copper resistors have resistivities lower than ITO, and are patterned beneath the chamber, as shown in Fig. 1. Since the integrated platinum sensor has linear characteristics, with a high temperature coefficient of resistance in the operating range from room temperature up to 100°C, it is favored as a thermal source [53]. The heating and cooling rates are 15°C/s and 3.5°C/s, respectively. Efficient and rapid cooling is also necessary for fast thermal cycling. Nitrogen gas purging or compressed air flowing,

is used when powerful cooling is required, and the achievable cooling rate is 10~20°C/s [28,50,51]. Fan blowing or water circulation can also be used for cooling.

Surface Modification and Reaction Additives

As mentioned above, a micro-PCR system has several advantages over conventional PCR systems; rapid thermal cycling, little sample consumption and a short analysis time, *etc.* However, there also exist some unintended disadvantages resulting from the miniaturization, such as high levels of non-specific adsorption of the reactants to the reactor due to the small volume and large surface-to-volume ratio. Particularly, chips made of hydrophobic polymer materials cause many problems. In many cases, the sample is a hydrophilic solution, and not easily introduced into the hydrophobic channel.

Choosing an appropriate material having a minimal affinity for the PCR mixture is required; however, non-specific adsorption of proteins and nucleic acids, and inhibitory effects, are inevitable to some extent. The most popular material, silicon, is known to be an inhibitor of PCR [18,20,25,46]. Therefore, appropriate surface (chamber or channel) modification is required for its successful application.

There are several ways to render the surface inert, including plasma treatment, as a physical method, and treatment with silicon powder, silanizing agents, polymers or acids, as a chemical methods [21,48,52]. Silicon dioxide coating [18,48], or addition of dynamic coating additives [52], during the reaction is another method for surface passivation. These make the surface hydrophilic, and consequently samples can be easily introduced to the channel or chamber [31]. Usually BSA (bovine serum albumin) is used as a dynamic coating agent to prevent non-specific adsorption, and importance of the BSA has been emphasized in many papers [18,31,48]. Since some materials used in micro-PCR are not suitable for enzymatic reactions, surface modification and condition optimization are indispensable [37].

Chamber-type Micro-PCR

Chamber-type micro-PCR resembles conventional PCR in many aspects. Conventional thermal-cyclers are based on a batch-process, and are usually used in array structures. Micro-PCR is much different in dimension, analysis time, and in its integration with other analytical tools. Some typical chamber-type micro-PCRs are listed in Fig. 3. A chamber is formed by chemical etching (silicon, glass or quartz) or physical punching (polymer materials) and cover plate bonding with the substrate in a blocked system are employed. Heaters and sensors are located underneath the chamber, and give rapid and efficient heating and cooling.

The arrays of fabrication materials are diverse, *e. g.* silicon [18,30,41,51], glass [27,50,52], silicon-glass [26, 48,49,54] glass-glass [27,28,55] and polymer-glass-bonding systems [31]. Other types using cartridge are also fabricated [56]. In many cases, micro-PCRs, made of glass or a glass a hybrid, are integrated with CE, or CGE, for product sizing. PDMS is a newly adopted transparent material for micro-PCR fabrication, and its chips are also feasible for integration with sizing and optical detection instruments as illustrated in Fig. 3 (a) [31]. Chamber-type micro-PCRs are often fabricated in an array, or a multi-chamber structure, for high-throughput analysis, as shown in Figs. 3 (c) and (d). The thermal cycling time is fast, with an average time of several minutes for dozens of cycles. The systems would require optimization of the reagents and temperature profile for even faster PCR.

The reaction volume in chamber-type micro-PCR varies from the nanoliter to the microliter range. However, a small volume does not always mean good performance, as evaporation problem can become serious as the reaction volume is reduced [49]. Therefore, determining the optimal reactor dimensions and reaction conditions are very important for successful micro-PCR.

Flow-type Micro-PCR

Flow-type micro-PCR chips are novel methods, and are quite different from conventional PCR and micro-chamber PCR chip techniques [45]. They resemble serpentine separation channels in shapes as shown in Figs. 4 (a) and (c). Although these types of micro-PCR are in their infancy, with few studies on their applications, compared with the chamber-type micro-PCR, they have as great a potential as the chamber-type systems. Their principle of operation is simple; the sample plug moves in a small channel extending through two or three individual temperature zones, whose temperatures are constant during the course of reaction. The performance of PCR is largely determined by the chip design: the channel dimension, number of channel cycles and the insulation between the thermal zones, *etc.* Controllable parameters for the enhancement of the performance of the PCR include, flow rates, surface modifications and additives. The incubation time in each PCR step are controllable by changing the flow rate (residence time), but the ratio of the incubation times in each zone is fixed, but some degree of variation can be attained by the precise and programmed control of the flow.

There are other factors affecting PCR performance, which are mainly due to the microfluidic characteristics and different heating strategies. Diffusion, and sample plug deformation in the channel are not issues in chamber-type micro-PCR. However, axial sample diffusion and plug deformation, due to the serpentine shape, are problems in flow-type micro-PCR. Chips must be elaborately designed to prevent their deformation. For the maintenance of the three different temperature zones, thermal insulation becomes important, and to this end, dam or air insulation boundaries are generated between the temperature zones, as shown in Figs. 4 (b) and (d) [46,53]. Since the thermal conductivity of stagnant air is about 0.0003 W/cm-K, air can act as a good insulator. In a silicon-glass hybrid chip, the glass can act as a thermal insulator between the silicon parts as indicated in

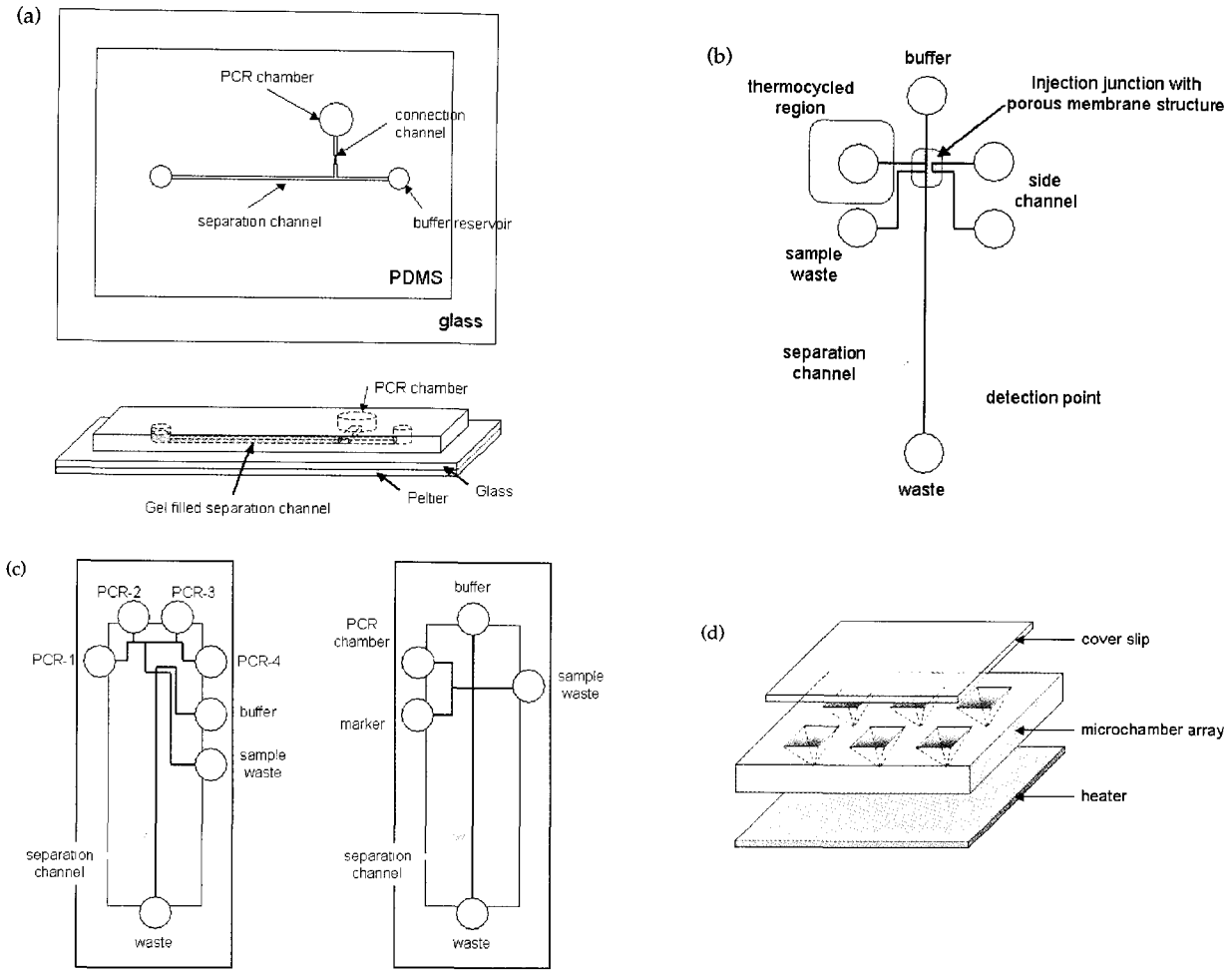

Fig. 3. Examples of the chamber-type micro-PCR (a) Chamber-type micro-PCR integrated with CGE (PDMS-glass chip), (b) Chamber-type micro-PCR integrated with CE and concentrating layer, (c) Modified chamber-type micro-PCR: multiplex PCR and co-running with marker, (d) Array-type micro-PCR.

Fig. 4 (b). The evaporation and bubble formation problems are serious as the total channel volume is in the tens of microliter range, and the denaturation zone is kept at over 90°C. Therefore, for a successful flow-type micro-PCR, the optimal dimensions and operation protocols are important.

One of main advantages of micro-PCR is the time reduction. In flow-type PCR the reaction time can be manipulated by varying the flow rate [17]. Flow-type properties provide for high-throughput analysis by the continuous injection of sample plugs, which can generated with media, such as air, gas and oil. It can be easily integrated with other high-throughput analytical tools, which are required with genome research or drug screening.

By changing the channel width or depth, the surface-to-volume ratios can be regulated much more freely with flow-type PCR than in conventional, or chamber-type, PCR. As the surface-to-volume ratio increases, the surface compatibility of the materials to the sample solution, and the proper surface modifications become more important. Some specific types of PCR are also easily applicable to flow-type PCR: for example, hot start PCR can be executed by the introduction of a buffer solution, containing polymerase, Mg^{2+} ion, dNPTs and primers, through the input channel located at the end of the initial denaturation zone. Possible problems with the flow-type PCR are: sample diffusion and deformation in the channel, which arises due to the microfluidic characteristics. Although these are not serious problems when only PCR is performed, they can become serious when the PCR is integrated with other analytical tools, or used for serial analysis.

CONCLUSION

Miniaturization of a PCR module provides various advantages, such as rapid thermal cycling, less sample requirement and less power consumption, and the miniaturized module can be easily integrated with other ana-

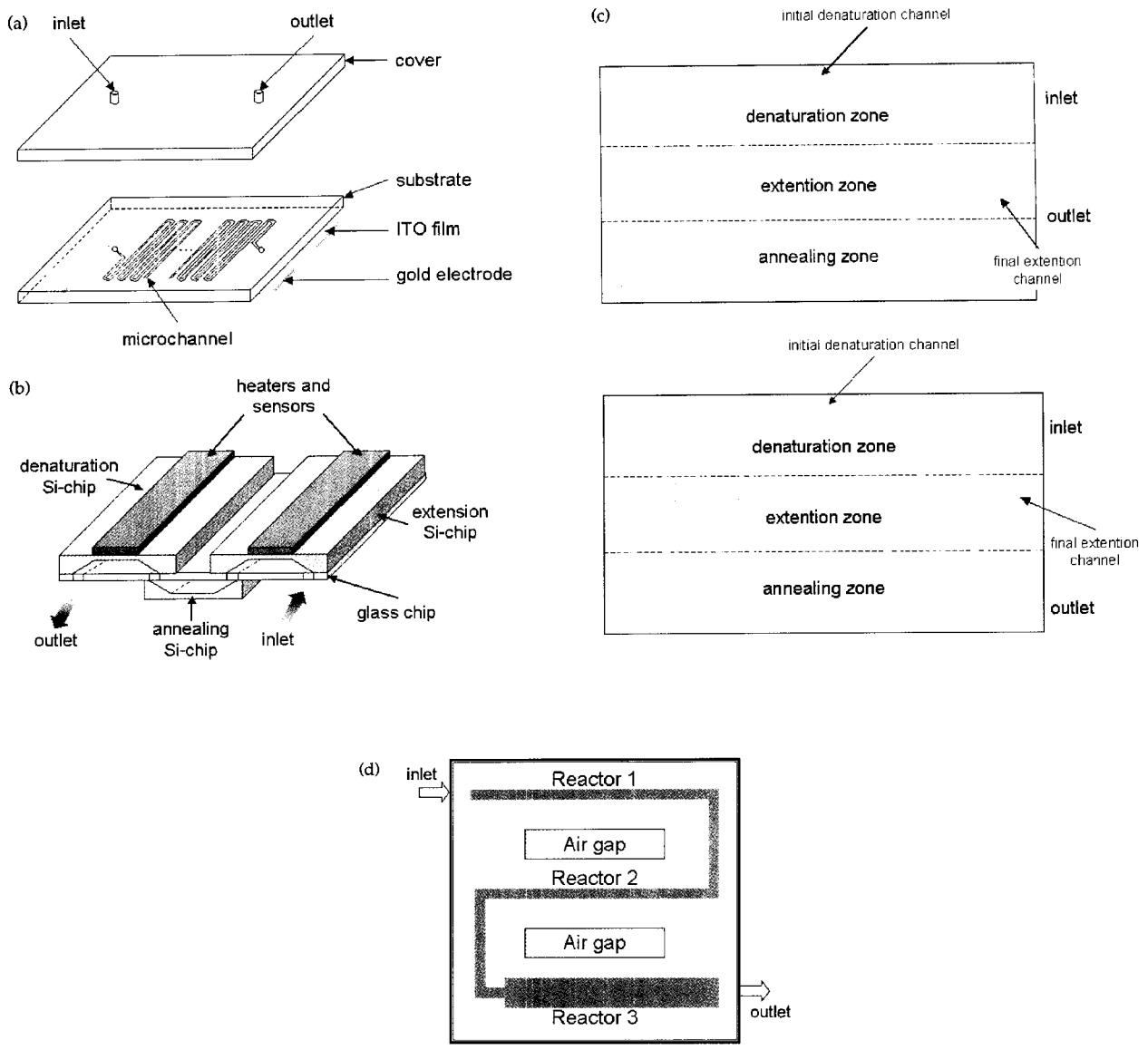

Fig. 4. Examples of the flow-type micro-PCR (a)Flow-type micro-PCR with two temperature zones made of quartz-glass, (b) Silicon-glass hybrid chip for flow-type micro-PCR, (c) Flow-type micro-PCR with three temperature zones and the extension zone modified type, (d) Silicon PCR chip with three reactors and air gaps.

lytical tools. However, the miniaturization also has some problems caused by more adsorption of the reactants to the surface, the very small amount of liquid being handled and the requirement of precise temperature control. More enzymes are required with the miniaturized PCR, as a greater amount of adsorption of the reactant to the surfaces arises due to the high surface-to-volume ratio. Evaporation of the sample solution may be critical due to the small reaction volume. Despite these obstacles, the potential of micro-PCR, as a diagnostic, high-throughput analysis chip, is still sufficiently attractive to take on the challenge of their development. Chamber- and flow-type micro-PCRs can be applied for various usages, either as stand-alone modules, or as an integrated form. For successful micro-PCR, several factors must be considered: firstly, appropriate material must be selected for their fabrication. The smart chip design, suitable for analytical purposes and the selection of efficient heating/cooling systems are other important factors. PCR-favorable surface modification and the addition of appropriate additives are important to increase the PCR efficiency.

Acknowledgement The authors wish to acknowledge the financial support of the Ministry of Science & Technology by nanotechnology research fund.

REFERENCES

[1] Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230: 1350-1354.

[2] Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 239: 487-491.

[3] Schnell, S. and C. Mendoza (1997) Theoretical description of the polymerase chain reaction. *J. Theor. Biol.* 188: 313-318.

[4] Kricka, L. J. (2001) Microchips, microarrays, biochips and nanochips: Personal laboratories for the 21st century. *Clin. Chim. Acta* 307: 219-223.

[5] Whitcombe, D., C. R. Newton, and S. Little (1998) Advances in approaches to DNA-based diagnostics. *Curr. Opin. Biotechnol.* 9: 602-608.

[6] Han, J. and H. G. Craighead (2000) Separation of long DNA molecules in a microfabricated entropic trap array. *Science* 288: 1026-1029.

[7] Chou, H.-P., C. Spence, A. Scherer, and S. Quake (1999) A microfabricated device for sizing and sorting DNA molecules. *Proc. Natl. Acad. Sci. USA* 96: 11-13.

[8] Khandurina, J., S. C. Jacobson, L. C. Waters, R. S. Foote, and J. M. Ramsey (1999) Microfabricated porous membrane structure for sample concentration and electrophoretic analysis. *Anal. Chem.* 71: 1815-1819.

[9] Hadd, A. G., D. E. Raymond, J. W. Halliwell, S. C. Jacobson, and J. M. Ramsey (1997) Microchip device for performing enzyme assays. *Anal. Chem.* 69: 3407-3412.

[10] Wang, J., A. Ibanez, M. P. Chatrathi, and A. Escarpa (2001) Electrochemical enzyme immunoassays on microchip platforms. *Anal. Chem.* 73: 5323-5327.

[11] Dunn, W. C., S. C. Jacobson, L. C. Waters, N. Kroutchinina, J. Khandurina, R. S. Foote, M. J. Justice, L. J. Stubbs, and J. M. Ramsey (2000) PCR amplification and analysis of simple sequence length polymorphisms in mouse DNA using a single microchip device. *Anal. Biochem.* 277: 157-160.

[12] Cheng, J., M. A. Shoffner, K. R. Mitchelson, L. J. Kricka, and P. Wilding (1996) Analysis of ligase chain reaction products amplified in a silicon-glass chip using capillary electrophoresis. *J. Chromatogr. A* 732: 151-158.

[13] Medintz, I. L., B. M. Paegel, and R. A. Mathies (2001) Microfabricated capillary array electrophoresis DNA analysis systems. *J. Chromatogr. A* 924: 265-270.

[14] Scherer, J. R., I. Kheterpal, A. Radhakrishnan, W. Ja William, and R. A. Mathies (1999) Ultra-high throughput rotary capillary array electrophoresis scanner for fluorescent DNA sequencing and analysis. *Electrophoresis* 20: 1508-1517.

[15] Ross, P. L., P. A. Davis, and P. Belgrader (1998) Analysis of DNA fragments from conventional and microfabricated PCR devices using delayed extraction MALDI-TOF mass spectrometry. *Anal. Chem.* 70: 2067-2073.

[16] Xue, Q., Y. M. Dunayevskiy, F. Foret, and B. L. Karger (1997) Integrated multichannel microchip electrospray ionization mass spectrometry: Analysis of peptides from on-chip tryptic digestion of melittin. *Rapid Commun. Mass Spectrom.* 11: 1253-1256.

[17] Kopp, M. U., A. J. Mello, and A. Manz (1998) Chemical amplification: continuous-flow PCR on a chip. *Science* 280: 1046-1048.

[18] Daniel, J. H., S. Iqbal, R. B. Millington, D. F. Moore, C. R. Lowe, D. L. Leslie, M. A. Lee, and M. J. Pearce (1998) Silicon microchambers for DNA amplification *Sens. Actuat. A* 71: 81-88.

[19] Chovan, T and A. Guttman (2002) Microfabricated devices in biotechnology and biochemical processing. *Trends Biotechnol.* 20: 116-122.

[20] Northrup, M. A., B. Benett, D. Hadley, P. Landre, S. Lehew, J. Richards, and P. Stratton (1998) A miniature analytical instrument for nucleic acids based on micromachined silicon reaction chambers. *Anal. Chem.* 70: 918-922.

[21] Shoffner, M. A., J. Cheng, G. E. Hvichia, L. J. Kricka, and P. Wilding (1996) Chip PCR. I. Surface passivation of microfabricated silicon–glass chips for PCR. *Nucleic Acids Res.* 24: 375-379.

[22] Jakeway, S. C., A. J. de Mello, and E. L. Russell (2000) Miniaturized total analysis systems for biological analysis. *Fresenius J. Anal. Chem.* 366: 525-539.

[23] Cheng, J., L. J. Kricka, E. L. Sheldon, and P. Wilding (1998) Sample preparation in microstructured devices. *Topics Curr. Chem.* 194: 215-231.

[24] Krishnan, M., V. Namasivayam, R. Lin, R. Pal, and M. A. Burns (2001) Microfabricated reaction and separation systems. *Curr. Opin. Biotechnol.* 12: 92-98.

[25] Wilding, P., L. J. Kricka, J. Cheng, G. Hvichia, M. A. Shoffner, and P. Fortina (1998) Integrated cell isolation and polymerase chain reaction analysis using silicon microfilter chambers. *Anal. Biochem.* 257: 95-100.

[26] Cheng, J., L. C. Waters, P. Fortina, G. Hvichia, S. C. Jacobson, J. M. Ramsey, L. J. Kricka, and P. Wilding (1998) Degenerate oligonucleotide primed–polymerase chain reaction and capillary electrophoretic analysis of human DNA on microchip-based devices. *Anal. Biochem.* 257: 101-106.

[27] Khandurina, J., T. E. McKnight, S. C. Jacobson, L. C. Waters, R. S. Foote, and J. M. Ramsey (2000) Integrated system for rapid PCR-based DNA analysis in microfluidic devices. *Anal. Chem.* 72: 2995-3000.

[28] Lagally, E. T., P. C. Simpson, and R. A. Mathies (2000) Monolithic integrated microfluidic DNA amplification and capillary electrophoresis analysis system. *Sens. Actuat. B* 63: 138–146.

[29] Woolley, A. T., D. Hadley, P. Landre, A. J. deMello, R. A. Mathies, and M. A. Northrup (1996) Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. *Anal. Chem.* 68: 4081-4086.

[30] Burns M. A., C. H. Mastrangelocarlos, T. S. Sammarco, F. P. Man, J. R. Webster, B. N. Johnson, B. Foerster, D. Jones, Y. Fields, A. R. Kaiser, and D. T. Burke (1996) Microfabricated structures for integrated DNA analysis. *Proc. Natl. Acad. Sci. USA* 93: 5556-5561.

[31] Hong, J. W., T. Fujii, M. Seki, T. Yamamoto, and I. Endo (2001) Integration of gene amplification and capillary gel electrophoresis on a PDMS-glass hybrid microchip. *Electrophoresis.* 22: 328-333.
[32] Soper, S. A., S. M. Ford, Y. Xu, S. Qi , S. McWhorter, S. Lassiter, D. Patterson, and R. C. Bruch (1999) Nanoliter-scale sample preparation methods directly coupled to polymethylmethacrylate-based microchips and gel-filled capillaries for the analysis of oligonucleotides. *J. Chromatogr. A* 853: 107-120.
[33] Waters, L. C., S. C. Jacobson, N. Kroutchinina, J. Khandurina, R. S. Foote, and J. M. Ramsey (1998) Microchip device for cell lysis, multiplex PCR amplification, and electrophoretic sizing. *Anal. Chem.* 70: 158-162.
[34] Anderson, R. C., X. Su, G. J. Bogdan, and J. Fenton (2000) A miniature integrated device for automated multistep genetic assays. *Nucleic Acids Res.* 28: e60.
[35] Belgrader, P., M. Okuzumi, F. Pourahmadi, D. A. Borkholder, and M. A. Northrup (2000) A microfluidic cartridge to prepare spores for PCR analysis. *Biosens. Bioelectron.* 14: 849-852.
[36] Burns, M. A., B. N. Johnson, S. N. Brahmasandra, K. Handique, J. R. Webster, M. Krishnan, T. S. Sammarco, P. M. Man, D. Jones, D. Heldsinger, C. H. Mastrangelo, and D. T. Burke (1998) An integrated nanoliter DNA analysis device. *Science* 282: 484-487.
[37] Taylor, T. B., E. S. Winn-Deen, E. Picozza, T. M. Woudenberg, and M. Albin (1997) Optimization of the performance of the polymerase chain reaction in silicon-based microstructures. *Nucleic Acids Res.* 25: 3164-3168.
[38] Ibrahim,M. S., R. S. Lofts, P. B. Jahrling, E. A. Henchal, V. W. Weedn, M. A. Northrup, and P. Belgrader (1998) Real-time microchip PCR for detecting single-base differences in viral and human DNA. *Anal. Chem.* 70: 2013-2017.
[39] Sun, K., A. Yamaguchi, Y. Ishida, S. Matsuo, and H. Misawa (2002) A heater-integrated transparent microchannel chip for continuous-flow PCR. *Sens. Actuat. B* 84: 283-289.
[40] Chen, Y and A. Pepin (2001) Nanofabrication: Conventional and nonconventional methods. *Electrophoresis* 22: 187-207.
[41] Poser, S., T. Schulz, U. Dillner, V. Baier, J. M. Kohler, D. Schimkat, G. Mayer, and A. Seibert (1997) Chip elements for fast thermocycling. *Sens. Actuat. A* 52: 672-675.
[42] Duffy, D. C., J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides (1998) Rapid prototyping of microfluidic systems in poly(dimethylsiloxane). *Anal. Chem.* 70: 4974-4984.
[43] McDonald, J. C., D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. Schueller, and G. M. Whitesides (2000) Fabrication of microfluidic systems in poly(dimethylsiloxane). *Electrophoresis* 21: 27-40.
[44] Fujii, T (2002) PDMS-based microfluidic devices for biomedical applications. *Microelectron. Eng.* 61-62: 907-914.
[45] Zhang, Q., W. Wang, H. Zhangc, and Y. Wang (2002) Temperature analysis of continuous-flow micro-PCR based on FEA. *Sens. Actuat. B* 82: 75-81.
[46] Chou, C. F., R. Changrani, P. Roberts, D. Sadler , J. Burdon, F. Zenhausern, S. Lin , A. Mulholland, N. Swami, and R. Terbrueggen (2002) A miniaturized cyclic PCR device-modeling and experiments. *Microelectron. Eng.* 61-62: 921-925.
[47] Lin, Y.-C., C.-C. Yang, and M.-Y. Huang (2000) Simulation and experimental validation of micro polymerase chain reaction chips. *Sens. Actuat. B* 71: 127–133.
[48] Lee, T. M. H., Hsing, I-M., A. I. K. Lao, and M. C. Carles (2000) A miniaturized DNA amplifier: Its application in traditional chinese medicine. *Anal. Chem.* 72: 4242-4247.
[49] Nagai, H., Y. Murakami, K. Yokoyama, and E. Tamiya (2001) High-throughput PCR in silicon based microchamber array. *Biosens. Bioelectron.* 16: 1015-1019.
[50] Lagally, E. T., I. Medintz, and R. A. Mathies (2001) Single-molecule DNA amplification and analysis in an integrated microfluidic device. *Anal. Chem.* 73: 565-570.
[51] Oda, R. P., M. A. Strausbauch, A. F. R. Huhmer, N. Borson, S. R. Jurrens, J. Craighead, P. J. Wettstein, B. Eckloff, B. Kline, and J. P. Landers (1988) Infrared-mediated thermocycling for ultrafast polymerase chain reaction amplification of DNA. *Anal. Chem.* 70: 4361-4368
[52] Giordano, B. C., E. B. Copeland, and J. P. Landers (2001) Towards dynamic coating of glass microchip chambers for amplifying DNA via the polymerase chain reaction. *Electrophoresis* 22: 334-340.
[53] Schneegaß, I. and J. M. Kohler (2001) Flow-through polymerase chain reactions in chip thermocyclers. *Rev. Mol. Biotechnol.* 82: 101-121.
[54] Cheng, J., M. A. Shoffner, G. E. Hvichia, L. J. Kricka, and Peter Wilding (1996) Chip PCR. II. Investigation of different PCR amplification systems in microfabricated silicon-glass chips. *Nucleic Acids Res.* 24: 375-379.
[55] Belgrader, P., W. Benett, D. Hadley, G. Long, R. Mariella, Jr., F. Milanovich, S. Nasarabadi, W. Nelson, J. Richards, and P. Stratton (1998) Rapid pathogen detection using a microchip PCR array instrument. *Clin. Chem.* 44: 2191-2194.

[Received January 14, 2003; accepted May 28, 2003]