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Research review paper

PCR microfluidic devices for DNA amplification

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Abstract

The miniaturization of biological and chemical analytical devices by micro-electro-mechanical-systems (MEMS) technology has posed a vital influence on such fields as medical diagnostics, microbial detection and other bio-analysis. Among many miniaturized analytical devices, the polymerase chain reaction (PCR) microchip/microdevices are studied extensively, and thus great progress has been made on aspects of on-chip micromachining (fabrication, bonding and sealing), choice of substrate materials, surface chemistry and architecture of reaction vessel, handling of necessary sample fluid, controlling of three or two-step temperature thermocycling, detection of amplified nucleic acid products, integration with other analytical functional units such as sample preparation, capillary electrophoresis (CE), DNA microarray hybridization, etc. However, little has been done on the review of above-mentioned facets of the PCR microchips/microdevices including the two formats of flow-through and stationary chamber in spite of several earlier reviews [Zorbas, H. Miniature continuous-flow polymerase chain reaction: a breakthrough? Angew Chem Int Ed 1999; 38 (8):1055–1058; Krishnan, M., Namasivayam, V., Lin, R., Pal, R., Burns, M.A. Microfabricated reaction and separation systems. Curr Opin Biotechnol 2001; 12:92–98; Schneegaβ, I., Köhler, J.M. Flow-through polymerase chain reactions in chip themocyclers. Rev Mol Biotechnol 2001; 82:101–121; deMello, A.J. DNA amplification: does 'small' really mean 'efficient'? Lab Chip 2001; 1: 24N–29N; Mariella, Jr. R. MEMS for bio-assays. Biomed Microdevices 2002; 4 (2):77–87; deMello AJ. Microfluidics: DNA amplification moves on. Nature 2003; 422:28–29; Kricka, L.J., Wilding, P. Microchip PCR. Anal BioAnal Chem 2003; 377:820–825]. In this review, we survey the advances of the above aspects among the PCR microfluidic devices in detail. Finally, we also illuminate the potential and practical applications of PCR microfluidics to some fields such as microbial detection and disease diagnosis, based on the DNA/RNA templates used in PCR microfluidics. It is noted, especially, that this review is to help a novice in the field of on-chip PCR amplification to more easily find the original papers, because this review covers almost all of the papers related to on-chip PCR microfluidics.

Keywords: Polymerase chain reaction (PCR); Microfluidics; DNA amplification

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Contents

1. Introduction

MEMS technologies are being developed in the semiconductor industry and the characteristic dimensions of those small structures are on the order of 1–1000 μm. Microfluidics devices manufactured by MEMS will represent a central technology in many miniaturized systems used for biological, chemical, and medical applications, whose advances promise to revolutionize many processes of detection of pathogens or environmental pollutants (Chow, 2002; Felton, 2003; Stone and Kim, 2001). Among the microfluidics, the miniaturized PCR instrument (or called PCR microfluidics) has become a very important tool. The PCR process is widely used as a molecular biological tool to replicate DNA, and can create copies of specific fragments of DNA by cycling through three temperature steps. Each temperature cycle can double the DNA, and so 20–35 cycles can produce millions of DNA copies. However, conventional PCR instruments usually achieve a ramping rate of about 1–2 °C/s in the temperature range relevant for PCR, where a complete PCR analysis needs approximately 1–2 h. The resulting lower ramping rate is due to the high thermal capacity of the material of the PCR reaction system, which seemingly cannot meet the need of fast DNA amplification in spite of the fact that the ramping rates may be improved for the current PCR instruments in the market. Fortunately, since the introduction of the first PCR chip (Northrup et al., 1993, 1995), all kinds of PCR microfluidic technologies have facilitated DNA amplification with much faster rates as the result of smaller thermal capacity and larger heat transfer rate between the PCR sample and temperature-controlled components, which have advantages of small sizes, fast ramping rates, low cost and high integration and so on. Here, we review the microfabrication, design, surface chemistry, temperature and fluid control, detection for PCR amplicons, integration with other functional components, and applications of PCR microfluidics. We begin with discussing the motivation for the development of PCR microfluidical systems. Next, we describe the aspects mentioned earlier in turn. It is noted here that we also emphasize the new substrate materials and the new ways to control the temperature cycling for PCR microfluidics, as well as the novel thermal convection-driven PCR thermocycling.

1.1. Motivation behind the development of PCR microfluidics

PCR microfluidics has a large potential for many applications, as will be discussed later. Miniaturization of PCR devices in these application fields leads to many improvements, mainly including decreased cost of fabrication and use; deceased time of DNA amplification; reduced consumption of biological sample necessary for PCR; reduced production of PCR dimer and other nonspecific products; increased portability and integration of the PCR device; acceptable disposal of the PCR reaction vessel; and avoidable cross-talk of the PCR reaction. In addition, large numbers of parallel amplification analyses on a single PCR microfluidic chip can lead to more accurate information and greater understanding necessary for some particular bioassays, which, however, are difficult, unpractical, or even impossible to perform on a macro-scale PCR device. Be-

sides, single molecule PCR can be easily performed in PCR microfluidics, starting with a single-copy sequence in the PCR mixture (Belgrader et al., 2003; Burns et al., 1996; Lagally et al., 2000, 2001b; Matsubara et al., 2005; Nagai et al., 2001a). Much smaller PCR reaction vessels can increase resolution while reducing the overall size of the PCR device, but effects related to the non-specific adsorption of biological samples to the surfaces of the vessel may become significant as a result of the increased Surface-to-Volume Ratio (SVR) upon miniaturization, which may inhibit PCR amplification. It is noted, here, that miniaturization of the PCR amplificaton is also needed to match microchip Capillary Electrophoresis (CE), which has to be employed to decrease the time required for the analysis of the amplicons to about several minutes or ever shorter times, along with very high sensitivity and the requirement of only 10^{-12} or 10^{-9} g sample levels. Though the time required for the DNA amplification is longer than that of the CE-chip separation in many cases, it has shown a strong trend to decrease so as to reduce the total time of a DNA analysis. In short, the concept of micro-Total Analysis Systems ("μ-TAS") has shown the possibility of performing all the steps of a bioassay on a single chip leading to significant advantages in terms of speed, cost and automation, which will ultimately promote the further development of PCR microfluidics.

1.2. Historical background of the development of PCR microfluidics

Let's trace the history of the PCR microfluidics development. About 30 years ago, the first miniaturized gas chromatograph was fabricated on a single silicon wafer at Stanford University (Terry, 1975). However, the response of the scientific community working on PCR (Mullis et al., 1986) research to this first micro-silicon device has not been immediate, maybe because of the lack of technological experience with the use of silicon chips. Until 1993, several years after the introduction of PCR, and about a full 20 years after the first silicon gas chromatograph microinstrument, the first silicon-based stationary PCR chip was described by Northrup et al. (1993). Since then, the gate to the microfluidics-based PCR chip had been opened and many research groups began to develop chip-based PCR devices. Presumably on the basis of the fact that the significant concept of μ-TAS was presented in 1990 (Manz et al., 1990) and the CE-chip was developed in the early period (Manz et al., 1992; Harrison et al., 1993), the first PCR-CE integrated microfluidics device was presented in 1996 (Woolley et al., 1996). Although the PCR and CE steps were not performed in a single chip, it led the way to further integration of PCR with other pre- or post-PCR processing on a single silicon or glass chip. In addition, the integration of PCR with DNA microarray hybridization on a monolithic chip also was reported in 2000 (Anderson et al., 2000). Besides, it should be pointed out that flow-through PCR chip microfluidics has attracted great deal of interest and is exhibiting a rapid development, in parallel with stationary chamber PCR microfluidics, since it was introduced for the first time in 1998 (Kopp et al., 1998). In short, PCR microfluidics is expected to show a very strong life force in spite of its very short developing history of about 10 years.

2. New materials for PCR microfluidics

The development of PCR microfluidics has occurred at an exponential rate. If we have a glance through the literature on PCR microfluidics in the 1990s and currently, we will find that almost all PCR microchambers (or microchannels) were constructed from silicon (Belgrader et al., 1998a,b, 1999a,b, 2001; Benett et al., 2000; Bu et al., 2003; Cheng et al., 1996, 1998; Chaudhari et al., 1998; Cui et al., 2001, 2002; Daniel et al., 1998; Erill et al., 2003, 2004; Felbel et al., 2004, 2002; Gulliksen et al., 2004; Higgins et al., 2003; Ibrahim et al., 1998; Iordanov et al., 2003; Ke et al., in press; Krishnan et al., 2004b; Lao et al., 2000; Lee et al., 2000, 2003a,b,c; Lin et al., 2000a,b,c, 2002; Lou et al., 2004; Marie et al., 2003; Mariella, 2003, 2001; Matsubara et al., 2005; Miao et al., 2003; Nagai et al., 2001a,b; Northrup et al., 1993, 1995, 1998; Panaro et al., 2004; Perch-Nielsen et al., 2003; Poser et al., 1997; Richards et al., 2000; Rodriguez et al., 2003; Ross et al., 1998; Schabmueller et al., 2000a,b,c, 2001; Shoffner et al., 1996; Taylor et al., 1997; Trau et al., 2002; Wang et al., 2004; Wilding et al., 1994, 1998; Woolley et al., 1996; Yang et al., 2004; Yoon et al., 2002; Zhang et al., 2002a; Zhao et al., 2001, 2003; Zou et al., 2003), or glass (Baechi et al., 2001; Burns et al., 1996; Burns et al., 1998; Dunn et al., 2000; Ferrance et al., 2003; Giordano et al., 2001a; Khandurina et al., 2000; Kopp et al., 1998; Krishnan et al., 2004b; Lagally et al., 2000, 2001a,b, 2003, 2004; Lee et al., 2003b; Liao et al., 2005; Mastrangelo et al., 1999; Namasivayam et al., 2004; Obeid et al., 2003; Oberd and Christopoulos, 2003; Oda et al., 2003; Pal and Venkataraman, 2002; Schneegaβ et al., 2001; Stern et al., 2002; Sun et al., 2002; Taylor et al., 1998; Wang and Gong, 2003; Wang et al., 2003; Waters et al., 1998a,b; Yuen et al., 2001; Zheng and Chen, 2001; Zhou et al.,

2004) used as the substrate material, with the exception of the non-chip PCR microfluidics made from glass microcapillary (Friedman and Meldrum, 1998; Hühmer and Landers, 2000; Kalinina et al., 1997; Lee et al., 2004a,b; Swerdlow et al., 1997), polytetrafluoroethylene (PTFE) capillary (Belgrader et al., 2003; Bruckner-Lea et al., 2002; Chiou et al., 2001; Curcio and Roeraade, 2003; Nakano et al., 1994), fused-silica capillary, (Park et al., 2003; Soper et al., 1999a,b; Zhang and Yeung, 1998; Zhang et al., 1999) the rectangular borosilicate glass block (Oda et al., 1998), and polypropylene (PP) pipette tips (Nakane et al., 2001). In practice, it is not surprising that these research groups have been employing silicon or glass as the substrate materials of the chip-PCR reaction well because the standard photolithography and chemical etching techniques could be used to conveniently and effectively construct these substrate materials to produce the microfluidic networks in order to perform the PCR and/or even the detection of the amplicons. In addition, the silicon material has the superior thermal conductivity (about 160 W/(m °C)) allowing for very fast ramping times. Furthermore, the various metal film heaters and sensors are easily patterned on its surface so as to provide a high degree of integration. However, silicon as a substrate material for the PCR chamber also is problematical. First, the bare silicon will inhibit the PCR reaction by reducing the amplification efficiency and even may not exhibit any amplification reaction at all. Second, the high conductivity of silicon has a negative effect in that a thermal insulation of the silicon substrate is usually needed to reduce the energy losses into the surroundings and the measures of thermal insulation will usually add the high complexity to the PCR microsystem. Third, the silicon substrate is not transparent, often limiting the application of real-time optical detection to the PCR microfluidic devices. Finally, the conductivity of silicon proves to be problematic when applying the high voltages necessary to induce Electro Osmotic Flow (EOF) if it is used as a substrate for CE chip microfluidics, and thus largely restricts the complete integration of PCR with CE on a single silicon wafer. All these inherent disadvantages of silicon substrate make complete integration difficult and an undesirable hybrid architecture is more often required. As seen from the literature listed above, glass has become an alternative substrate material for PCR microfluidics from the later 1990s to now and consequently an increasing number of PCR microfluidic systems were constructed from glassy material. Glass possesses some beneficial characteristics such as well-defined surface chemistries, superior optical transparence, and good EOF characteristic, which has triggered the integration of PCR and CE separation on a monolithic glass chip or on a hybrid silicon/glass chip. However, the PCR microfluidics made from silicon or glass material aren't disposable due to the higher cost of fabrication. Furthermore, the majority of problematical issues in silicon/glass micriofabrication processing may ultimately hinder their wide use in commercial applications.

Although no single substrate material can offer a preferable solution to all these restrictions including cost, ease of fabrication, disposability, biocompatibility, optical transparence, etc., polymers might show their superiority over silicon/glass and become the very promising substrate materials for PCR microfluidics. Currently, many researcher groups have taken a great interest in the development of polymer-based PCR microfluidics, and so all kinds of polymer-based PCR microfluidics have been reported, including polydimethylsiloxane (PDMS) (Cady et al., 2005; Fukuba et al., 2002, 2004; Hong et al., 2001; Liu et al., 2002a, 2003; Shin et al., 2003; Yu et al., 2003; Zhao et al., 2002), polycarbonate (PC) (Anderson et al., 2000; Chartier et al., 2003; Hupert et al., 2003; Liu et al., 2004, 2002b; Mitchell et al., 2003;Yang et al., 2002), polymethylmethacrylate (PMMA) (Hupert et al., 2003; Lee et al., 2003a, 2004a), polyimide (PI) (Giordano et al., 2001b), polyethylene terephathalate (PET) (Zou et al., 2002), SU-8 (El-Ali et al., 2004; Lao et al., 2003; West et al., 2002), poly(cyclic olefin) (Fan et al., 2003; Koh et al., 2003), epoxy (Grodzinski et al., 2001; Sethu and Mastrangelo, 2004; Yu et al., 2000), and Gene Frame® double-sided tape (Shen et al., 2005). During only 4 or 5 years, about ten categories of polymers have been successfully applied to the fabrication of PCR microfluidics and they are also believed to have a perfect future as the substrate materials of choice for PCR microfluidics.

Besides, some researchers have used other materials as a substrate for PCR microfluidics, such as ceramics (Chou et al., 2002, 2003; Sadler et al., 2002, 2003) and even aluminum wells coated with PTFE (Sasaki et al., 1997). Although these materials haven't been widely recognized as the very ideal substrates for PCR microfluidics if compared with silicon, glass and polymers, they nonetheless have broadened the scope of substrate materials for PCR microfluidics. In addition, the substrate materials pointed out above relate to the substrate material of the PCR reaction chamber. Of course, many researchers have taken full advantage of the respective properties of silicon, glass or polymers and have investigated PCR microfluidics based on hybrid substrate materials, for instance silicon/glass

hybrids (Krishnan et al., 2004b; Rodriguez et al., 2003; Woolley et al., 1996), polymers/silicon (West et al., 2002), and polymers/glass (Hong et al., 2001; Shen et al., 2005). With these substrate materials for the PCR microfluidics and their corresponding basic background in mind, the following section describes the technologies available for the microfabrication of the PCR microfluidics.

3. Fabrication of chip PCR microfluidics

It has to be stressed that the fabrication methods are chosen if the substrate material of the PCR microfluidics is given. As a whole, the diversity of fabricating methods available for the PCR microfluidics can be classified into silicon/glass-based and polymer-based microfabricating methods. The former have greatly contributed to the advance in the early work on the PCR microfluidics and until now have grown into the more mature technologies, whereas the latter is continuously developing and all kinds of different polymer-based microfabricating methods are coming forth. Here, we will briefly introduce some silicon-based microfabricating technologies applied to the PCR microfluidics, and great importance will be taken into the description of the polymer-based micromachining applied to the PCR microfluidics. Meanwhile, the issues about the bonding/sealing of the chip-PCR microfluidics are also discussed in detail. It is pointed out, however, that in addition to the silicon, glass and polymer substrate materials, low-temperature cofired ceramic material which is based on the vertical integration of multi-layer ceramic technology has also been utilized as a substrate for PCR microfluidics (Chou et al., 2002, 2003; Sadler et al., 2002, 2003).

3.1. Fabrication for silicon/glass

The basic silicon micromachining technology mainly includes: photolithography, thermal growth of silicon oxide, chemical etching, electrochemical etching, ion etching, chemical vapor deposition (CVD), physical vapor deposition, epitaxy, and anodic bonding, which have all been applied to microfabricate silicon-based PCR microfluidics. The organic combination of these micromachining tools has formed the silicon bulk micromachining, surface micromachining and bonding of silicon wafer. At the same time, the micromachining technologies based on lithography and etching have also been perfectly applied to fabricate glass-based PCR microfluidics. Table 1 shows the main micromachining technologies used for the silicon/glass-based PCR microfluidics. It is noted that the fabrication of a silicon/glass-based PCR microfluidics usually involves a series of micromachining processes such as photolithography, film deposition, wet etching, etc. In addition, the lift-off technique is also used to micromachine all kinds of metal film resistive heaters and sensors (Burns et al., 1996; Daniel et al., 1998; Lao et al., 2000; Lee et al., 2003a,b,c; Liao et al., 2005; Perch-Nielsen et al., 2003; Yoon et al., 2002; Zhao et al., 2003; Zheng and Chen, 2001). Also, in most cases, among the film deposition methods, both evaporation and sputtering techniques are also indispensable processes for the microfabrication of the PCR microfluidics, which will however not be discussed in detail here.

3.2. Fabrication for polymers

3.2.1. Material properties of polymers

It is necessary to introduce some basic properties of polymer materials before we will describe micromachining methods of polymers applied to the PCR microfluidics. As a rule, polymers are macromolecular substrates with a high molecular weight. They may be separated into three groups according to their properties: thermoplastics, elastomers and thermosets. Thermoplastics consist of unlinked or weakly linked chain-like molecules that at a temperature above the glass transition temperature (T_g) become plastic and can be molded into specific shapes, which will cure after cooling to temperatures below T_g. Elastomers are weakly cross-linked polymers which can be stretched in the presence of an external force but relax and come back to the original state once the external force vanishes. Finally, thermosets are heavily cross-linked polymers which are usually hard, fragile and intractable, which soften very little when the temperature reaches their decomposition temperature. To our knowledge, to date no thermosets have been used as a substrate material of the PCR microfluidics or other biochip microfluidics.

3.2.2. General comparison of polymer fabrication methods

All methods available for fabricating polymers can typically be divided into two groups: replication methods and direct fabrication methods. On the one hand, replication methods, mainly including hot embossing, injection molding, casting, soft lithography, normally make use of a template or master from which many identical polymer microstructures can be made with precision. On the other hand, direct fabrication methods, such as laser ablation,

Table 1
Main micromachining methods of silicon/glass and their applications in the PCR microfluidics

Substrate material	Micromachining technology			Applications in the PCR microfluidics
Silicon	Lithography	Photolithography		Belgrader et al., 1998a,b; Cheng et al., 1996; Daniel et al., 1998; Erill et al., 2003; Krishnan et al., 2004a,b; Lao et al., 2000; Lee et al., 2003a,b,c; Lin et al., 2000a,b,c, 2002; Nagai et al., 2001a,b; Schabmueller et al., 2000a,b,c; Schneegaβ et al., 2001; Shoffner et al., 1996; West et al., 2002; Wilding et al., 1998; Woolley et al., 1996; Yoon et al., 2002; Zhao et al., 2003
	Etching	Oxidization	Thermal oxidation	Lao et al., 2000; Lee et al., 2003a,b,c; Schabmueller et al., 2001; Zhao et al., 2003; Zou et al., 2003
			Dry oxidation	Erill et al., 2003
			Wet oxidation	Schabmueller et al., 2000a; West et al., 2002; Yoon et al., 2002
		CVD	Atmospheric pressure vapor deposition	Erill et al., 2003
			Low pressure chemical vapor deposition (LPCVD)	Belgrader et al., 1998a,b; Erill et al., 2003; Lao et al., 2000; Lee et al., 2003a,b,c; Ke et al., in press; Perch-Nielsen et al., 2003; Schabmueller et al., 2000a,b,c; Woolley et al., 1996
			Plasma enhanced chemical vapor deposition (PECVD)	Burns et al., 1996; Daniel et al., 1998; Zou et al., 2003
		Wet etching	Anisotropical etching	Chaudhari et al., 1998; Daniel et al., 1998; Ke et al., in press; Lee et al., 2003a,b,c; Lin et al., 2000a,b,c, 2002; Nagai et al., 2001a,b; Poser et al., 1997; Schabmueller et al., 2001; Schneegaβ et al., 2001; Taylor et al., 1997; West et al., 2002; Wilding et al., 1998; Yoon et al., 2002; Zhao et al., 2003; Zou et al., 2003
			Electrochemical etching	Lin et al., 2000a,b,c, 2002
			Buffered oxide etching	Lao et al., 2000; Zheng and Chen, 2001
		Dry etching	Plasma etching	Lao et al., 2000; Lee et al., 2003a,b,c; Ke et al., in press; Krishnan et al., 2004b
			RIE	Belgrader et al., 1998a,b; Burns et al., 1996; Daniel et al., 1998; Erill et al., 2003; Schabmueller et al., 2000a,b,c; Wilding et al., 1998; Woolley et al., 1996; Zou et al., 2003
			Deep reactive ion etching	Miao et al., 2003; Yoon et al., 2002
Glass	Lithography	Photolithography		Dunn et al., 2000; Ferrance et al., 2003; Khandurina et al., 2000; Lagally et al., 2000, 2001a,b, 2004; Obeid et al., 2003; Oberd and Christopoulos, 2003; Liao et al., 2005; Sun et al., 2002; Waters et al., 1998a,b; Zheng and Chen, 2001; Zhou et al., 2004
	Etching	CVD	LPCVD	Lagally et al., 2000, 2001b
			PECVD	Lagally et al., 2000, 2001a; Zheng and Chen, 2001
		Wet chemical etching		Burns et al., 1996; Dunn et al., 2000; Fukuba et al., 2004; Ferrance et al., 2003; Khandurina et al., 2000; Krishnan et al., 2004b; Lagally et al., 2000, 2001a,b; Obeid et al., 2003; Oberd and Christopoulos, 2003; Sun et al., 2002; Waters et al., 1998a,b; Zheng and Chen, 2001; Zhou et al., 2004
		Isotropical etching		Schneegaβ et al., 2001
		RIE		Lagally et al., 2001a,b, 2004

plasma etching, X-ray lithography, stereo-lithography, SU-8 LIGA (Lithographie, Galvanoformung, Abformung), layering, etc., can directly fabricate the individual polymeric surfaces so as to form the microsystem structures. Here, we only briefly summarize and compare the most common micromachining techniques applied to fab-

ricate polymer materials. A more extensive and detailed review of different polymer microfabrication techniques may be found in some review articles (Becher and Gärtner, 2000; Becher and Locascio, 2002; deMello, 2002; McDonald et al., 2000; Rossier et al., 2002). It is noted, furthermore, that the new micromachining techniques associated with polymer substrates are continuously being further developed with the advance of state-of-the-art MEMS technique. As a special example, a novel technique that is called the "shadow mask" concept has been reported to form metallized features on plastics without the use of lithography or photoresist, which is essential to device integration of cell lysis, CE, PCR, resistive temperature detection (RTD), and heaters on a single device platform (Smekal et al., 2002).

3.2.3. Fabrication of current polymer-based PCR microfluidics and their features

The essential elements required for satisfactory application of polymer substrates to the PCR microfluidics fabrication are as follows: First, they must possess high thermal stability with a T_g above 95 °C, because the PCR reaction is a temperature-controlled reaction with a series of temperature cycles in the range of about 55–95 °C. Secondly, they have good chemical resistance to some nonaqueous solvents (including the cleaning steps) necessary for a successful PCR reaction, because PCR is a reaction of high sensitivity and specificity and even minute adverse environmental changes will largely affect the PCR amplification efficiency. Thirdly, they have also a good dimensional stability which should remain intact at temperatures up to 100 °C in the presence of water. Fourthly, they must consist of a PCR-compatible material that can't inhibit the PCR reaction and/or eliminate PCR reagents by adsorption onto the surfaces of the PCR reaction chamber. In addition, it is important, if possible, that they should be transparent to allow for the incorporation of other functional components such as fluorescence detection of the PCR products. Currently, some polymer substrates have been successfully applied to the PCR microfluidics. Table 2 lists several polymer substrate materials and their corresponding micromachining technologies that have been applied to PCR microfluidics. In Table 2, we also refer to the volume and/or other relevant dimensions of the PCR reaction chamber, as well as the resultant heating/cooling rates (or cycles for the flow-through PCR microfluidics). This table illustrates that polymer-based PCR microfluidics in terms of ramping speed are comparable with silicon/glass-based PCR microsystems in spite of their slow response times due to their lower thermal conductivity and slow heat transfer through the polymers between the heating element and the PCR solution, in the presence of state-of-the-art heating/cooling methodology. Furthermore, polymer-based micromachining techniques allow for the volume of the PCR reaction chamber to be as small as nanoliter or submicroliter, and thus parallel biochemical analysis on a monolithic polymer-based PCR microfluidics can be carried out.

3.2.4. Master fabrication of replication methods for polymer-based PCR microfluidics

In the micromachining process of replication techniques for polymer-based PCR microfluidics, the fabrication of the master is the first and also a very important step because it defines whether the fabrication of the PCR microfluidics is desirable or not. Here, we will limit ourselves to a summary of the most common master fabrication methods based on the polymer-based PCR microfluidics. More extensive details of master fabrication methods were presented by Becher and Gärtner (2000).

3.2.4.1. SU-8 master. Nowadays, many researchers have created SU-8 master with a thickness of 100–150 μm on silicon or glass wafers by an SU-8 thin film photolithographical technique (Cady et al., 2005; Fukuba et al., 2002, 2004; Hong et al., 2001; Liu et al., 2002a; Shin et al., 2003; Zhao et al., 2002). Furthermore, some of them have also treated the master (Hong et al., 2001) or coated silicon wafers (Fukuba et al., 2002, 2004) with fluorocarbon (CHF_3) in a reactive ion etching (RIE) system with the purpose to facilitate the easy release of the PDMS replica from the master after curing. Others have also pretreated the silicon wafer with hexamethyldisilazane (HMDS) (Liu et al., 2002a). Sometimes, a two-level SU-8 mold has been fabricated after the formation of the channel in order to easily pour the PDMS microchannel (Zhao et al., 2002). With this technique, 2-D patterned layers are usually manufactured. However, two or more 2-D patterned layers need to be stacked together and aligned with precision when 3-D structures are to be realized. Furthermore, the limitation in height of SU-8 as a conventional photoresist also becomes a problem in the microfabrication of deep reaction chambers.

3.2.4.2. Silicon master. In order to overcome the disadvantages of the SU-8 master, some researchers have made attempts to use a silicon master (Lee et al., 2003a, 2004a,b; Sethu and Mastrangelo, 2004; Yu et al., 2003; Zou et al., 2002). In fact, silicon itself has preferable material properties for a master such as high stiffness

Table 2
Polymer-based PCR microfluidics and their corresponding characteristics

Polymer material	Micromachining technology	Volume or dimensions of PCR chamber	Heating/cooling rates (chamber) or cycles (flow-through)	Reference
PDMS	Injection molding, drilling;	30–50 μL	2–4/1–3 °C s^{-1}	Hong et al., 2001
	Injection molding	25 nL	Shorter cycling times	Yu et al., 2003
	Injection molding	~2 μL		Zhao et al., 2002
	Injection molding	2 μL	2/1.2	Shin et al., 2003
	Soft lithography	100 μm in depth and width, 3033 mm long	30 cycles	Fukuba et al., 2002, 2004
	Soft lithography		3.1/3.1 (35 min for 40 cycles)	Cady et al., 2005
	Multilayer soft lithography	Only 12 nL volume of sample	Adjustable cycles (~17 s/circle)	Liu et al., 2002a
	Multilayer soft lithography	3 nL		Liu et al., 2003
PC	Convention computer-controlled machining	0.25–1.5 mm in depth, 0.25–10 mm in length		Anderson et al., 2000
	CO_2 laser ablation	Serpentine channel with 0.25 mm in depth, 1.5 mm in width (40 μL)	7–8/5–6 °C s^{-1} (18–19 s/circle)	Yang et al., 2002
	CO_2 laser ablation	Serpentine channel with 0.25 mm in depth and width	7.9/−4.6 °C s^{-1} (−19 s/circle)	Liu et al., 2002b
	Conventional computer-controlled machining	0.3–1.2 mm in depth, 1–5 mm in width		Liu et al., 2004
	Mechanical machining or hot embossing	Square shaped grooves with a 500 μm side and 700 μm pitch		Chartier et al., 2003
	Hot embossing	Spiral channel with 50 μm wide and 150 μm deep		Hupert et al., 2003
	Hot embossing	Spiral channel		Mitchell et al., 2003
PMMA	Hot embossing	~200 nL	>80/>60 (~7 min for 30 cycles)	Lee et al., 2003a, 2004a,b
	LIGA	1 mm in diameter and 100 μm deep		Ueda et al., 2000
Epoxy	casting	1.5 μL PCR mixture	2.4/2.0	Yu et al., 2000; Grodzinski et al., 2001; Sethu and Mastrangelo, 2004
SU-8	Standard MEMS technique	~20 μL (7 mm × 7 mm × 0.4 mm)	~50/~20	El-Ali et al., 2004
	Coating and patterning	~1 μL	16/9.6	Lao et al., 2003
	Silicon bulk micromachining and EPON SU-8Rapid prototyping	180 μm deep, 1000 μm wide annular channel (4.5 mm internal radius)	92 s rev^{-1}	West et al., 2002
PET	Thermal forming	20 μL	34–50/23–31	Zou et al., 2002
PI	Laser ablation	2 mm (wide) × 5.5 mm (long) × 150 μm (deep), 1.65 μL	10/10	Giordano et al., 2001b
Poly(cyclic olefin) resin	Compressing molding	29/84 nL	~12/~2	Koh et al., 2003; Fan et al., 2003
Gene Frame® tape	Punching	25 μL	8/8	Shen et al., 2005

and thermal conductivity. Importantly, a wide diversity of silicon bulk and surface micromachining techniques exists. The inductively coupled plasma (ICP) etching can create a deep 3-D silicon master to avoid cross-contamination among chambers (Yu et al., 2003). Also, a layer of parylene C with a 0.2-μm thickness, which is a chemically inert polymer, can be deposited to aid in the release of the cast part from the master (Sethu and Mastrangelo, 2004).

3.2.4.3. Nickel (Ni) master. For master fabrication, the electroplating step is the most commonly utilized technique, which can result in a replication master structured from Ni (Grodzinski et al., 2001; Hupert et al., 2003; Mitchell et al., 2003) or other metal layer (Koh et al., 2003). An alternative method of Ni electroplating over microcasted PDMS master substrates has been developed to address the difficult issue of post-plating photoresist removal (Grodzinski et al., 2001). The surface roughness of a Ni master is usually very small and furthermore the Ni master has a good surface chemistry for most polymers. However, its drawbacks involve the rather slow growth rate of Ni in the electroplating process, the high stress in thick Ni layers, and the radial dependency of the growth rate (Becher and Gärtner, 2000).

3.3. Bonding/sealing for complete PCR microfluidics

All microfabrication methods described above require the bonding/sealing of the microchannel or microchamber to form an enclosed structure. As the substrate materials for PCR microfluidics differ, a large variety of bonding or sealing techniques (such as fusion bonding, anodic bonding, hydrofluoric acid (HF) bonding, adhesive bonding, etc.) are applied to realize the complete PCR microfluidics.

3.3.1. Anodic bonding

The anodic bonding technique is a method of hermetically and permanently joining glass to silicon in the presence of an electrostatic field. This technique has been widely applied to the sealing of microfluidics due to its intimate contact and high stability at the interface of silicon and glass. Presently, most silicon-based PCR microfluidics were anodically bonded to a glass cover to obtain a closed PCR system (Chaudhari et al., 1998; Cheng et al., 1996; Cui et al., 2002; Erill et al., 2003; Krishnan et al., 2004a,b; Lee et al., 2003a,b,c, 2000; Lin et al., 2000a,b,c, 2002; Pal and Venkataraman, 2002; Rodriguez et al., 2003; Schabmueller et al., 2000a,b,c; Taylor et al., 1997; Wilding et al., 1998; Yoon et al., 2002; Zou et al., 2003). In a few cases, glass PCR microfluidics were bonded to a silicon cover by anodic bonding (Krishnan et al., 2004a,b; Schneegaβ et al., 2001).

3.3.2. Fusion (thermal) bonding

When the PCR microfluidics are made from glass, closed PCR microsystem can be fabricated by using fusion bonding with a glass cover (Ferrance et al., 2003; Lagally et al., 2000, 2001a,b, 2003; Obeid et al., 2003; Oberd and Christopoulos, 2003; Stern et al., 2002; Waters et al., 1998a,b; Zheng and Chen, 2001; Zhou et al., 2004). In addition, polycarbonate (PC)-based PCR microfluidics can also be sealed with another thin PC layer (Hupert et al., 2003; Liu et al., 2002a; Mitchell et al., 2003; Ueda et al., 2000; Yang et al., 2002) using a solvent-assisted thermal bonding technique in which acetone is first applied on one side of the thin layer (Liu et al., 2004).

3.3.3. Adhesive bonding

Adhesive bonding is a process in which two similar or non-similar materials are solidly and permanently assembled using an adhesive. This method has been widely used to close silicon, glass or polymer-based PCR microfluidics due to its many advantages such as simplicity and automation, low cost, uniform stress distribution, as well as its impermeability to air and water. Importantly, adhesive materials of varying types have been chosen to seal PCR microfluidics, including epoxy (Dunn et al., 2000; Lao et al., 2000; Lee et al., 2004a,b; Waters et al., 1998a,b; West et al., 2002; Zou et al., 2002), potassium silicate (Khandurina et al., 2000), UV glue (Ke et al., in press; Liao et al., 2005; Trau et al., 2002), PP tape (Zou et al., 2002), gene Frame® double-sided tape (Shen et al., 2005), or others (Burns et al., 1996; Hong et al., 2001; Liu et al., 2002b; Poser et al., 1997).

3.3.4. O2 plasma irreversible bonding of PDMS

This bonding technique has been successfully applied to enclose PDMS-based PCR microfluidics with PDMS covers so as to form a reaction channel or chamber where all walls are made from the same material (Cady et al., 2005; Fukuba et al., 2002, 2004; Shin et al., 2003; Yu et al., 2003). PDMS–PDMS bonding is usually performed by activation of the PDMS surface with O_2 plasma in a RIE machine in order to achieve irreversible bonding. PDMS contains repeating units of –O–Si$(CH_3)_2$–. Exposing a PDMS replica to the O_2 plasma will introduce polar groups, namely silanol groups (Si–OH), on the surface

at the expense of methyl-groups (Si–CH_3). These silanol groups can condense with appropriate groups (for instance OH, COOH) on another surface when the two layers are in close contact with each other (McDonald et al., 2000). One of the important characteristics of this irreversible bonding method is that devices fabricated in this way can withstand higher pressure, compared with the PDMS–PDMS reversible bonding.

3.3.5. Reversible bonding of PDMS

The sealing of PDMS can also occur in a reversible way, namely, conformal sealing with a flat surface such as glass (Zhao et al., 2002), or SU-8 (Lao et al., 2003). It should be pointed out that the bonding of SU-8 and glass can be performed with thin coatings of PDMS (El-Ali et al., 2004). This sealing takes place because PDMS is flexible and can conform to minute imperfections in a "plane" surface leading to Van de Waals contact with this surface. The advantages of this bonding include high watertightness, fast rates, the desired occurrence of bonding at room temperature, and the simplicity of PDMS removal from the plane surface without any degradation or residue. However, one of its disadvantages is the poor resistance to high pressure.

3.3.6. Lamination

This bonding method has been widely used in the macroworld for encapsulation of paper and polymers in a polymer film and performs well for macro-channels. However, some researchers have employed the lamination process for the enclosure of PCR microfluidics because of its ease and the absence of any alignment requirement (Chartier et al., 2003; Fan et al., 2003; Grodzinski et al., 2001; Koh et al., 2003; Sethu and Mastrangelo, 2004; West et al., 2002; Yu et al., 2000). In fact, this process can be accomplished with certain types of commercially available film, for example negative dry resist film (Chartier et al., 2003), polybutylene terephthalate-polycarbonate (PBT-PC) film (Chartier et al., 2003), poly (cyclic olefin) film (Fan et al., 2003; Koh et al., 2003) within a short time in a laminator. However, this bonding technique will easily lead to blocking of the channel resulting from the used adhesive, and the inhomogeneous interface between chamber and cover wafer due to the difference between the materials used.

3.3.7. HF bonding

HF bonding is a novel bonding technique, which can perform the bonding between silicon and silicon, silicon and glass, glass and glass (Sun et al., 2002), silicon dioxide and silicon dioxide, silicon dioxide and silicon, etc. in the presence of HF at room temperature. This low-temperature bonding method may provide a good solution to residual stress.

3.3.8. Ultrasonic welding

Ultrasonic welding is the fusion of two polymer layers where a localized melting of the polymer is brought about by the energy density of an ultrasonic wave. This technique has been successfully used for the bonding between PC and PC (Anderson et al., 2000).

4. Designs for PCR microfluidics

Since the introduction of the thermostable thermos aquatics (Taq) DNA polymerase as a substitute for the Klenow fragment of *Escherichia coli* DNA polymerase I, considerable efforts have been made to promote the automation of PCR amplification. Furthermore, the miniaturization of PCR devices can offer an opportunity to improve them further in terms of shorter amplification times, higher sample throughput, and minimum human/world-to-PCR intervention and contamination. Up to now, PCR microfluidics of varying design have been developed for effective and fast DNA amplification by many research groups, comprising chamber stationary PCR and flow-through PCR, as well as thermal convection-driven PCR.

4.1. Chamber stationary PCR microfluidics

The chamber stationary PCR microfluidics work in the same manner as described for the conventional PCR devices, where the PCR solution is kept stationary and the temperature of the PCR reaction chamber is cycled between three different temperatures. After completion of the PCR reaction, the amplification products are recovered from the chamber for off-line detection or are detected in an on-line way. This format of PCR microfluidics can be separated into two groups: single/multi chamber stationary PCR microfluidics, which will be described below.

4.1.1. Single chamber stationary PCR microfluidics

A number of PCR microfluidics have been developed for faster thermal cycling by reducing the thermal capacity of the PCR system. The first PCR microfluidics was reported by Northrup et al. (1993), where a microwell cavity structure acts as an appropriate PCR reaction chamber fabricated by using silicon anisotropical wet etching. An amplification of 20 cycles was carried out in a 50-μL microwell, four-fold faster than

in a conventional PCR device, along with a much lower consumption of power. Since then, single chamber stationary PCR microfluidics were widely investigated and improved by other groups (Anderson et al., 2000; Burns et al., 1996; Cady et al., 2005; Cheng et al., 1996; Cui et al., 2001, 2002; Daniel et al., 1998; El-Ali et al., 2004; Erill et al., 2003, 2004; Fan et al., 2003; Felbel et al., 2002, 2004; Fermér et al., 2003; Ferrance et al., 2003; Giordano et al., 2001a,b; Grodzinski et al., 2001; Gulliksen et al., 2004; Hong et al., 2001; Hühmer and Landers, 2000; Ke et al., in press; Khandurina et al., 2000; Koh et al., 2003; Krishnan et al., 2004a,b; Lagally et al., 2003, 2004; Lao et al., 2000, 2003; Lee et al., 2003a,b,c, 2004a,b, 2000; Lin et al., 2000a,b,c, 2002; Liu et al., 2004, 2002b; Marie et al., 2003; Oda et al., 1998; Orrling et al., 2004; Pal and Venkataraman, 2002; Poser et al., 1997; Rodriguez et al., 2003; Sasaki et al., 1997; Schabmueller et al., 2000a,b,c, 2001; Sethu and Mastrangelo, 2004; Shen et al., 2005; Shin et al., 2003; Shoffner et al., 1996; Soper et al., 1999a,b; Swerdlow et al., 1997; Waters et al., 1998a,b; Wilding et al., 1998; Woolley et al., 1996; Yang et al., 2002; Yoon et al., 2002; Yu et al., 2000; Zhang and Yeung, 1998; Zhao et al., 2001, 2002, 2003; Zhou et al., 2004). They can perform very well in terms of fluidical and thermal control, and present beneficial properties such as reduction of thermal and fluidic cross-talk between PCR reaction microchambers. However, a single chamber stationary PCR microfluidics is not suitable for high-throughput PCR. Instead, many sequential PCR experiments will be needed in case of a number of DNA samples to be amplified to meet the detection requirement. Furthermore, there is the risk of carry-over from experiment to experiment so as to enlarge the possibility of incomplete amplification or partial failure if these PCR amplifications are performed in the same PCR microfluidics.

4.1.2. Multi chamber stationary PCR microfluidics

In order to improve the PCR throughput and reduce the analysis time, as well as the required labor (due to the introduction of a robot to inject the sample), multi-chamber stationary PCR microfluidics on a single chip has been explored by many groups (Chartier et al., 2003; Chaudhari et al., 1998; Daniel et al., 1998; Deshpande et al., 1999; Dunn et al., 2000; Friedman and Meldrum, 1998; Iordanov et al., 2003; Lagally et al., 2000, 2001a,b; Liao et al., 2005; Liu et al., 2003; Matsubara et al., 2005; Miao et al., 2003; Nagai et al., 2001a,b; Nakane et al., 2001; Poser et al., 1997; Stern et al., 2002; Taylor et al., 1997, 1998; Trau et al., 2002; Wang and Gong, 2003; Wang et al., 2003; Yu et al., 2003; Yuen et al., 2001; Zhang et al., 1999; Zou et al., 2002, 2003). Nevertheless, special care must be taken in the thermal design of a chamber array in order to acquire temperature uniformity between chambers. Without a careful design to assure temperature uniformity and the elimination of any possible differences in amplification, the reliability, repeatability, sensitivity, efficiency and specificity of PCR amplification across the different chambers may be compromised. In addition, handling and processing of small sample volumes in the case of the increasing number of PCR reaction chambers on a single chip pose some potential challenges. The resulting issues are loss of sample on the walls of transferring devices, loss by evaporation, loss of components from the sample during manipulation, possible loss of sample resulting from the immediate contact of the PCR solution with chamber walls. In addition, further miniaturization of the reaction chamber will lead to a significant increase of SVR, and consequently special attention has to be paid to the nonspecific adsorption of the PCR samples on the walls of the reaction chamber, especially for the reaction chamber with a nanoliter volume.

4.2. Flow-thought PCR microfluidics

The above-discussed chamber stationary PCR microfluidics lack the flexibility to change the reaction rate, resulting in more cycling and heating time. However, another important configuration of the PCR microfluidics is ingeniously realized by flow-through PCR with a "time–space conversion" concept. Instead of being stationary in a chamber, the PCR solution is continuously and repeatedly flowing through the three different temperature zones necessary for PCR amplification. The attractive and interesting features of this type of PCR microfluidics comprise the following aspects: (1) its very rapid heat transfer and high thermal cycling allowing for the achievement of total run times of the order of minutes; (2) its low possibility of cross-contamination between samples allowing for very specific amplification; (3) its high potential for further development of μ-TAS by incorporating many functionalities; (4) its facility for integration with the diversity of microfluidical liquid transport such as magneto-hydrodynamic (MHD) actuation (West et al., 2002). A significant limitation of this approach is the fixed cycle number which is dictated by the channel layout. To the authors' thinking, the flow-through PCR microfluidics may be divided into three categories, as described below.

4.2.1. Single straight capillary based flow-through PCR microfluidics

This type of flow-through PCR microfluidics consists of a capillary tube, heater zones, an optical window, etc., and obviously doesn't belong to on-chip PCR microfluidics. This novel PCR microfluidics was recently reported by Chiou et al. (2001), where three heat blocks define denaturation, annealing and extension, and 30 cycles of a 500 base-pair (bp) product were performed in 23 and even 2.5 min with 78% amplification efficiency. In fact, this new simple flow-through PCR microfluidics was developed earlier (in 1994) by Nakano et al. (1994), where the amplification efficiency was half of that required by a conventional PCR device and the total reaction time was from 12 to 18 min. The speed of Nakano's PCR microfluidics could be varied by increasing the travel speed of the sample but its efficiency decreases as speed increases. Chiou's PCR microfluidics can amplify a DNA sample in as little time as possible with an efficiency comparable to that of conventional PCR devices. Furthermore, the total number of cycles and the ratios between the denaturation, annealing and extension times are flexible. Currently, a reusable flow-through PCR microfluidics was also reported for the continuous monitoring of infectious biological agents, where the copy number of amplification ranged from 0.3 to 30,000, along with an amplification efficiency of about 96.5% and a good elimination of carryover from sequential runs (Belgrader et al., 2003). This type of PCR microfluidics had also been reported elsewhere (Bruckner-Lea et al., 2002).

4.2.2. On-chip serpentine rectangular channel based flow-through PCR microfluidics

Compared with the single capillary PCR microfluidics, the flow-through microfluidics with rectangular flow channel on chip has become central in the research of current flow-through PCR microfluidics. This significant new development in the world of PCR microfluidics was first reported by Kopp et al. (1998). In this work, the PCR chip was etched in Corning 0211 glass with channel dimensions of 40 μm deep, 90 μm wide and 2.2 m long. The channel passes 20 times through three temperature zones of 95 °C, 60 °C and 77 °C, respectively. With flow rates between 5.8 and 72.9 nL/s, the transition time between two temperatures was less than 100 ms and the total amplification time was between 18.8 and 1.5 min. Since the pioneer invention, this type of flow-through PCR microfluidics has undergone different kinds of improvements, all with different properties in terms of the structure and SVR of microchannel (Bu et al., 2003; Felbel et al., 2002; Fukuba et al., 2002, 2004; Kopp et al., 1998; Obeid et al., 2003; Oberd and Christopoulos, 2003; Schneegaβ and Köhler, 2001; Schneegaβ et al., 2001; Sun et al., 2002; Zhang et al., 2002a). However, with respect to these novel flow-through PCR microfluidics, to date their potential and feasibility of integration with pre-PCR sample preparation or post-PCR on-line detection for PCR product haven't been made full use of. To the best of our knowledge, they haven't been integrated with pre/post-PCR functional components to form a "real" μ-TAS although the microfluidic device with an integration of three stages of dielectrophoresis-field flow fractionation (DEP-FFF) cell separator, cell isolator and lysis, flow-through PCR and detection, has recently been brought forward for malaria detection (Gascoyne et al., 2004). Another application, i.e. the flow-through PCR microfluidics based on a circular arrangement of three temperature zones (as will be discussed bellow) has been incorporated into bioelectronic DNA detection microarray for genotyping (Chou et al., 2003; Sadler et al., 2002, 2003).

4.2.3. "Circular" arrangement of three temperature zones for flow-through PCR microfluidics

The arrangement of the three temperature zones on most of the on-chip rectangular serpentine channel PCR microfluidics is: denaturation temperature→extension temperature→annealing temperature. One attractive advantage of such an arrangement is that a rather smooth temperature gradient is established on a monolithic chip, without the need of a forced cooling process or the inherent complex structure. In this case, however, the melted single-stranded DNA sample is very likely to form double strands with the template strands or their complementary fragments when passing through the extension zone, which will result in a decreased amplification efficiency. In order to circumvent this problem, a novel temperature arrangement is exploited, consisting of a "circular" arrangement of the three zones to generate the sequence of denaturation, annealing, and extension (Park et al., 2003; Curcio and Roeraade, 2003; Liu et al., 2002a; West et al., 2002). It is acknowledged, however, that the PCR reaction channel for these flow-through PCR microfluidics can also consist of either capillary tubes (Park et al., 2003; Curcio and Roeraade, 2003) or an on-chip annular channel (West et al., 2002; Liu et al., 2002a). Furthermore, a serpentine channel on a single chip was laid out having the liquid repeatedly pass through the cyclic sequence of three temperature zones. In this set-up of flow-through PCR microfluidics the proper insulation

of the thermal zones was achieved with the aid of air gaps (Chou et al., 2002, 2003; Sadler et al., 2002, 2003) or by utilizing glass chips with a low thermal conductivity of the material (Schneegaβ and Köhler, 2001; Zheng and Chen, 2001). Recently, a novel spiral channel configuration was also used to perform flow-through PCR on a single PC wafer with a circular arrangement of three temperature zones, allowing for a compact footprint and a minimal number of heaters for temperature control (Hupert et al., 2003; Mitchell et al., 2003). The main purpose of highlighting this type of flow-through PCR microfluidics from the ones mentioned above is to emphasize the underlying advantages of a "circular" arrangement of the three temperature zones. The single straight capillary based flow-through PCR microfluidics was singled out because of its wide applicability, not necessarily in chip applications and its flexibility regarding the three temperature zones. It needs to be noted that the classification proposed here for flow-through PCR microfluidics is tentative. For example, an innovative droplet-based oscillating flow-through PCR microfluidics, similar to not only the single straight capillary flow-through PCR microfluidics but also the on-chip serpentine rectangular channel-based PCR microfluidics, has recently been reported (Wang et al., 2004). Alternatively, flow-through PCR microfluidics may also be simply classified into two categories: unidirectional PCR and oscillatory-flow PCR (Chen et al., 2004).

4.3. Thermal convection-driven PCR microfluidics

Convectively driven PCR microfluidics, although only in its infancy, have been proven by three research groups to be capable of rapid DNA amplification. In the October 25, 2002 issue of *Science*, a convection-driven PCR microfluidics, a significant new development, was reported by Krishnan et al. (2002). Rayleigh-Bénard convection was used to perform PCR amplification inside a 35-μL cylindrical cavity, and the temperature cycling was achieved as the flow continuously shuttles fluid vertically between the two temperature zones of annealing/extension (top, 61 °C) and denaturation (below, 97 °C). Subsequently, a laminar convection PCR microfluidics was designed by Braun et al. (2003; Braun, 2004; Braun and Libchaber, 2004). A chamber of 5 mm diameter and 1 mm thickness is heated in the center of the chamber by an infrared (IR) source. In this way, a layered PCR is induced allowing for a cheap reaction chamber with a high reaction speed using a non-contact heating method. The described laminar convection PCR cycles a volume of 20 μL and achieves a 1,000,000 fold λ-DNA amplification within 25 min with a power consumption of only 75 J. Recently, Wheeler et al. (2003, 2004) reported a closed PCR microfluidic heating pipe with a "0" shape where the fluid is heated on one side (94 °C) and cooled on another side (57 °C). The PCR solution is placed in a PP thin bag sandwiched between the two circuit boards. One desirable advantage of such a design is that the whole fluid in the pipe passes through both temperature zones avoiding any dead zones not being amplified. However, the parabolic velocity distribution of the fluid flow results in an uneven distribution of convection times, which causes variability in amplification times within the same volume. It was illustrated that the sample volume of 75 μL was cycled for 30 min with a cycle time of about 24 s with a very low power consumption of about 866 J/run. A similar loop-based convection-driven PCR was also presented more recently by Chen et al. (2004) and Krishnan et al. (2004a).

The three designs for convection-driven PCR microfluidics mentioned above, all employ two fixed temperature zones to facilitate the convection-driven sample flow, resembling flow-through PCR microfluidics. However, they differ from flow-through PCR microfluidics in terms of the driving force of the sample. They use buoyancy forces to drive the sample fluid between the temperature zones. There is no need of an external force to drive the fluid through the different temperature zones. As compared with the flow-through or chamber stationary PCR microfluidics, these convection-driven PCR microfluidics have the advantages of simplicity in design, cheapness of fabrication, and much faster temperature transition speed. Furthermore, a much simpler facility is possible, only requiring a cavity/loop and specific thermostable zones, and no electronic control components to perform dynamic temperature cycling.

5. Temperature kinetics, heating and temperature sensing methods, and simulation for PCR microfluidics

PCR is a typical temperature-controlled and enzyme-catalyzed biochemical reaction system that consists of the periodical repetition of three different temperatures (melting, annealing and extension temperature). Alternatively, only two temperatures can be applied, combining annealing and extension temperature thus further reducing the complexity of the thermal cycling profile and increasing the speed and efficiency of the PCR reaction (Belgrader et al., 1998a). As a rule, the PCR system contains a master mix (magnesium salts, pH

buffer, and potassium salts), DNA substrate(s), oligonucleotide primers, polymerase enzyme, and dNTPs. Because of the temperature-sensitivity of the PCR system a minor temperature difference may significantly affect the efficiency of DNA amplification, especially in PCR microfluidic systems. Therefore, research on the effects of temperature on enzyme kinetics, on heating and temperature measuring methods, and on temperature field simulation of PCR microfluidics aiming at a better understanding of PCR kinetics in microfluidics is a key issue of great interest.

5.1. General description of temperature kinetics associated with PCR microfluidics

The requirements and characteristics of temperature cycling kinetics associated with PCR systems (including PCR microfluidics) are usually explained as follows:

(1) Denaturation temperature of the DNA template. This is a temperature leading to the melting of double-stranded DNA into single-stranded DNA for subsequent amplification. If melting is not complete, the PCR reaction will only be partially successful. However, the temperature should not exceed the acceptable temperature of DNA melting, or it will reduce the activity of DNA polymerase. The range of denaturation temperatures for DNA is usually from 90 to 95 °C.
(2) Annealing temperature of primers After completion of DNA denaturation, the temperature should decrease to a lower value necessary for annealing of DNA primers serving as starting molecules for DNA synthesis and flanking the DNA sequence to be amplified from both sides. The temperature for primer annealing depends on their inherent nucleic acid sequence and is optimized for each primer pair. The annealing temperature can to a great extent affect the PCR yield and specificity, which is usually around 55 °C.
(3) Extension temperature of primer extension is performed by increasing the lower annealing temperature to a medium temperature. The extension temperature is dependent on the optimal temperature for DNA polymerase, but usually is 72 °C at which the extension rate of Taq polymerase is 60–100 nucleotides/s.

Therefore, for the amplification DNA to succeed and to obtain amplification products with high throughput and high specificity, the cycling temperatures in PCR microfluidics must be precisely controlled so as to acquire the desirable temperature kinetics for DNA amplification.

5.2. Various heating methods for PCR microfluidics

The choice of a heating method for PCR microfluidics is of importance for achieving faster temperature ramping rates. The diversity of material exhibiting differences in thermal mass may require different heating methods. Presently, the heating methods for PCR microfluidics can be divided into two categories: contact and non-contact heating. The contact heating methods utilize the electrothermal conversion to heat the PCR solution, in which the thermal components embedding the heating element are in direct contact with the components of the PCR amplification (in fact, the heating methods of this type may be considered as the indirect utilization of joule heat for the PCR solution.). In the case of non-contact heating, the heating part is not in direct contact with the PCR amplification components.

5.2.1. Contact heating methods for PCR microfluidics

Among the contact heating methods, thin film heating elements and metal/other heating blocks are the two main formats. The former are mainly micromachined to form the film heating elements by using thin film deposition techniques. The latter mainly consist of inserting the cartridge heater into the metal blocks or of utilizing Peltier elements.

5.2.1.1. Thin film heating elements. There are two main materials used to fabricate microheaters, platinum and polysilicon. So far, most thin film heating elements for PCR microfluidics are made from metallic platinum (Pt) (Bu et al., 2003; Cui et al., 2001, 2002; Daniel et al., 1998; El-Ali et al., 2004; Felbel et al., 2004; Lagally et al., 2001a, 2004; Lao et al., 2000; Lee et al., 2004a,b, 2003a,b,c, 2000; Liao et al., 2005; Schabmueller et al., 2000a,b,c, 2001; Schneegaβ et al., 2001; Smekal et al., 2002; Trau et al., 2002; Wang et al., 2004; Yoon et al., 2002; Zhao et al., 2001, 2003) and nonmetallic polysilicon (Belgrader et al., 2003; Erill et al., 2003, 2004; Iordanov et al., 2003; Northrup et al., 1993, 1995; Woolley et al., 1996; Yang et al., 2004). Pt is the most commonly used material due to its best temperature vs. resistance relationship, ability to withstand high temperatures, good chemical stability, high antioxidation and purity, as well as easy micromachining. When Pt thin film deposition is performed to fabricate microheaters and microsensors, a thin layer of titanium is often functioning as an adhesion layer for Pt. However, this titanium thin layer exhibits a high diffusion rate at

high temperatures, which will deteriorate the Pt (Yang et al., 2004). Polysilicon is a polymer consisting of many of single-crystal particles, which is known to be compatible with high temperature processing and which interfaces very well with thermal silicon dioxide. Therefore, it has been widely used to fabricate thin film resistors in PCR microfluidics. The electrical characteristic of polysilicon thin films depends on its doping. Heavier doping results in a lower resistivity in single crystal silicon, whereas polysilicon is more resistive than single crystal silicon for any given level of doping because the grain boundaries in polysilicon hamper carrier mobility. Common dopants for polysilicon include arsenic, phosphorus, and boron, whose different concentrations in polysilicon have led to a large range of Temperature Coefficients of Resistance (TCR) of polysilicon thin films.

In addition to platinum and polysilicon, some other metals, alloys or inorganic compounds have been used as thin film heaters in PCR microfluidics, such as aluminum (Burns et al., 1996; Rodriguez et al., 2003; Zou et al., 2002, 2003), Ni (Zheng and Chen, 2001), tungsten (Liu et al., 2002a), silver/graphite inks (Fan et al., 2003; Koh et al., 2003), silver/palladium (Chou et al., 2002; Sadler et al., 2002, 2003), Ni/chromium (Poser et al., 1997), chromium/aluminum (Zou et al., 2003), Al_2N_3 (Belgrader et al., 2001) and indium-tin-oxide (ITO) (Friedman and Meldrum, 1998; Fukuba et al., 2002, 2004; Sun et al., 2002). It is noted here that ITO thin film has presented great potential as a heating element for PCR microfluidics due to its advantageous properties including low resistivity, strong adhesion to glass substrates and good transmittance in visible regions (>80%) (Sun et al., 2002). Furthermore, in terms of its optical transparency, the ITO thin film is superior to the Pt thin film because the Pt film isn't transparent. In addition, ITO is also an ideal candidate for a sensitive electrochemical detection of the hybridization event with the silver-enhanced gold nanoparticle approach as a result of its inherent low background silver deposition characteristic (Lee et al., 2003c).

5.2.1.2. Metal or other heating blocks. To date, along with the thin film heaters, the metallic heating blocks and Peltier-effect-based Thermo-Electric (TE) ceramic heating blocks have also been widely applied in temperature control of PCR microfluidics in spite of their having a larger thermal mass, slower temperature ramping rates, and being non-transparent (Anderson et al., 2000; Bruckner-Lea et al., 2002; Cady et al., 2005; Chaudhari et al., 1998; Erill et al., 2004; Grodzinski et al., 2001; Gulliksen et al., 2004; Hong et al., 2001; Khandurina et al., 2000; Lin et al., 2000a,b,c, 2002; Liu et al., 2004, 2002b; Marie et al., 2003; Matsubara et al., 2005; Shoffner et al., 1996; Taylor et al., 1997; West et al., 2002; Wilding et al., 1998; Yang et al., 2002; Yuen et al., 2001; Zhou et al., 2004; Chiou et al., 2001; Kopp et al., 1998; Nagai et al., 2001b; Obeid et al., 2003; Oberd and Christopoulos, 2003; Park et al., 2003; Shin et al., 2003; Zhang et al., 2002a). In the application of TE heating to PCR microfluidics, some interesting and important issues are worth noting: First, it is usually hard to achieve fast thermal transition from 55 to 95 °C in the presence of a single TE device. In order to achieve reasonable thermal cycling rates, two (Bruckner-Lea et al., 2002; Khandurina et al., 2000; Liu et al., 2002b; Yang et al., 2002; Zhou et al., 2004) and even more (Matsubara et al., 2005) TE devices were coupled to thermally cycle the PCR solution. Moreover, the top TE device in the scheme of dual TE devices may not be placed in direct contact with the PCR chip. The advantages of this scheme are its flexible and reliable thermal cycling performance (elimination of the potential for boiling off PCR solution at denaturation temperatures), reduction of the possibility of contamination of the PCR mixture. Besides, the PCR procedures is simplified because there is no need of PCR chamber sealing and consequent problems related to reaction inhibition by sealing adhesives and thermally induced fluid pumping (Khandurina et al., 2000). However, all these advantages are achieved at the cost of substantially increasing the power consumption. Second, in order to ensure good thermal contact between the TE element and the cycled region of the chip, some thermal conduction-supporting substances such as mineral oil (Khandurina et al., 2000; Zhou et al., 2004) or metallic thin wafer (Gulliksen et al., 2004) can be added onto the interface of the TE element and the cycled region. The most obvious quality of these added substances is their higher thermal conductivity. Third, a TE cell consists of an array of parallel P–N junctions and each parallel P–N junction establishes its own temperature differential for a given voltage, and consequently a radial temperature gradient on the hot surface of the TE cell will unavoidably be created so as to cause nonhomogeneity of surface temperature of the TE cell which will compromise the efficiency of PCR amplification. In order to overcome the nonuniform temperature distribution, an oxygen-free thin copper wafer is necessary to redistribute the surface temperature to achieve a homogenous temperature distribution across the surface of the TE cell (Erill et al., 2004).

Apart from thin film heating, TE heating and metallic block heating methods some other reliable heating solutions can also be used, for example commercial thin film resistance heaters (Koh et al., 2003; Hupert et al., 2003; Mitchell et al., 2003), thermal component constructed by using the single-sided Flexible Printed Circuit (FPC) technology (Shen et al., 2005), resistive heater coils (Curcio and Roeraade, 2003; Grodzinski et al., 2001; Sethu and Mastrangelo, 2004; Yu et al., 2000), and even more convenient commercial thermal cyclers (Krishnan et al., 2004a,b; Liu et al., 2003; Waters et al., 1998a,b; Yu et al., 2003). These methods have been proven to be efficient and robust for achieving faster PCR cycling in contrast to conventional PCR devices. It is believed that more and more desirable contact heating methods for PCR microfluidics will be developed based on MEMS or non-MEMS techniques using electrothermal Joule heating blocks in the future. In any case, the thermal-cycling rate is limited by the absolute thermal mass of the heating element itself and of the whole PCR system. Moreover, in the case of an external contact device, localized heating is ultimately limited in lateral resolution by thermal conductivity of the substrate material for on-chip PCR microfluidics, and fabricating devices with multiple independent heating points is not feasible. In the case of on-chip integrated heaters, these devices still need some tedious and complicated micromachining processes, which restrict the flexibility to easily change the PCR microfluidic design (Slyadnev et al., 2001). Consequently, great efforts have been made to develop non-contact or other heating methods, as will be discussed later.

5.2.2. Non-contact heating methods for PCR microfluidics

Contact-mediated temperature cycling for PCR as discussed above has a number of inherent problems, the most obvious of which is thermal mass. Contacting a chamber containing a PCR solution of smaller volume with a heating source, whether it be a thin-film-based microheater or a block based macroheater, adds thermal mass to the PCR amplification assembly. This will inevitably hinder fast thermal transitions during PCR amplification. Furthermore, when the integration of the PCR amplification with an electrophoresis microchip is taken into consideration, it is very difficult to regard the contact resource as part of the PCR chip and not as part of the electrophoresis chip itself on a single chip wafer in the presence of a contact-mediated heating method. These restrictions have triggered much interest in the development of non-contact heating approaches.

5.2.2.1. Non-contact heating based on hot air cycling.

Early on in the development of PCR cycling, the non-contact heating for PCR cycling based on hot air cycling was developed by Wittwer et al. (1989, 1990). Temperature cycling with a small volume sample was performed without physical contact between the heating source and the reaction sample. This non-contact heating was utilized to carry out rapid thermal cycling by rapidly switching streams of air of the desired temperature. Due to the lower thermal mass of air, a higher temperature ramping rate can be obtained, which, in recent years, has been further improved by several research groups (Lee et al., 2004a,b; Soper et al., 1999a,b; Swerdlow et al., 1997; Wang and Gong, 2003; Wang et al., 2003; Zhang et al., 1999; Zhang and Yeung, 1998). Specifically, an integrated capillary microfluidics was reported by Swerdlow et al. (1997), where the hot-air-cycling-based thermal cycling, purification of the reaction mixture, in-line loading, and CE were combined in a single instrument. In their system, a complete 25-cycle protocol took about 8 min for amplification of a M13mp18 DNA template and the rapid PCR, purification and analysis were accomplished within 20 min, which confirms that the non-contact heating based on hot air cycling is an effective method. A similarly integrated assembly was also presented elsewhere (Zhang and Yeung, 1998). Subsequently, a highly automated and integrated capillary microfluidics of high-throughput DNA analysis using hot air non-contact thermal cycling was also described (Zhang et al., 1999; Soper et al., 1999a,b). More recently, hot air-mediated heating has been successfully applied in thermal cycling control of high-throughput PCR chip microfluidics (Wang and Gong, 2003; Wang et al., 2003). However, the control and application of hot air for thermal cycling on a single chip may not be easily accomplished without an impact on other structures and possibly other reactions to be carried out on the chip (Oda et al., 1998).

5.2.2.2. Non-contact heating based on IR light radiation.

In the search for a non-contact heating method that will be amenable to chip-based PCR microfluidics, a novel non-contact heating method based on IR light radiation was first developed by Oda et al. (1998). A single and inexpensive tungsten lamp was utilized as a non-contact heat source for heating the glass microchambers with rapid temperature ramping rates of 10 °C/s (heating) and 20 °C/s (cooling), respectively. Hühmer and Landers reported an IR-mediated fused-silica capillary cycling with nanoliter volumes (160 nL), which requires not only less expensive Taq polymerase but

also less thermal cycling time to produce a detectable amount of PCR product, with heating and cooling rates of 65 and 20 °C/s, respectively (Hühmer and Landers, 2000). Also, a novel PI PCR microchip used in connection with IR-mediated thermocycling was described, where amplification of a 500 bp λ-phase DNA fragment was accomplished in a 1.7-μL chamber, with a total time of only 240 s for 15 cycles (Giordano et al., 2001a). Almost at the same time, this research group reported and discussed many buffer additives and absorbed coatings as alternatives to silanization of inner surfaces of glass PCR microchips for effective amplification using IR-mediated PCR (Giordano et al., 2001b).Recently, IR-mediated PCR amplification of a β-globin fragment followed by electrophoretic analysis on a single glass chip was presented for the first time. Less than 15 min were needed for 35 cycles demonstrating a clear-cut reduction of time required for the analysis of Duchenne Muscular Dystrophy (DMD) diagnosis (Ferrance et al., 2003). The cases described above have fully proven that non-contact IR-mediated heating is a very effective and robust solution to achieve rapid thermal transitions in PCR amplification. Furthermore, the integration of PCR with other functional components such as electrophoretic separation on a single chip was shown to be possible in combination with IR-mediated heating methods. This may turn out to be a nice approach to match the required amplification time with that of an electrophoretic separation and even that of sample preparation.

5.2.2.3. Non-contact heating based on laser-mediated heating. The IR-mediated non-contact heating approach was not combined with a flow system. Moreover, the tungsten lamp is a non-coherent and non-focused light source leading to a relatively large focus projection, which limits the heating efficiency when applied to microchips with a much smaller cross-section. In order to overcome these problems, a laser-mediated non-contact heating method was demonstrated to perform temperature control of a chemical reaction in a glass microchip. It used the photothermal effect produced by a diode laser to heat an absorbing target of a black ink point, which was placed on top of the microchip cover plate above the reaction channel (Tanaka et al., 2000). Subsequently, this group has reported on an integrated glass microchip with non-contact IR laser-mediated heating of the solvent, based on a photothermal effect. This method was capable of fast and localized temperature control under flowing conditions with ultrafast heating and cooling rates of 67 and 53 °C/s, which is allegedly 30 times faster than a conventional system and 3~6 times faster than electrothermal miniaturized thermocyclers (Slyadnev et al., 2001). This method may be a very attractive and desirable heating method due to its high resolution of spatially localized heating, its easy and flexible movement along the chip, and its property of being a point light source. Unfortunately, this effective heating method so far hasn't been applied for temperature control in PCR microfluidics.

5.2.2.4. Non-contact heating based on halogen lamp light source. Most recently, a non-contact heating method using an inexpensive halogen lamp as a low power radiation source for rapid temperature ramping in a silicon microreaction chamber was described, where a rate of 4 °C/s for heating and 4 °C/s for cooling were achieved. This cycling time is shorter than that required for the same PCR mixture in a silicon microreactor when heated by using a Peltier block heating method. It is for the first time that this non-contact heating approach was successfully applied to thermally cycle a silicon microchamber, although it had been demonstrated previously for glass or polymer-based PCR reaction microsystem (Ke et al., in press).

5.2.2.5. Non-contact heating based on induction heating. Among those non-contact heating methods using light radiation, lenses and filters are usually needed to eliminate wavelengths that could interfere with the PCR reaction. Accurate positioning of the reaction mixture is also required at the appropriate focal distance for the reaction to occur. Moreover, in the lamp heating method the power transfer efficiency is not very high, which complicates the design and development of battery-powered portable PCR microfluidics. Based on these thoughts, induction heating as a new and efficient non-contact method of thermocycling was first reported by Pal and Venkataraman (2002). The induction heating can lead to much simpler fabrication steps, and fast heating and cooling rates of 6.5 and 4.2 °C/s by optimizing the heater dimensions and heating frequency. This method has the advantage that accurate positioning of the reaction mixture with respect to the heater is not necessary, thin-film deposition steps to pattern thin-film heaters on the chip are not required, and elaborate percentage/integrator/differentiator (PID) control is not needed. The only requirement is a simple on-off electric temperature control which gave a temperature stability of ±0.2 °C. Importantly, the low power consumption makes it very suitable for portable battery-operated microchip PCR applications. Subsequently, Lao et al. have also described a similar non-contact thermocycling system, which could achieve

heating and cooling rates of over 16 and 9.6 °C/s, respectively, using programmable closed loop control of a Digital Signal Processor (DSP) based servo controller to control the required PCR temperatures (Lao et al., 2003).

5.2.2.6. Non-contact heating based on microwave irradiation. Presently, a novel non-contact heating method with focused microwave irradiation as the heat source for PCR amplification was first demonstrated by Fermér et al. (2003), where a single-mode microwave cavity was used to heat a 100 μL PCR mixture in a 0.5 mL PP tube with a 60 min for 25 cycles. This microwave heating method for PCR amplification had the following properties (Fermér et al., 2003): First, microwave-based PCR can reach the same efficiency as conventional PCR, while maintaining Taq polymerase activity even after 25 cycles using microwave heating. Second, the irradiation energy is used to heat only the PCR solution and not the block or the tubes, and so the ramping time may be substantially shortened. Third, the required temperature is reached almost instantaneously and simultaneously, allowing for shortening of the incubation time. In addition, temperature gradient within the samples are small or even absent, which offers the possibility to perform PCR on a larger scale, which may be of importance in situations where large quantities of DNA are needed. Most recently, this group has reported microwave-induced, milliliter-scale PCR, which has led to a further study for this novel non-contact heating method (Orrling et al., 2004).

5.2.3. Other heating methods for PCR microfluidics

Detailed discussions about contact and non-contact heating methods for PCR amplification are presented above, so we had a comprehensive profile of heating methods for PCR microfluidics in mind. Below a description will be given for some other heating methods for PCR amplification, which have been proven very effective.

5.2.3.1. Heating methods based on chemical and physical processes. More recently, a new integrated temperature control system for PCR microfluidics was introduced using chemical and physical processes to locally regulate temperature. Cooling of a microchannel was realized by the evaporation of acetone as an endothermic process, whereas heating of a microchannel was achieved by dissolution of concentrated sulfuric acid in water as an exothermic process (Guijt et al., 2003). Within this approach, the thermal effect was initiated by the mixing of two components in the case of a vacuum used as the driving force of the system, the extent of which can be controlled by either the flow ratio of the two components, or by selection of the components based on their chemical or physical properties. The main benefits from this method include no need of external components or of convection to cool the microfluidics, the simplicity and the lower cost of microfabrication, the decrease of the footprint of the device, and thus the possibility of further integration.

5.2.3.2. Heating method based on alternating-electric-current induced buffer joule heating. To overcome the issues of large thermal mass of the heating device in the contact heating methods and to facilitate heat transfer to the samples, a new alternating-electric-current induced joule heating method was proposed to perform the PCR within a defined microchannel segment (Stern et al., 2002). Electric current is directly transferred into the PCR solution, leading to rates of 15 °C/s for heating and 15 °C/s for cooling. This method utilizes in-channel Pt electrodes to transfer the alternating current (AC) heating current into the buffer under conditions of minimal oxidation reduction and water electrolysis. The key features of this approach include nanoliter-level reaction volumes for decreased sample consumption, rapid thermocycling for better PCR performance, and simple and full integration of reaction buffer joule heating with conductivity-based direct temperature sensing for accurate temperature control. However, although buffer conductivity has a positive temperature coefficient which can be exploited to determine its temperature, this direct temperature monitoring by electrical resistance measurement is restricted especially for the samples with varied electrical conductivity (Slyadnev et al., 2001).

5.2.3.3. Heating method based on a flowing fluid of water. In case of the large SVR of the pipette tip PCR reaction vessel geometry, water as a flowing fluid medium can be ideal for thermal cycling (Nakane et al., 2001). Water possesses a higher thermal stability and uniformity due to its high specific heat. To achieve fast thermal ramps, three separate water baths are maintained at the temperatures required during the PCR. Computer-controlled valves/pumps determine which bath is selected as the water source to flow through the container housing the tips (Nakane et al., 2001). This method resembles the hot air non-contact heating method, and may be regarded as a water-mediated non-contact heating method.

5.2.4. Summary of heating methods for PCR microfluidics

The contact and non-contact heating methods represent the very important and widely acceptable solutions to the realization of thermal cycling for PCR microfluidics. All of them have the potential to achieve the desired temperature cycling in the presence of active/passive cooling approaches. However, it is noted that contact heating can also achieve the same performance and thermal cycling effect as the non-contact heating methods. For example, Nagai et al. have realized rapid PCR thermal cycling by moving the PCR microchamber system between three heating blocks of different temperatures. This system comprised a number of PCR microchambers arranged to reduce the heat capacity of the PCR solution allowing for heating/cooling rates of ~16 °C/s (Nagai et al., 2001b). The main characteristic of this configuration is that the heating/cooling rates are affected mainly by the heat capacity of the PCR solution and the reaction chamber itself. In addition, among most of the contact thin film heaters, the heaters and temperature sensors are located outside the PCR reaction chamber, impeding precise temperature measurement of the PCR solution, reducing the heating effect thus causing higher power consumption. In order to overcome the shortcomings of this arrangement, some researchers have microfabricated the PCR chambers in a new configuration, where the microheaters and sensors are located inside the reaction chamber resulting in more effective heating/cooling performance and more precise temperature measurement (Lagally et al., 2003; Lee et al., 2003b; Liao et al., 2005). Also, utilizing the same material as the heating/sensing elements in most of the thin film resistors for the temperature control in PCR microfluidics can significantly simplify the design task.

5.3. Various methods of temperature measurement for PCR microfluidics

In PCR microfluidics, it is very important to select methods for temperature measurement to accurately control temperature during PCR cycling. Presently, the temperature measurement methods are usually divided into two categories: contact and non-contact temperature measurement. The former includes thin-film-type temperature sensing and non-thin-film-type temperature sensing. The thin film temperature sensors comprise Pt (Bu et al., 2003; Cui et al., 2001, 2002; Daniel et al., 1998; El-Ali et al., 2004; Felbel et al., 2004; Fukuba et al., 2004; Lagally et al., 2001a, 2004; Lao et al., 2000; Lee et al., 2003a,b,c, 2004a,b, 2000; Liao et al., 2005; Poser et al., 1997; Schabmueller et al., 2000a,b,c, 2001; Schneegaβ et al., 2001; Smekal et al., 2002; Trau et al., 2002; Wang et al., 2004; Zhao et al., 2001, 2003), aluminum (Burns et al., 1996; Rodriguez et al., 2003; Zhao et al., 2003; Zou et al., 2002, 2003), ITO (Friedman and Meldrum, 1998; Fukuba et al., 2002), polysilicon (Erill et al., 2003, 2004), and even copper temperature sensors (Shen et al., 2005). The non-thin-film temperature sensors generally include thermocouples (Belgrader et al., 2003; Chiou et al., 2001; Chou et al., 2002; Ferrance et al., 2003; Giordano et al., 2001a,b; Grodzinski et al., 2001; Gulliksen et al., 2004; Hong et al., 2001; Hühmer and Landers, 2000; Hupert et al., 2003; Khandurina et al., 2000; Koh et al., 2003; Lagally et al., 2000, 2001b; Lao et al., 2003; Liu et al., 2002b; Mitchell et al., 2003; Nakane et al., 2001; Oda et al., 1998; Pal and Venkataraman, 2002; Sethu and Mastrangelo, 2004; Shin et al., 2003; Soper et al., 1999a,b; Wang and Gong, 2003; Wang et al., 2003; West et al., 2002; Woolley et al., 1996; Yang et al., 2002; Yu et al., 2000; Zhou et al., 2004), Pt100 electrical-resistance thermometers (Curcio and Roeraade, 2003; Ke et al., in press; Kopp et al., 1998; Lin et al., 2000a,b,c; Obeid et al., 2003; Oberd and Christopoulos, 2003; Sun et al., 2002), semiconductor electrical-resistance thermometers (thermistors) (Belgrader et al., 2001; Bruckner-Lea et al., 2002; Cady et al., 2005; Chaudhari et al., 1998; Liu et al., 2002a; Shoffner et al., 1996), and diode thermometers (Iordanov et al., 2003; Sadler et al., 2002, 2003). The thin film temperature sensors are usually made from some metallic, nonmetallic or oxide materials by using thin film deposition techniques, which can provide the PCR microfluidics with a higher degree of integration, small footprint and good biocompatibility. The design principle for these thin-film temperature sensors may be found in references (Lao et al., 2000; Lee et al., 2000). To utilize some non-thin-film temperature sensors to measure the temperatures may lead to adverse problems such as biocompatibility and/or integration. However, they are still widely used in PCR microfluidics because of their lower cost and convenience. Whatever the contact temperature sensors' nature, they will add their own thermal mass to the PCR system, which ultimately adversely affect the thermal cycling performance of PCR microfluidics. Additionally, the contact temperature measurement techniques can yield temperature data only at a few discrete points or lines and only indirectly reflect the temperature of the PCR solution, and so the precision and accuracy of temperature measurement is limited. Although direct contact between the tempera-

ture sensors and the PCR solution may lead to a more accurate temperature measurement, the presence of the sensor may cause side effects on the PCR and increase the risk for sample cross-contamination. Moreover, the utilization of metallic lines of the thermocouple on the inner walls of reaction chambers is not feasible as metal lines can inhibit the PCR by inactivating the Taq polymerase through irreversible adsorption (Oda et al., 1998). In order to overcome these problems, some researchers have made attempts to develop non-contact temperature measurement techniques for PCR microfluidics, such as IR thermometry (Chou et al., 2002; Liao et al., 2005; Matsubara et al., 2005; Nagai et al., 2001b; Zou et al., 2003). The advantages of this type of temperature measurement technique include rapid response, continuous temperature readings, higher spatial resolution, and no interference with the object observed. However, IR thermometry has also disadvantages such as a precision lower than that of contact measurements. Also, only information about the two-dimensional surface temperature of the IR-absorbing substrate is obtained, which can be easily affected by the intermediate medium such as vapor and carbon dioxide.

To directly acquire the spatial and real temperature distribution of the PCR solution in PCR microfluidics which the above-mentioned contact or non-contact techniques are incapable of, great interest has been taken in developing other novel temperature measure techniques in PCR microfluidics, such as transient Thermochromic Liquid Crystal (TLC) thermometry (Chaudhari et al., 1998; Liu et al., 2002a; Mitchell et al., 2003), temperature-dependent fluorescence dye-based thermometry (Guijt et al., 2003; Ross et al., 2001; Slyadnev et al., 2001), and temperature measurement by measuring the fluorescence spectra of the aqueous solution (Sun et al., 2002). TLCs are materials that change their optical properties with temperature within ranges specific to their chemical composition. Within the active range for a given TLC, the wavelength-dependent reflectivity varies strongly with temperature leading to a strong temperature dependence of the apparent color. This color–temperature response can then be readily captured by a color charge-coupled devices (CCD) camera, formulated into a calibration curve of color versus temperature and used to transform a color measurement system into very effective TLC-based thermometry. Different formulations of liquid crystals can be utilized to give measurements of high precision (0.1 °C) over various temperature intervals. Unfortunately, these encapsulated TLC beads suspended in liquids have dimensions of the order of tens of micrometers and so are impractical when used in micrometer-sized microfluidic structures (Ross et al., 2001). Temperature measurements using a temperature-dependent fluorescent dye constitute a simple technique for measuring temperature in microfluidic structures with high spatial and temporal resolution, by making use of the temperature dependence of the fluorescence intensity of a dilute fluorophore such as rhodamine B (Guijt et al., 2003; Ross et al., 2001) or rhodamine 3B (Slyadnev et al., 2001. Although as yet this technique has not been really applied for measuring temperature fields in PCR microfluidics, it is very promising due to its better precision as well as high spatial and temporal resolution. It is noted, additionally, that a melting curve experiment with the PCR products using SYBR green I as a fluorescent dye which fluoresces only in the presence of dsDNA, can be performed to test how accurate the temperature is controlled within the reaction chamber of PCR microfluidics (El-Ali et al., 2004).

Besides, some researchers have developed some additional techniques to measure temperature in PCR microfluidics. For instance, a cantilever-based temperature sensor (Marie et al., 2003), temperature measurement by an IR pyrometer or by a fluoroptic probe (Orrling et al., 2004), and buffer conductivity-based temperature sensing (Stern et al., 2002) were described. The cantilever-based temperature sensors have several clear-cut advantages such as robustness and compactness in design, ease of electrical connections to the chip, stable encapsulation of the electrical lines on the chip, and tightness in reaction chamber for PCR solution (Marie et al., 2003). The IR pyrometer-based temperature measurement is a kind of non-contact technique somewhat similar to the above-mentioned IR thermometry.

5.4. Correlative temperature control methods for PCR microfluidics

As already described above, PCR is a typical temperature-controlled reaction system, so temperature control of the PCR solution is a key issue. Within current PCR microfluidics, the most commonly used temperature control algorithm is accomplished through a PID module (within a certain software program) (Chaudhari et al., 1998; Chiou et al., 2001; Chou et al., 2002; Curcio and Roeraade, 2003; El-Ali et al., 2004; Gulliksen et al., 2004; Hühmer and Landers, 2000; Hupert et al., 2003; Khandurina et al., 2000; Kopp et al., 1998; Lagally et al., 2000, 2001a,b; Lee et al., 2003c; Lin et al., 2000a,b,c, 2002; Mitchell et al., 2003; Obeid et al., 2003; Oberd and Christopoulos, 2003; Rodriguez et al., 2003; Shin et al., 2003; Sun

et al., 2002; Wang and Gong, 2003; Wang et al., 2003; Zhang et al., 2002a; Zhao et al., 2003; Zheng and Chen, 2001; Zou et al., 2002). PID control is one of the earliest developed control strategies, which has been widely used for the control of industrial processes.

However, in order to meet the requirements of temperature control for PCR microfluidics, some researchers may want to make some modifications to the PID control strategy and adopt alternative temperature control algorithms such as proportional control (Oda et al., 1998; Daniel et al., 1998), PI control (Lao et al., 2000; Lee et al., 2000; Sadler et al., 2002, 2003; Yoon et al., 2002), PD control (Soper et al., 1999a,b), and PD–PID control (Erill et al., 2003, 2004). In addition to PID control, some other temperature control strategies were also successfully developed for the PCR microfluidics, such as a neural networks "predictive control" method (Liao et al., 2005), a Modular Laser Diode Controller through an RS 232 interface (Shoffner et al., 1996), a DSP-based servo controller (Lao et al., 2003), and only on-off control (Pal and Venkataraman, 2002).

5.5. Numerical simulation of temperature fields for PCR microfluidics

In general, the thermal cycling performance for most PCR microfluidics has greatly improved due to their smaller thermal capacity resulting from the choice of substrate material, choice of heating and temperature sensing methods, the decreased volume of the PCR chamber and so on. However, with respect to the design of most PCR microfluidics, there is still a significant challenge in the improvement of thermal cycling ramps and temperature uniformity.

The temperature cycling rates and homogeneity in PCR microfluidics depend on many factors, including heating and cooling approaches, used substrate material, architecture and arrangement of heating elements, structural and dimensional design of PCR microfluidics itself, etc. Although the temperature pattern for these PCR microfluidics can be effectively achieved by appropriate experimental methods mentioned above, these methods are usually so complicated that it is very difficult and impractical to investigate the temperature pattern of PCR systems with different materials at the same time. Furthermore, expensive illumination and imaging systems are usually necessary for these experiments, and complex calibration procedures are to be performed since the images obtained from these experiments depend strongly on the illumination technique and the properties of materials within the optical path. Most importantly, these experiments cannot provide any static and/or dynamic information on temperature before actual microfabrication of PCR microfluidics has taken place. So, in order to acquire the 2/3-D static/dynamic temperature pattern and optimized structures and dimensions of PCR chambers or heating elements before their fabrication, numerical simulation is regarded as a very effective means, and an opportunity to assess the details of the flow, thermal and even chemical processes that take place in PCR microfluidics. At present, numerical simulation methods applied to PCR microfluidics comprise Finite Element Analysis (FEA) (Bu et al., 2003; Chou et al., 2002, 2003; El-Ali et al., 2004; Iordanov et al., 2003; Lee et al., 2004a,b; Lin et al., 2000c; Miao et al., 2003; Mitchell et al., 2003; Poser et al., 1997; Sadler et al., 2002, 2003; Shin et al., 2003; Shen et al., 2005; Wang et al., 2004; Zhang et al., 2002a; Zou et al., 2002, 2003), commercial MEMS software (IntelliSuite™) (Cui et al., 2001, 2002; Zhao et al., 2001), lumped thermal capacity model (Chaudhari et al., 1998; Daniel et al., 1998; Iordanov et al., 2003; Wang et al., 2004), and few Finite Volume Method (FVM) (Krishnan et al., 2004a; Lao et al., 2000; Yoon et al., 2002). In addition, the predictive CAD design analysis was applied to observe the contamination between neighboring wells on a PCR chip during temperature cycling (Deshpande et al., 1999); a novel SystemC design environment is utilized as system performance evaluation for two PCR microfluidic systems: flow-through systems and droplet-based systems (Zhang et al., 2002b, 2004). Also, the CFD (Computation Fluid Dynamics)–ACE (Advanced Computational Engineering)+MEMS developed by CFD Research Corporation (CFDRC) as multi-physics numerical code for high-fidelity thermo-fluid simulations of MEMS, has been extended to include the chemical reactions that take place during PCR (Athavale et al., 2001). The lumped thermal capacity model can be used for transient determination of the PCR thermal time constant in which the temperature difference between the heater and reaction chamber is measured as a function of the ramping rate. A thermal time constant which is not too big is required to ensure rapid cooling and not too small to keep the input power low (Daniel et al., 1998). However, the temperature-uniformity-assumption-based lumped thermal capacity is an approximate model, which sometimes will lead to an inaccurate temperature analysis. Recently, a quasi multi-system lumped thermal capacity model has been advanced to accurately simulate the whole chip's thermal cycling performance, where the chip is divided into several subsystems based on its different physical functions, and the linkages between the

subsystems are based on energy conservation (Wang et al., 2004). The FEA is a very useful mathematical simulation tool which can carry out thermal, electric field, structure analysis and so on. The most obvious advantage of the FEA method is its flexibility to irregular regions, but the workload of an FEA computation is usually larger than that of FVM. Moreover, while solving the problems concerning flow and heat transfer, the FEA is not as mature as the FVM in terms of the discrete treatment of a convective phase and the solution of incompressible fluids by a primitive variable method. The discrete equations deduced by the FVM method may be considered conservative, with coefficients that have specific physical meaning, so the FVM is the most widely used method among the numerical computations of fluid and heat transfer. Recently, the steady-state flow patterns within the cavities and loop structures in the convection-driven PCR microfluidics were successfully simulated using commercial Fluent software based on FVM (Krishnan et al., 2004a).

6. Flow control methods of microfluidics for PCR microfluidics

In PCR microfluidics, especially flow-through PCR microfluidics, the flow of the DNA sample within microchannels must be precisely controlled for successfully performing the PCR. Convection-driven PCR microfluidics does not need an external force to drive the fluid through the different temperature zones. Nevertheless, the sample needs to be carefully introduced into the microchannels which must be accurately sealed to prevent bubble formation that is a significant concern as the presence of bubbles in miniature pipes can adversely affect or even block sample flow (Chen et al., 2004). In the case of chamber stationary PCR microfluidics, the solution is fixed within one or multiple reaction chambers to underdog the PCR after the sample is introduced into the system through the inlet. So here, we have limited ourselves to the flow-through PCR microfluidics to illustrate the effect of flow control on the PCR performance. Other aspects of convection-driven and chamber stationary PCR microfluidics have consciously been omitted.

For flow-through PCR microfluidics, the sample flow may greatly affect PCR amplification, and comprises the flow rate, flow stability and serial flow mode of PCR solution within microchannels. The maintenance of flow stability is a crucial factor for the reproducibility of the residence time in each cycle. The formation of bubbles in the denaturation zone might be critical. The flow rate of the sample through the channel, together with the cross-section and the length of the channel in each zone, can determine the residence time in each zone and the cycling times of total PCR. The possible generation of a serial flow mode of sample plugs is also one of the most attractive features in the flow-through PCR because of the enhancement of the sample number capacity of the device (Schneegaβand Köhler, 2001a). However, the sample flow through the channel has an intimate relation with the flow control methods of microfluidics, as will be discussed below.

6.1. Flow control by constant flow pump

6.1.1. Syringe pump

A syringe pump generally consists of a cylinder that holds the mobile phase which is expelled by a piston which is advanced by a motor connected through worm gears, to provide smooth pulseless flow. This type of pump has some obvious characteristics such as precision in sample injection rate, simplicity, built-in intelligence and automation in process, as well as a wide range of flow rates (0.1–400 mL/h), and so is very suitable for flow control of biological samples within microchannels. Presently, many researchers have applied the syringe bump to control the flow of PCR solution through microchannels of flow-through PCR microfluidics (Belgrader et al., 2003; Curcio and Roeraade, 2003; Fukuba et al., 2002; Hupert et al., 2003; Kopp et al., 1998; Mitchell et al., 2003; Nakano et al., 1994; Obeid et al., 2003; Oberd and Christopoulos, 2003; Park et al., 2003; Schneegaβ et al., 2001; Sun et al., 2002; Zhang et al., 2002a,b; Zheng and Chen, 2001). Generally, different channel systems for flow-through PCR need to adopt different flow rates. The residence times within a channel usually differ even when the same flow rate is applied by the syringe pump, and primarily depends on the cross-section and the length of the channel in each zone. However, the cycle time of one PCR depends on the synthesis rate of the polymerase and diffusion time, whereas the diffusion time is almost negligible owing to the small cross-section of channel. So, the flow rate can theoretically be accommodated up to the synthesis rate of the polymerase (Schneegaβ et al., 2001). The influence of various flow rates on the amplification efficiency in the presence of flow control by a syringe pump was investigated by several groups in order to minimize the demand of time for the whole PCR (Fukuba et al., 2004; Kopp et al., 1998; Park et al., 2003; Schneegaβ et al., 2001). For a given PCR system, there exists an optimized flow

rate below or above which a decrease of PCR products is observed. The lower flow rate will result in a longer residence time of polymerase at the denaturation zone, which may inactivate the polymerase leading to lower PCR products over time, whereas the PCR process won't be fully and completely performed at the higher flow rate (Schneegaβ et al., 2001). It is noted that pressure-driven flow by a syringe pump is sometimes chosen over electrically driven flow in the case of extensive length of the flow channel (Hupert et al., 2003). However, its low integration and much larger footprint make it difficult to realize a "real" μ-TAS in spite of its simplicity and ease of flow control.

6.1.2. Peristaltic pump

Peristaltic pumps possess the properties of precision of flow control, negligible contamination, and convenience in use and maintenance, and have been widely utilized in such fields as food, drug production, and genetic research. Sample flow control for PCR amplification is likewise achieved by using a peristaltic pump (Chou et al., 2002; Sadler et al., 2002). The use of this type of routine peristaltic pump in PCR microfluidics will cause a larger footprint and a lower degree of integration. More recently, a microbi-peristaltic pump has been integrated onto a flow-through PCR microfluidics to implement the flow control of sample. This device is actuated by three piezoelectric discs located in a recess etched in the glass and is designed on the basis of plate and shell theory (Bu et al., 2003).

6.2. Flow control by pressure differential across the channel

In flow-through PCR microfluidics, the pressure differential across the channel is often used to control sample flow in the microchannels. An air pressure differential and some precise hydrophobic patches are used to isolate and move drops of <1 nL within the channels, where air pressure can be supplied by an external source or by on-chip expansion chambers (Burns et al., 1998). The oil reservoirs to which the ends of capillary reaction channel are attached are connected through computer-controlled solenoid valves introducing pressure differentials across the reservoirs of 5 psig, 7.5 psig and atmospheric pressures that drive the sample plug in the capillary (Chiou et al., 2001). A similar flow control by pressure differentials for flow-through PCR is also reported elsewhere (Wang et al., 2004). For flow control of sample by pressure differentials, it is usually difficult to determine the accurate location of the sample along the microchannel. Nowadays, diode detectors (Burns et al., 1998) and photodiodes (Chiou et al., 2001) are usually used to accurately control the location of sample in the channel in order to automatically control the heating process.

6.3. Flow control by rotary pump

The diffusivity of DNA in the sample is very small and the diffusion constant of a 1-kbp long DNA molecule is ~10 $\mu m^2/s$ (Bader et al., 1999), and so it will take up to 1000 s for these DNA molecules to diffuse an area of 100×100 μm^2. It is very important for the integrated microfluidics to rapidly perform on-chip mixing to bring about reactions between samples and reagents. In order to overcome the diffusion limit during the handling of a microfluidic sample, Chou et al. have introduced a new key component: a rotary pump, where solutions are actively pumped into a circulating loop (Chou et al., 2001). Recently, this type of rotary pump was first integrated onto a PCR microfluidics by Liu et al. (2002a,b). The integration of a rotary pump onto PCR microfluidics for flow control has led to the possibility of conveniently performing both stationary and flow-through PCR with sample volumes as small as 12 nL.

6.4. Flow control by MHD

The MHD micropump is a new type of non-mechanical micropump which uses Lorentz force as the pumping mechanism to control the flow of a biological sample within the channel. The related basic theory may be described as follows (Lemoff and Lee, 2000): the Lorentz force is produced when an electric current is applied across a channel filled with conducting solution in the presence of a perpendicular magnetic field. The flow rate for a rectangular cross-section channel in the action of this Lorentz force is expressed as $Q = \frac{IBw^3h^2}{8\mu L(w+h)^2}$, where I is electric current across the channel (measured in amperes), B is the magnetic field (measured in Tesla), w is the distance between the electrodes, h is the height of the electrode, L is the total length of the channel, and μ is the viscosity of the fluid. An AC MHD micropump has some obvious advantages: First, it can produce a continuous flow and it is suitable for solutions containing biological samples. Second, the voltage required for the AC MHD micropump is lower than most of other micropumps, which is compatible with biological systems. Third, the multiple, independently controlled AC MHD micropumps can be integrated on a single chip, thus enabling complex microfluidic systems. Recently, West et al. have developed a novel flow-through PCR

microfluidics using AC MHD actuation to control sample flow, in which a two-step PCR reaction with a total of 20 cycles was chosen for successful amplification of a 142 bp *rpoB* gene fragment of *Mycobacterium tuberculosis* (West et al., 2002). However, when operating at elevated temperatures electrolysis occurs in spite of the application of considerably lower voltages, resulting in blocking of channel by the formation of bubbles and electrolysis by-products that could interfere with the PCR reaction (West et al., 2002). The circulation of fluids by using MHD actuation for PCR amplification needs further development for fulfilling the requirements of better PCR chemistry.

7. Surface chemistry (surface inactivation) of PCR microfluidics

7.1. Necessity of surface passivation for PCR microfluidics

For PCR, biocompatibility may be the most sensitive and delicate issue because a PCR solution is in permanent contact with a certain amount of the materials. Currently, the PCR microfluidics have been mostly micromachined from silicon, glass or polymer substrate materials. Early work concerning silicon/glass PCR microfluidics have revealed the problems of deleterious surface interactions (Wilding et al., 1994, 1995; Shoffner et al., 1996), which are mainly caused by the following two factors. First, the multicomponent PCR will lead to the significant probability of at least one of these components binding onto the inner surface of the reaction chamber/channel. The concentrations of several necessary PCR components usually need to be maintained in a fairly narrow range and to be maintained in a sensitive balance with respect to one another so as to produce an optimized PCR reaction. Second, the PCR solution is subject to a significant increase in SVR in PCR microfluidics and the inner surface chemistry of the reaction chamber/channel begins to become a significant component in the reaction and can pose a dominating position, which further increases the possibility of deleterious interactions between the inner surface and the components of PCR solution. In addition, the wide range of inhibitors present with target DNAs from many sources may interfere with polymerase activity (Wilson, 1997), although some novel microfluidics have been developed to removing PCR inhibitors, such as the DEP selective filter (Perch-Nielsen et al., 2003), weir-type based silicon microfilter (Wilding et al., 1998; Yuen et al., 2001) and microchip for solid phase extraction of DNA (Ferrance et al., 2003). Inhibition of PCR by substrates introduced during microfabrication (i.e. chromium) has been presented previously for glass microchips (Taylor et al., 1998). Most recently, Panaro et al. have evaluated the biocompatibility of several common plastics, commercially available plastic tubing, disposable syringes and gasket materials which might be useful in the construction of PCR microfluidics, and it is thought that the initial effects of surfaces and of surfaces repeatedly exposed to reagents and washing procedures is an important consideration in the overall assessment of the properties of a platform construction material (Panaro et al., 2004). All these facts result in the great complexity in the design and architecture for PCR microfluidics. Fortunately, however, some surface passivation techniques have been successfully developed to produce PCR-friendly inner surfaces for PCR microfluidics. More recently, Kricka and Wilding have reviewed the surface chemistry problems presented in the PCR microfluidics and discussed the surface passivation strategies developed for the solution to the difficulties concerned (Kricka and Wilding, 2003).

7.2. Two surface passivation techniques applied for PCR microfluidics

The surface passivation techniques applied in PCR microfluidics may be classified into two categories:

(1) *Static passivation:* In surface passivation of this type, the inner surface of PCR microfluidics is precoated by using a PCR-friendly substance during the fabrication of PCR microfluidics or before starting the PCR chemistry. In most silicon/glass hybrid PCR microfluidics, a thin layer of silicon oxide surface coating is deposited to enhance PCR compatibility. Sometimes, this type of surface coating technique can also be used to deposit the inner surfaces of plastic substrates for PCR microfluidics (Lee et al., 2004a,b). An obvious advantage of the silicon oxide layer method is that the passivation process is accomplished during chip fabrication and the subsequent sealing of the chips with glass wafer by an anodic bonding technique is not intervened with (Wilding et al., 1994). Furthermore, deposition of oxide surfaces is a standard industry procedure that is reproducible and inexpensive and can be accomplished in a batch production setting (Shoffner et al., 1996). Another commonly used static passivation procedure is chemical silanization of inner surfaces which is performed by filling the reaction chamber/channel with a silanizing agent and incubating the filled

chip for a period of time, followed by removal of the excess silanizing agent and then drying and washing of the silanized chip. However, silanization is a time-consuming and complex labor-intensive process, and after silanization the chips need to be stored in liquids to protect the silane film from damage, which could be a serious problem for practical applications (Lee et al., 2003b). It is noted, additionally, that the inner surfaces of the reaction chamber may be silanized to a certain extent by silanization of the master which is used for the fabrication of polymer-based PCR microfluidics (Lagally et al., 2000, 2001b; Zhao et al., 2002). An overall summary of static passivation and dynamic passivation as discussed below is presented in Table 3, whereas the most commonly used silanizing agents in PCR microfluidics is also summarized in Table 4.

(2) *Dynamic passivation:* This type of passivation procedure occurs during the practical operation of PCR microfluidics and is realized by adding the passivation agent to PCR solution. For this passivation technique, the most frequently used passivation agents include a competing protein adjuvant-bovine serum albumin (BSA), polymer solutions such as polyethylene glycol (PEG) and polyvinylpyrrolidone (PVP), and the nonionic surfactant Tween 20. BSA is often included into the PCR solution to stabilize the polymerase enzymes and to reduce undesired adsorption of polymerase onto the inner surfaces of reaction chamber. On the one hand, while the addition of BSA occasionally improves reaction fidelity, it is not necessary if the surface of the reaction chamber is adequately passivated (Northrup et al., 1998; Oda et al., 1998). On the other hand, BSA dynamic passivation may prove to be insufficient if the volume of the reaction chamber is in the low-microliter to nanoliter scale where the SVR has increased by more than 1000-fold (Giordano et al., 2001a). For example, multiplex PCR shows a higher degree of failure if the inner diameter of the capillary chamber decreases, when the T4 gene 32 protein need to be added into the PCR solution to acquire an effective PCR (Zhang et al., 1999). For the PEG, PEGs with different molecular weights (e.g. PEG 400, PEG 1000, PEG 8000, etc.) had been included into the PCR solution respectively and the effect of their addition on the PCR had been tested. The best results were achieved by addition of PEG 8000 at a 0.75% (w/v) concentration (Yang et al., 2002). Similar cases can be found elsewhere (Giordano et al., 2001a,b; Panaro et al., 2004). With respect to the PVP, the addition of only PVP may not have a significant effect on PCR in the PC microfluidics, regardless of its concentration (Yang et al., 2002). It may need to be utilized in combination with some other passivation techniques (Kopp et al., 1998; Zhou et al., 2004). The PCR buffer is completed by using Tween 20 as an additive which is found to be effective for PCR in chip microfluidics. Tween 20, which acts on the relaxation of the surface tension of solutions and is often utilized in fields of protein and nucleic acid handling, serves as a dispersant, emulsifier and solubilizer in protecting the enzyme (Schneegaβ et al., 2001).

7.3. Mechanism of PCR inhibition by silicon-related materials and their corresponding passivation solutions for PCR microfluidics

Presently, most of PCR microfluidics are still fabricated from silicon substrate material with a silicon chamber covered with a glass wafer although several kinds of polymer materials have been applied for the fabrication of PCR microfluidics. Therefore, silicon and glass are the two main materials for the development of PCR microfluidics. However, there are also some obvious disadvantages in terms of their utilization, especially regarding their PCR-friendliness. Although the two surface passivation techniques have been developed for acquiring PCR-friendly inner surfaces almost since the PCR chip microfluidics was introduced, the underlying mechanisms that govern the inhibition of PCR by silicon-related materials are still not clearly understood. Two complementary mechanisms have been proposed to account for the inhibition of PCR: straight chemical inhibition and surface adsorption due to the significantly increased SVR (Erill et al., 2003). Recently, several research groups have further studied and probed into the inhibition mechanism and corresponding passivation techniques in the case of silicon/glass PCR chips (Erill et al., 2003; Felbel et al., 2002, 2004; Krishnan et al., 2004b). By systematically analyzing the material-related inhibition and adsorption phenomena in glass/silicon PCR chips, Erill et al. have reported that the previously reported inhibition of PCR by silicon-related materials results mainly from the adsorption of Taq polymerase onto chip walls due to the increased SVR and not from a straight chemical action of silicon-related materials on the PCR solution. In contrast to Taq polymerase, DNA is not adsorbed in noticeable amounts (Erill et al., 2003). These findings may provide an ideal starting point for the further exploration of

Table 3
The surface passivation techniques applied for the PCR microfluidics

Surface passivation technique	Reference
Static passivation	
Deposition of silicon oxide	Cheng et al., 1996, 1998; Chaudhari et al., 1998; Cui et al., 2001, 2002; Ke et al., in press; Lee et al., 2004a,b; Lin et al., 2002; Schabmueller et al., 2000a,b,c; Shoffner et al., 1996; Yoon et al., 2002; Yuen et al., 2001; Zhao et al., 2001, 2003
BSA	Burns et al., 1996; Cady et al., 2005; Rodriguez et al., 2003
Deposition of silicon oxide+BSA	Daniel et al., 1998
Chemical silanization	El-Ali et al., 2004; Felbel et al., 2004; Fukuba et al., 2002, 2004; Gulliksen et al., 2004; Hühmer and Landers, 2000; Oda et al., 1998; Park et al., 2003; Shoffner et al., 1996; Wang et al., 2004
Epoxy-polydimethylacrylamide	Ferrance et al., 2003; Giordano et al., 2001a
PP liner	Belgrader et al., 1998a, 1999a,b, 2001; Ibrahim et al., 1998; Northrup et al., 1998; Ross et al., 1998; Woolley et al., 1996
PDMS	Poser et al., 1997
Parylene-C	Anderson et al., 2000; Chartier et al., 2003; Shin et al., 2003
Teflon	Sasaki et al., 1997
Deposition of silicon oxide+Teflon	Krishnan et al., 2004a,b
PVP surface film (Teflon AP film)+chemical silanization	Felbel et al., 2002
Dynamic passivation	
BSA	Dunn et al., 2000; Friedman and Meldrum, 1998; Lagally et al., 2000; Liu et al., 2004; Liu et al., 2002b; Waters et al., 1998a,b; Shen et al., 2005; Swerdlow et al., 1997; Yang et al., 2002; Yu et al., 2000; Zhang et al., 1999; Zhang and Yeung, 1998
BSA+Tween 20	Felbel et al., 2002; Oberd and Christopoulos, 2003
PEG	Giordano et al., 2001a,b; Panaro et al., 2004; Yang et al., 2002
PVP	Giordano et al., 2001a; Yang et al., 2002
Sing-walled carbon nanotubes (SWCNTs)	Cui et al., 2004
T4 gene and 23 protein	Zhang et al., 1999
Combination of static and dynamic surface passivation	
Deposition of silicon oxide+BSA	Erill et al., 2003, 2004; Lee et al., 2003c; Lin et al., 2000a,b; Nagai et al., 2001a,b; Taylor et al., 1997
Deposition of silicon oxide+BSA (static)+PVP	Matsubara et al., 2005
BSA+BSA	Khandurina et al., 2000; Koh et al., 2003; Hong et al., 2001; Hupert et al., 2003
PVP+BSA	Zhou et al., 2004
Chemical silanization+BSA	Trau et al., 2002
Chemical silanization+Tween 20	Sun et al., 2002
Deposition of silicon oxide+chemical silanization+BSA	Lee et al., 2000
Chemical silanization+PVP+Tween 20	Kopp et al., 1998
Chemical silanization+BSA+Tween 20	Obeid et al., 2003; Schneegaβ et al., 2001
Chemical silanization+Acrylamide polymerization+BSA	Lagally et al., 2000, 2001a,b

adsorption effects in PCR microfluidics. In high SVR SiO_2 microstructures, Krishnan et al. have also studied the problem of reaction nonuniformity within the microchannels and found that the observed reaction nonuniformity along the microchannels may be related to the special adsorption of magnesium ions onto the negative-

Table 4
Some silanizing agents used in the PCR microfluidics

Silanizing agent	Reference
SurfaSil™	Shoffner et al., 1996
SigmaCote™	Gulliksen et al., 2004; Shoffner et al., 1996
BTMSTFA	Hühmer and Landers, 2000; Oda et al., 1998
DDMS	El-Ali et al., 2004; Felbel et al., 2002, 2004; Kopp et al., 1998; Obeid et al., 2003; Sun et al., 2002; Wang et al., 2004
Chlorodimethyloctylsilane	Giordano et al., 2001a
CTMS	Felbel et al., 2002, 2004; Lee et al., 2000
HMDS	Felbel et al., 2002, 2004; Schneegaβ et al., 2001
TCPS	Felbel et al., 2002, 2004
Trimethoxymethylsilane	Trau et al., 2002
DMF solution (0.02 M TMS+0.04 M imidazole)	Park et al., 2003
2-Methacryloyloxyethyl phosphorylcholine (MPC) with trimethoxysilil group as silane coupler	Fukuba et al., 2002, 2004

ly charged SiO_2 surface. They also found that the polymerase needs to have a higher concentration in microchannels of higher SVR to achieve comparable PCR amplification efficiency (Krishnan et al., 2004b). The need of more polymerase to effectively compensate the surface adsorption phenomena is also described elsewhere (Cady et al., 2005; Chou et al., 2002; Erill et al., 2004; Taylor et al., 1997; Zhang and Yeung, 1998). The effect of polymerase adsorption in the case of higher SVR can also be counteracted by the addition of BSA because BSA may stick to the SiO_2 surface competitively with DNA polymerase and DNA as a result of the charge characteristic of BSA which is similar to that of DNA under the same conditions (Nagai et al., 2001a). However, the concentration of BSA usually needs to be properly optimized because high concentrations of BSA may cause lower PCR products than the control and can shut down the reaction at very high concentrations (Taylor et al., 1997). In addition, Felbel et al. have also systematically compared surface passivation effects of several commonly used silanizing agents such as dichlorodimethylsilane (DDMS), Chlorotrimethylsilane (CTMS), HMDS and trichloropropylsilane (TCPS) on the PCR amplification efficiency and studied the stability of protective surface films of chemical surface modification by wetting (i.e. contact angle) experiments (Felbel et al., 2002, 2004). To sum up, in spite of the undesirable and even self-contradictory results with respect to the passivation of silanizing agents, the inhibitive effect displayed in the silicon, glass and polymer based PCR microfluidics can be effectively overcome using a wide scope of methods based on understanding the PCR inhibition mechanisms.

8. Integration of PCR with other analytic steps on a single microfluidics

Since the onset of the "μ-TAS" concept in the early 1990s, great interest has been taken in the development of the μ-TAS technique by many research groups. Microfluidic analytical components, such as micropump, microvalve, microheater, microsensor, microflowmeter, microdetector, etc., have been constructed onto silicon/glass/polymer substrate materials using MEMS technology in order to integrate the steps constituting the analytical process (for example sample preparation, chemical reaction and analytical detection) as applied in the biological and chemical fields. The μ-TAS technique offers some obvious advantages in terms of: greatly decreased analytical cost, enhanced sensitivity and speed of detection and accurate accomplishment and automation of almost all processes from sample preparation to the display of reaction results within a short time, which will effectively overcome the errors resulting from some manual operations. Although the "real" "μ-TAS" seems to have been confined to the laboratory stage, partially integrated "μ-TAS" devices have been developed, which has strongly necessitated studies on the feasibility of adapting traditional biochemical analytical instruments to the microscale environment. The PCR microfluidics per se is the main component of the μ-TAS, and presently, partially integrated PCR microfluidics are increasingly developed, including the integration of PCR with CE, DNA microarray hybridization, and/or sample preparation, respectively, on single microfluidic devices, as will be discussed below.

8.1. Integration of PCR with CE/capillary gel electrophoresis (CGE) separation on a single microfluidics

The most common operational component integrated with PCR on a single microfluidic may be CE/CGE, which forms the PCR-CE/CGE integrated microfluidics allowing for the on-line separation and detection of PCR products. The theoretical advantages of performing CE in a miniaturized channel (or capillary) may be evaluated by the single determinants of separation efficiency: $\frac{N}{t}\frac{1}{d^2}$, where N is the so called 'number of theoretical plates', t is the analysis time, d is the capillary diameter, which means that the separation efficiency per unit time is inversely proportional to the square of the capillary diameter, indicating the potential of microfluidic technologies to realize great improvements in performance (Becher and Locascio, 2002). Early in 1996, a hybridized DNA analytical device that carries out PCR amplification in a silicon heater containing a PP tube and electrophoresis analysis in a glass microchip was reported for the first time (Woolley et al., 1996). Rapid thermal cycling ramps (10 °C/s for heating and 2.5 °C/s for cooling) on microfabricated PCR devices and high-speed DNA separations (<120 s) provided by microfabricated CE chips were achieved and the total DNA analysis time was under 20 min. Although this type of integrated PCR-CE microdevice wasn't accomplished on a single chip, its presence is an important step toward complete integration of DNA analyses on a single microdevice, with a complete absence of manual sample transfer. After that, all kinds of PCR-CE/CGE microfluidic devices with a certain degree of functional integration have been developed and improved on monolithic glass (Dunn et al., 2000; Ferrance et al., 2003; Khandurina et al., 2000; Lagally et al., 2000, 2001a,b, 2003, 2004; Lee et al., 2003b; Waters et al., 1998a,b; Zhou et al., 2004),

polymers such as poly(cyclic olefin) (Fan et al., 2003; Koh et al., 2003) and PMMA (Ueda et al., 2000) microchips, hybridized microchips such as silicon/glass (Burns et al., 1998; Mastrangelo et al., 1999; Rodriguez et al., 2003) and PDMS-glass hybrid (Hong et al., 2001) and non-chip capillary (Swerdlow et al., 1997; Zhang et al., 1999; Zhang and Yeung, 1998) by many research groups. In the case of higher integration of PCR-CE/CGE microfluidics, there are several aspects to be noted: First, the microheater and/or temperature microsensor can be coupled to the PCR microchips leading to a decreased thermal mass of the system, as compared with commercial thermocyclers used in PCR-CE microfluidics (Dunn et al., 2000; Waters et al., 1998a,b; Zhou et al., 2004), allowing for the rapid heating rates of over 10 °C/s (Khandurina et al., 2000; Lagally et al., 2000; Rodriguez et al., 2003; Woolley et al., 1996). Furthermore, the microfabricated resistance temperature detector can be placed within the reaction chamber allowing for a more accurate temperature measurement (Lagally et al., 2001a). Second, parallel DNA amplification (Dunn et al., 2000; Lagally et al., 2000, 2001a,b; Waters et al., 1998a; Zhang et al., 1999) or multiplex DNA amplification (Ferrance et al., 2003; Lagally et al., 2004; Waters et al., 1998b; Zhang et al., 1999; Zhou et al., 2004) followed by CE separation and detection can be performed on an integrated microdevice to further save the total DNA analysis time. Third, the sensitivity for PCR amplification of the total systems can be enhanced to the level of detecting a single DNA copy, which will facilitate studies of expression from individual cells and genetic heterogeneity (Lagally et al., 2000, 2001a). Fourth, other functional components have been incorporated into PCR-CE systems to form a more "complete" analytical microfluidic, such as sample loading by valves and hydrophobic vents (Lagally et al., 2000, 2001a,b), cell lysis (Waters et al., 1998a) and on-chip DNA concentration through a porous, semipermeable polysilicate membrane allowing to further reduce the analysis time by decreasing the number of thermal cycles required (Khandurina et al., 2000). In addition, inexpensive polymeric materials may have taken an increasingly important role in fabricating PCR-CE/CGE integrated microfluidics (Ueda et al., 2000; Hong et al., 2001; Koh et al., 2003; Fan et al., 2003), taking advantage of the superiority of polymeric materials in optics, electrical insulation, microchannel surface charge and fabrication cost and so on. In the PCR-CE chip microfluidics, the electrical driving method of the liquid is mostly used for the realization of the driving and positioning of sample and has displayed the great development potential. However, this type of driving technology of sample can't be used to drive non-electrolytes and belongs to the nonhomogeneous fluid control, which lacks the flexibility and potential to be further developed. The microsyringe pump is so expensive that it is undesirable for applications in PCR-CE/CGE microfluidics, and in general it is only used in the laboratory. Versatile and feasible driving technology for microfluidic samples has become an outstanding issue. Among the integrated PCR-CE/CGE microfluidics, Burns's research group has developed PCR-CE functional microfluidics with a high degree of integration having achieved extraordinary progresses in many aspects, including sample loading, drop metering, mixing, thermal reaction, gel loading and electrophoresis onto a monolithic microdevice. No external lenses, heaters, mechanical pumps are necessary for complete sample processing and analysis (Burns et al., 1998; Mastrangelo et al., 1999).

8.2. Integration of PCR with DNA microarray hybridization on a single microfluidics

DNA hybridization reaction is a selective reaction taking place between target DNA/RNA sample and a DNA probe. The probe is complementary in sequence to the fragment of DNA/RNA to be detected, and thus it can selectively hybridize to the correct fragment of DNA/RNA by Watson-Crick base pairing if that fragment is present in the test sample. The principle of DNA microarrays is that the advances in microfabrication technology have made it possible to miniaturize the DNA probe detection method. One obvious advantage of DNA microarrays is to allow thousands of specific DNA/RNA sequences to be detected simultaneously on a very small silicon/glass/polymer wafer.

The fragment of DNA/RNA to be detected is usually first fluorescence-labeled and PCR amplified, and then hybridized with the probe by sample loading or microfluidic technology. To take advantages of the superiority of PCR microfluidics and DNA microarrays, a highly integrated microfluidic device has been developed on an $8 \times 40 \times 70$ mm PC wafer smaller than a credit card, which is capable of extracting and concentrating nucleic acids from milliliter aqueous samples and performing microliter chemical reactions, serial enzymatic reactions, metering, mixing and nucleic acid hybridization (Anderson et al., 2000). When PC is selected as a substrate material, the two ways have been taken into consideration: not only can it provide excellent thermal isolation to meet the requirement of independently controlling different temperature zones on a single chip but it can also be fabricated inexpensively as a low cost disposable

thus avoiding any possibility of cross-talk contamination. Subsequently, the PCR-DNA microarray hybridization integrated microfluidics are also developed on a PC (Liu et al., 2004, 2002b), silicon (Trau et al., 2002), or ceramic (Chou et al., 2003; Sadler et al., 2002, 2003) chip by other research groups. In Liu's PCR-DNA microarray hybridization microfluidics, a planar serpentine PCR channel design is selected due to requirements of functional integration and ease of fluid transfer; the thickness of the PC wafer is optimized to achieve reasonable thermal cycling rates (7.9 °C/s for heating and ~4.6 °C/s for cooling); carrying out DNA hybridization in microfluidic channels facilitates the integration of a DNA hybridization with a sample preparation functional unit; the new coupled bonding protocol was adopted that the hybridization channels are enclosed by two-sided adhesive tape bonding whereas the PCR reaction chamber is sealed through thermal bonding, thus avoiding problems resulting from a single bonding method (Liu et al., 2002b). The PCR-DNA microarray integrated microfluidics developed most recently by Liu et al. has some obvious characteristics as follows (Liu et al., 2004): the device is completely self-contained, eliminating possible sample contamination and simplifying device operation; cavitation microstreaming is implemented to enhance target cell capture from whole blood samples using immunomagnetic beads and accelerate DNA hybridization reaction; sample preparation (including magnetic bead-based cell capture, cell preconcentration and purification, and cell lysis) and electrochemical (EC) detection are also coupled onto the single chip; thermally actuated paraffin-based microvalves are developed to regulate flows leading to ease of integration of continuous multiple analysis processes. Trau et al. have developed a novel micro-DNA amplification and analysis device consisting of multiple PCR microreactors with integrated DNA microarrays on a single silicon chip, where the four PCR microreactors with different samples of 3 μL internal volume allow to perform parallel analysis of DNA sample, and furthermore the oligonucleotide probes are printed on the bottom wall of each microreactor so that no buffer exchange or sample transfer is needed, thus leading to reduction of assay time and of contamination risk (Trau et al., 2002). Notably, the flow-through PCR microdevice has been integrated with a bioelectronic DNA microarray for genotyping on different substrate materials such as ceramics and plastics, which includes PCR amplification, DNA hybridization, and bioelectronic detection (Chou et al., 2003; Sadler et al., 2002, 2003).

The PCR-DNA microarray hybridization integrated biomicrofluidics is the organic coupling of informational-biochip and functional-biochip, which not only can realize such functional operations as sample preparation (Anderson et al., 2000; Liu et al., 2004) and PCR amplification by controlling the fluid of sample within microchannels or microreactors, but also can acquire information about the amount, sequence and even source of thousands of specific DNA/RNA target sequences through DNA microarray hybridization. In practice, this type of PCR-DNA microarray hybridization integrated microfluidics is a great progress in the development of function-biochip.

8.3. Integration of PCR with sample preparation on a single microfluidics

Among the integration of PCR microfluidics with CE or microarray hybridization, the function of sample preparation has also been integrated into these devices in order to circumvent the possible inhibition of amplification or decrease of the success of PCR analytical instruments by chemical interference. Although an on-line sample pre-preparation step is not always necessary for successful PCR amplification, it is usually required when using environmental or otherwise complex samples such as whole blood samples since a variety of contaminants can inhibit PCR. It is noted, however, that only few attempts have been made to run PCR directly from crude blood and cell samples (Zhang et al., 1999), lessening the need to integrate the sample preparation functionality with the PCR microfluidic module. On-line sample preparation is superior in speed and sample consumption over off-line manual sample preparation which is time-consuming, poorly portable and which requires multiple laboratory instruments. However, it still takes a longer time to perform on-line sample pre-preparation on a microfluidics chip, compared to performing the PCR and CE/CGE on a microfluidics chip. And thus the pre-PCR sample preparation may become a "bottleneck" which restricts the further development of "μ-TAS" integrated PCR analysis. In 1998, an integrated dual-purpose microfluidics for performing cell isolation and PCR was developed in glass-silicon hybrid chips, in which a series of 3.5 μm feature-sized coiled/linear weir-type-based silicon microfilters with short flow paths were used to effectively separate white from red blood cells. The weir-type filter chip was then used for the amplification of a 202 bp sequence of exon 6 of the dystrophin gene directly from genomic DNA in filtered white blood cells (Wilding et al., 1998). However, drawbacks of this system were that sample injec-

tion, cell isolation, and PCR amplification were not carried out on a single chip, thus resulting in the increased risk of sample loss or contamination. Moreover, some manual operations were still required for the analysis (Yuen et al., 2001). Subsequently, this research group has designed and constructed a Plexiglas-based microchip module for the integration of blood sample preparation and PCR. They pointed out that the microchip module could provide a convenient means to simplify nucleic acid analyses and that the microchip-scale module proved to be a simple way of assessing microfluidics prior to microchip fabrication (Yuen et al., 2001). In the same year, Baechi et al. reported on high-density microvalve arrays (up to 300 valve s/cm^2) for sample processing in PCR chips, where the microvalves and photodiodes are integrated onto a microchannel network allowing for dynamic parallel processing of nanoparticles with very high throughput. It is for the first time that practical signal sensors were integrated onto microfluidics, which can provide a superiority in the choice of working fluids and particle size that can be handled over the electrophoresis systems. More importantly, the integrated heaters can be used to control the temperature in the fluidic channels which can meet requirements for PCR thermal cycling (Baechi et al., 2001). Most recently, a monolithic DNA purification/real-time silicon-based PCR microfluidics has been proposed, where an automated real-time fluorescence detection platform was used, with integrated microprocessor, multiple-channel syringe pump, valves, thermocycler and miniaturized fluorescence detection modules (Cady et al., 2005).

The several methods described above for pre-PCR sample pre-preparation are all based on 3-D mechanical filtering, which belongs to the non-electrophoretic methods. Another way of filtering is the use of electrical fields. More recently, the dielectrophoresis (DEP) effect has been used as a selective filter for holding cells in a microsystem, so that the PCR inhibitors can be washed out of the system to increase the sensitivity of the PCR amplification (Perch-Nielsen et al., 2003). This method has been successfully used for the preparation of parasitized cell microsamples related to malaria before (Gascoyne et al., 2002). Furthermore, the novel DEP-Field Flow Fractionation (FFF) method for washing and isolating malaria-infected cells was most recently also applied to a novel microfluidic device. This system was regarded as a proof-of-concept system to demonstrate malaria detection in a miniaturized format, where the DEP–FFF cell separation, cell isolation and lysis, and flow-through PCR and detector were integrated onto the same chip for diagnosis of malaria (Gascoyne et al., 2004). The DEP approach to selective filtration has several advantages: (1) there is no need of antibody and hence no changes of biological properties due to the antibody reaction will occur; (2) the effect of the applied alternating electric fields on the cells is nondestructive and the nature of cell growth and splitting won't alter; (3) the easy control of electric field strength, frequency and phase can facilitate the automation of the process; (4) DEP can be coupled with other methods such as FFF (Gascoyne et al., 2004) to acquire an optimized separation effect. However, DEP also has many disadvantages such as the limits imposed on conductivity and pH of the fluid carrying the cells; cell lysis or sample heating that may occur when applying the electric field; adhesion of cells to the electrodes (Perch-Nielsen et al., 2003).

9. Detection of PCR amplification products for PCR microfluidics

As is seen from the development history of PCR microfluidics, another "bottleneck" blocking the realization of a truly integrated DNA analyzer may be a portable detection module for on-line PCR product detection. The most common detection scheme is off-line or on-line CE separation of the PCR product (see above), usually followed by laser-induced fluorescence detection or in some cases by EC detection (Zhao et al., 2002). However, optical detection systems are difficult to miniaturize onto a monolithic microanalytical system. Furthermore, the electrophoretic separation and detection technique can't provide data on the sequence of the PCR product since it mainly serves to separate DNA fragments of different sizes from a mixture of DNA fragments. To acquire information concerning the sequence of a PCR product, the DNA microarray hybridization, which is a sequence-based detection method, has been integrated into PCR microfluidics platforms (see above). However, the use of DNA microarrays has some problems in terms of reproducibility and reliability due to the fact that the DNA probes are fixed on electrodes (Oda et al., 2003). In addition, DE (delayed extraction)–MALDI (matrix-assisted laser desorption/ionization)–TOFMS (time-of-flight mass spectrometer) has also been shown to be a precise, accurate and versatile analysis tool for the detection of DNA fragments from conventional and microfabricated PCR devices (Ross et al., 1998). However, the challenges of PCR product analysis by mass spectrometry still exist al-

though it exhibits a number of desirable features. In this section, several integrated detection techniques for PCR products different from the CE/CGE detection or microarray hybridization detection for PCR product will be introduced in detail, including the fluorescence-based end-point and real-time detection of PCR products (for example TaqMan, SYBR green/ethidium bromide (EtBr) dye-based and molecular beacon technique), EC and electrochemoluminescence (ECL) detection. Fluorescence-based detection is a powerful and important detection method for PCR microfluidics. A fluorescence dye/probe is added at the start of the PCR process. The probe must be compatible with PCR and must not inhibit the PCR reaction. Table 5 lists several different detection methods for PCR products.

9.1. End point vs. real-time detection for PCR product among PCR microfluidics

The end point detection method of PCR products may be defined as a fluorescence measurement after completion of the PCR by which it can be established whether the sample DNA is successfully amplified or not. This approach is simple and doesn't involve real-time observation of PCR amplification kinetics, and it is therefore sometimes used for special fields of PCR microfluidics. For example, the change in fluorescence intensity before and after the PCR in a standard PCR solution can be used to validate the biocompatibility of the PCR reaction chamber (Nagai et al., 2001a). Any PCR reaction inhomogeneity (i.e. location-specific amplification signal intensity) in high SVR SiO_2 microchannels (Krishnan et al., 2004b) or capillary microchannels (Kalinina et al., 1997) can also be illustrated in terms of the fluorescence-based end point detection technique. Furthermore, single initial template molecules can be detected in the presence of the TaqMan end point detection technique (Kalinina et al., 1997; Matsubara et al., 2005; Nagai et al., 2001a). However, it is difficult to obtain reliable results if the initial concentrations of target DNA in the PCR system are calculated only on the basis of the ultimate amplified PCR products in the presence of the end point detection technique.

Real-time detection can be realized by kinetically measuring the fluorescence signals resulting from the interaction between the fluorescent dye/probes and the increasing amount of double-stranded DNAs, where one can observe the yields of the PCR process as soon as the yields of PCR products reach the detection threshold, rather than waiting until all the cycles necessary for the total PCR process have been completed. The number of cycles needed to reach this threshold cycle (C_t) is proportional to the negative logarithm of the initial concentration of target DNA on condition that no cross-reactivity and no inhibitors exist in the PCR reaction system. The different C_t values from different initial concentrations of target DNA are used to calculate the PCR efficiency (Belgrader et al., 2003; Braun et al., 2003; Lin et al., 2002) and/or to verify the performance of the PCR microfluidics (e.g. to determine the detection limit) (Belgrader et al., 1998a, 1999a,b, 2003; Braun et al., 2003; Ibrahim et al., 1998). There are several obvious advantages for the real-time detection of PCR products. First, the initial concentration of the target DNA fragment in the PCR solution before thermal cycling can be easily obtained from the recorded time history of fluorescence intensity. Second, real-time detection can greatly simplify the instrumentation required for the detection of PCR product as a result of the elimination of numerous manual steps necessary for the electrophoretic separation and detection. Third, real-time detection can greatly reduce the assay time because there is no need of cycle completion or of subsequent processing steps such as gel/capillary electrophoresis.

Table 5
Several detection techniques for PCR product in the microfluidics platform

Detection technique	Reference
End point	
TaqMan probe	Kalinina et al., 1997; Krishnan et al., 2004b; Matsubara et al., 2005; Nagai et al., 2001a,b
SYBR green dye	Braun et al., 2003; Braun, 2004; Braun and Libchaber, 2004; Cui et al., 2002; Kalinina et al., 1997; Lee et al., 2003a, 2004a,b; Namasivayam et al., 2004; Zhao et al., 2003
Real-time	
TaqMan probe	Belgrader et al., 1998a, 1999a,b, 2001, 2003; Higgins et al., 2003; Ibrahim et al., 1998; Liu et al., 2002a; Mariella, 2001, 2003; Northrup et al., 1998; Richards et al., 2000; Taylor et al., 1997, 1998
SYBR green I dye	Belgrader et al., 1998b; Braun et al., 2003; Braun, 2004; Braun and Libchaber, 2004; Cady et al., 2005; Higgins et al., 2003; Lee et al., 2004a,b; Lin et al., 2002; Liu et al., 2002a; Namasivayam et al., 2004; Wang and Gong, 2003
EtBr dye	Northrup et al., 1998
Molecular beacon	Gulliksen et al., 2004
EC/ECL	
EC	Lee et al., 2003c; Oda et al., 2003
ECL	Hsueh et al., 1996

9.2. TaqMan vs. SYBR green I dye based detection for PCR product among PCR microfluidics

As is seen from Table 5, the TaqMan probe or SYBR green I dye are the most commonly used probes/dyes for the end point or real-time detection of PCR products in PCR microfluidics. The TaqMan probe, which is completely complementary in sequence to the middle part of the target template to be amplified, is labeled with a fluorescence reporter dye at the 5′ end and a fluorescence quencher dye at the 3′ end, where the fluorescence from the reporter dye is largely quenched by the quencher dye as a result of fluorescence resonance energy transfer (FRET). During the PCR process, the target probe is cleaved between the reporter and the quencher by the 5′-nuclease activity of Taq DNA polymerase. The separation of the reporter and the quencher in solution results in an increase of fluorescence, which is proportional to the amount of PCR product that is generated. Some obvious advantages of the TaqMan probes are that they have high specificity, sensitivity, speed and quantification, as well as ease of use. The use of TaqMan probes designed to hybridize to specific regions of the target DNA sequence can obviously reduce background signal and improve signal-to-noise ratios (SNR) (Higgins et al., 2003). The sensitivity of the TaqMan detection can be improved to allow for detection of single nucleic acid molecules if the volume of the reaction chamber is reduced to nanoliter scale (Kalinina et al., 1997; Matsubara et al., 2005; Nagai et al., 2001a). The specificity of the TaqMan detection can be designed to rapidly detect single-base differences in two different targets (Ibrahim et al., 1998). However, the TaqMan probes can be more expensive than DNA binding dyes, and require prolonged testing and optimization periods before the ideal reaction conditions are determined (Higgins et al., 2003). Furthermore, the TaqMan detection requires longer thermal cycles (Northrup et al., 1998).

Presently, the SYBR green I dye is the most sensitive double-stranded-DNA-binding fluorescent dye and the intensity of the fluorescence signal is significantly enhanced as soon as the SYBR green I dye binds to the double-stranded DNAs. In addition to the high sensitivity, another advantage of using the SYBR green I dye is that unbound SYBR green I dye has only a weak fluorescence and hence exhibits a very low background intensity compared to EtBr staining (Lin et al., 2002). However, high concentrations of matrix chemicals along with SYBR green I dye may inhibit the PCR amplification (Lin et al., 2002). The non-specific fluorescence signals can sometimes result from the binding of SYBR green I dye to primer dimers and other amplification artifacts (Higgins et al., 2003), which may adversely affect the detection results. Recently, it has been found that the interaction of SYBR green I with the PDMS surface of the reaction chamber may lead to a decrease of the initial fluorescence intensity of the reaction, and that the C_t for a positive result must be less than 40 cycles. This is quite common for real-time PCR reactions using SYBR green and is thought to be due to the formation of primer-dimers and non-specific amplification products (Cady et al., 2005).

Here, it is noted that the molecular beacons such as the TaqMan system can specifically detect the target DNA of interest, the sensitivity of which may be enhanced by elaborate design and optimized conditions. However, each molecular beacon probe must be solely and carefully designed before it can be used to detect some given target DNA. Furthermore, its performance strongly depends on purity and working performance. Importantly, the measurement of fluorescence intensity must be carried out at the correct temperature. Based on the above reasons, molecular beacons so far have not been applied for the detection of PCR products in PCR microfluidics although they have been successfully utilized for miniaturized real-time nucleic acid sequence-based amplification (NASBA) in microchips with nanoliter volumes (Gulliksen et al., 2004).

9.3. Optical apparatus applied for fluorescence-based end point or real-time detection among PCR microfluidics

The fluorescence-labeling PCR experiment requires fluorescence signal measurement during PCR amplification for end point or real-time analysis. It is vital to ensure that the emitted fluorescence signal is accurately acquired, detected and analyzed after each PCR cycle. The quality of the fluorescence signal mainly depends on the choice of the excitation light source and on the optical detection apparatus, as well as on the use of the corresponding interference filters. Currently, the optical detection apparatus applied to PCR microfluidics usually consists of a CCD camera, photomultiplier tube (PMT), photodiode, or laser scanning microscope, while the excitation sources have tungsten, mercury, or Xenon lamps, and light emitting diode (LED), as well as external argon lasers, as shown in Table 6.

Table 6
Excitation source and fluorescence detection apparatus used currently for the PCR microfluidics

Excitation source	Fluorescence detection	Reference
Tungsten light source	CCD camera	Taylor et al., 1997, 1998; Wang and Gong, 2003
	CCD camera	Braun et al., 2003; Braun, 2004; Braun and Libchaber, 2004
Mercury lamp	CCD camera	Krishnan et al., 2004b
	CCD camera	Liu et al., 2002a
	CCD camera	Matsubara et al., 2005
Mercury lamp	PMT	Lin et al., 2002
	PMT	Braun et al., 2003; Braun, 2004; Braun and Libchaber, 2004
LED	Miniaturized PMT	Cady et al., 2005; Gulliksen et al., 2004; Higgins et al., 2003
LED	Photodiode	Belgrader et al., 1998a, 1999a,b, 2001, 2003; Ibrahim et al., 1998; Namasivayam et al., 2004; Northrup et al., 1998; Schabmueller et al., 2001
External argon laser	Laser scanning microscope	Kalinina et al., 1997
Xenon bulb	Miniature spectrometer	Lee et al., 2004a,b

9.3.1. Excitation light source

In the field of fluorescence detection for PCR microfluidic devices, most devices utilize bulky bench-top excitation sources such as tungsten, mercury, or xenon lamps, and lasers. The tungsten/mercury/xenon lamps belong to the general class of high intensity light sources and are often used in connection with CCDs (see Table 6). Xenon lamps, which have been recently used as the excitation light source for real-time PCR (Lee et al., 2004a,b), can provide a high intensity emission light with a continuous and homogeneous emission spectrum for fluorescence excitation but they are usually expensive and nondurable. Mercury lamps are often utilized on fluorescence microscopes and offer a useful optical system for fluorescence detection within PCR microfluidics (Krishnan et al., 2004b). However, the light of the mercury lamp isn't as intensive as that of the xenon lamp. In addition, the mercury lamp cannot supply a continuous spectrum for fluorescence detection. In contrast to the above general light sources, an argon laser is not only more stable in wavelength and energy but also larger in power, and thus is very suitable for fluorescence excitation on biochips. However, its drawbacks are their relative bulkiness in volume and high-cost. To our knowledge, it has not yet been used for fluorescence excitation in chip-based PCR microfluidics although it was used for fluorescence excitation within a glass capillary based PCR microfluidics (Kalinina et al., 1997).

The common drawback of the different excitation light sources mentioned above is their relatively large footprint which has severely inhibited the portability of the PCR microfluidics. So far there have been relatively few reports on miniaturized excitation light sources for PCR microfluidics. In the search for a miniature fluorescence excitation source, LEDs have been used as excitation sources for PCR microfluidics by several groups (see Table 6). There are several obvious advantages in using LEDs: low cost, high efficiency, small footprint, simple operation and considerable durability. Due to its low power consumption, LED-based excitation is highly useful for portable analytical devices (Cady et al., 2005). For example, a portable handheld advanced nucleic acid analyzer, which weighs less than 1 kg, has recently been reported, where LEDs have been utilized as an excitation source, combined with miniature PMT detectors (Higgins et al., 2003). Another notebook-sized, compact, real-time PCR instrument, which used LED as excitation source and a photodiode detector, was reported in the early period. This instrument weighed 3.3 kg, measured $26 \times 22 \times 7.5$ cm, and could run continuously on internal batteries for 4 h (Belgrader et al., 2001).

9.3.2. Optical detection device

As can bee seen from Table 6, CCDs have been widely used for fluorescence imaging of the amplification over time during real-time PCR processes (Braun et al., 2003a; Braun, 2004; Braun and Libchaber, 2004; Liu et al., 2002a; Taylor et al., 1997, 1998; Wang and Gong, 2003) or for fluorescence imaging of the products after completion of PCR amplification (Braun et al., 2003; Braun, 2004; Braun and Libchaber, 2004; Krishnan et al., 2004b; Matsubara et al., 2005). Presently, the CCD technology has been increasingly improved and the sensitivity has been greatly enhanced, to the extent that CCD is approaching the PMT in sensitivity (see below). Furthermore, commercial CCDs utilize Peltier elements to cool the CCD chip down to $-40\ ^{\circ}C$ leading to a great reduction in dark current which decreases the noise level and improves the SNR. However, the microscope-based CCD camera systems generally occupy a larger footprint, which has become an obstacle to the development of compact, portable, and real-time PCR microfluidics. In contrast to the CCD system, both PMTs and photodiodes have smaller footprints and thus contribute to compactness and portability of real-time PCR microfluidics, especially when combined with

LEDs for fluorescence excitation. As far as the PMT is concerned, it has three obvious advantages: high gain (up to 10^7), high sensitivity, and linear output in a wide range of optical densities. The setting of gain in the PMT can allow for the detection of adequate photons and the maximum fluorescence signal usually utilizes the maximum dynamic range. In order to obtain fluorescence images of high quality, two factors should be taken into consideration with respect to the choice of the PMT: sensitivity and SNR. One should select a PMT with a high SNR and high sensitivity to the fluorescence wavelength to be detected. A monolithic DNA purification/real-time PCR microfluidics with an LED as excitation source and a miniaturized PMT, has most recently been described, which measures $36 \times 28 \times 15$ cm and has a weight of 4 kg (Cady et al., 2005). A microfabricated PCR microfluidics with a microfabrication-compatible integrated photodiode and optical fibres for in situ detection of fluorescence emission has been investigated, in which optical fibres and a photodiode are used for the delivery and capture of fluorescence light within the PCR chamber (Schabmueller et al., 2001). However, an actual PCR process has not been demonstrated in this microsystem. Most recently, a novel integrated detection device with an on-chip miniaturized photodiode and interference filter was presented, in which a high sensitivity (detection limit of 0.9 ng/μL of DNA), low noise (SNR~100/1) and enhanced quantum efficiencies (>80%) over the entire visible spectrum have been achieved by enhancing photocarrier generation in the active region and increasing the collection efficiency of the generated photocarriers (Namasivayam et al., 2004). This is a significant innovation in exploiting photodiode-based detection schemes for real-time or end-point detection of DNA products in PCR microfluidics. In addition to the photodiode and the PMT, a miniature spectrometer has most recently been proposed for fluorescence detection within a novel real-time PCR microfluidics, with the purpose of overcoming the drawbacks of fluorescence sensing using photodiodes in commercial real-time PCR machines. In this system, the spectrometer enables one to detect a continuous fluorescence spectrum and thus more labeling dyes can be used in the PCR mixture for real-time PCR (Lee et al., 2004a,b).

9.4. EC/ECL detection for PCR product among PCR microfluidics

In the fluorescence-based detection scheme for PCR products mentioned above, the optical systems are necessary for excitation and detection of fluorescence. Although the relatively miniaturized LEDs and PMTs can offer the miniaturization and portability for real-time PCR microfluidics, they may have some difficulty in being further miniaturized and integrated onto a monolithic microfluidic system. Towards the goal of achieving fully integrated miniature PCR microfluidics, there are urgent needs for developing the alternative microfabrication-compatible detection techniques. The EC detection method is a commonly used detection method in microfluidic devices and can offer considerable promise for such PCR microfluidics with inherent features that include high sensitivity, adjustable selectivity, miniaturization and portability, low power and low cost. Importantly, EC detection also has the ability of obtaining sequence information, which is accomplished by utilizing hybridization between a DNA probe immobilized on an electrode transducer surface and the target DNA segment (Lee et al., 2003c). Commonly used EC detection systems include voltammetry, conductivity and potentiometry. With respect to sensitivity, voltammetry is superior to both conductivity and potentiometry. Recently, the microfabricated silicon/glass hybrid PCR microfluidics with functionalities of simultaneous PCR amplification and sequence-specific voltammetry EC detection has been developed. A silicon microchamber with a volume of 8 μL is sealed with a glass wafer and the electrode materials were patterned onto the glass substrate and served as an EC detection platform onto which DNA probes were immobilized (Lee et al., 2003c). The voltammetric EC detection device can be significantly miniaturized and integrated onto the chip, where the detected target DNA sample needn't be labeled and only the presence of an electrochemically active substance is necessary. However, the fabrication of the electrodes for EC detection is usually elaborate and difficult. Even now, the EC detection method is superior to other detection methods in terms of low-consumption, portability and miniaturization.

Another easily miniaturized and integrated detection method is ECL detection, which is accomplished by an electrogenerated, chemiluminescence reaction by using tri-propylamine (TPA) and tris (2,2′-bipyridyl) ruthenium (II) (TBR). The high ECL efficiency of TBR, together with its low oxidation potential, makes it very attractive for microsensor applications. Furthermore, the emitted light is in the visible range, allowing for integration of a silicon-based photodetector (Hsueh et al., 1996). Early in 1996, a microfabricated ECL cell was proposed for the detection and quantification of amplified DNA products, where the cell was fabricated on a silicon substrate bonded onto a glass wafer, containing a thin-film gold cathode and a thin film ITO

anode. The anode is transparent, enabling the detection of the luminescence by an external photodetector (Hsueh et al., 1996). Unfortunately, as yet the ECL detection method hasn't been widely developed for detection of DNA product in the PCR microfluidics.

10. Applications of PCR microfluidics

Seen from the reported literature concerning PCR microfluidics, a significantly wide range of target DNA samples has been amplified within the PCR microfluidics with a variety of architectures, which indicates that the PCR microfluidics have a beautiful future of wide applications although few chip-based PCR microfluidics have been commercialized. Presently, as far as the amplified target DNAs are concerned, PCR microfluidics have had a great potential for biomedical and bioanalytical applications such as microbial detection including *E. coli* (Fukuba et al., 2004; Higgins et al., 2003; Sun et al., 2002; Yang et al., 2002), biological agents (Belgrader et al., 1999a,b, 2001, 2003), and disease diagnosis including infectious diseases (for example, human immunodeficiency virus (HIV) (Anderson et al., 2000; Northrup et al., 1998), human papillomavirus (HPV) (Gulliksen et al., 2004), hepatitis virus (Lin et al., 2000a,b,c, 2002; Zhao et al., 2003), *Salmonella typhimurium* (Erill et al., 2003, 2004), *M. tuberculosis* (West et al., 2002), malaria (Gascoyne et al., 2004), hereditary diseases (for example, hereditary sideroblastic anemia (Yu et al., 2003), hereditary hemochromatosis (Belgrader et al., 2001), cystic fibrosis transmembrance conductance regulator (CFTR) gene (Curcio and Roeraade, 2003)) and other diseases (Liu et al., 2002b; Obeid et al., 2003, Oberd and Christopoulos, 2003; Schneegaβ and Köhler, 2001; Shin et al., 2003; Yang et al., 2002). However, it should be pointed out that the applications of PCR microfluidics are not limited to the fields mentioned here. In principle, the PCR microfluidics can been applied to any field where minute amounts of nucleic acid DNA/RNA need to be amplified and subsequently analyzed.

11. Outlook and conclusions

The PCR microfluidics, in particular microchip-based PCR microfluidics, have been developed and nowadays have become an important domain of application of miniaturization technology. The organic combination of both science and technology in terms of MEMS has contributed to advances in many aspects of PCR microfluidics, including new substrate materials and correspondingly adopted microfabrication technologies, various architectures for PCR microfluidics, heating and temperature sensing, integration of on-chip PCR with other device components into one analytical system, and feasible miniaturization of detection functionality of PCR products and so on, with the potential to greatly improve the performance, sensitivity, miniaturization, portability and cost of biological assay procedures. The driving force behind the increasing development of PCR microfluidics is certainly the commercialization of other microfluidics with their wide applications in biology, chemistry and other disciplines, strongly demanding for the emergence of PCR microfluidics of higher performance.

What are the main trends in the field of PCR microfluidics? First, the new polymer substrates with low cost, disposability, ease of microfabrication and desirable biocompatibility will be further utilized in chip-base PCR microfluidics, at the expense of the silicon, glass and ceramics materials. Second, the flow-through or convection-driven PCR microfluidics will be further developed due to their clear advantages over stationary chamber PCR microfluidics. Especially, with respect to convection-driven PCR microfluidics, challenges still exist in such aspects as implementation of on-chip convective PCR, realization of parallel and multiplex convective PCR, integration of convective PCR with other analytical functionalities in a monolithic format. Third, more attention will be directed to the integration of PCR with sample preparation and/or detection of PCR product (including on-line CE separation, fluorescence and non-fluorescence detection) with the aim of the development of a "real" miniaturized system. In conclusion, we have only seen the beginning of many aspects of PCR microfluidics, and commercialization will prove a tremendous stimulus. We also believe that the "real" PCR microfluidics with many integrated functionalities will certainly become "household" items in the coming future.

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