Eur Biophys J (2010) 39:979–986
DOI 10.1007/s00249-009-0466-5

ORIGINAL PAPER

Effect of materials for micro-electro-mechanical systems on PCR yield

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Received: 2 February 2009 / Revised: 27 April 2009 / Accepted: 29 April 2009 / Published online: 20 May 2009

Abstract In this study we analyzed the surface properties of different silicon-based materials used for micro-electro-mechanical systems (MEMS) production, such as thermally grown silicon oxide, plasma-enhanced chemical vapor deposition (PECVD)-treated silicon oxide, reactive-ion etch (RIE)-treated silicon oxide, and Pyrex. Substrates were characterized by atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) to define the surface chemical and morphological properties, and by fluorescence microscopy to directly assess the absorption of the different polymerase chain reaction (PCR) components. By using microchips fabricated with the same materials we investigated their compatibility with PCR reactions, exploiting the use of different enzymes and reagents or proper surface treatments. We established the best conditions for DNA amplification in silicon/Pyrex microdevices depending on the type of device and fabrication method used and the quality of reagents, rather than on the passivation treatment or increment in standard Taq polymerase concentration.

Keywords PCR · Silicon microchip · Surface interactions · Taq polymerase adhesion

Abbreviations

MEMS	Micro-electro-mechanical systems
PCR	Polymerase chain reaction
PECVD	Plasma-enhanced chemical vapor deposition
RIE	Reactive-ion etch

Introduction

During the last decade we have witnessed a big boost in the miniaturization of conventional medical and biotechnological methods, in particular in the development of micro-analysis systems that include sample preparation, separation, and detection on a small, single chip (Kricka and Wilding 2003; Sun and Kwok 2006). Such systems are capable of saving time and labor and are minimally invasive, being very promising for research as well as forensic analysis (Bienvenue et al. 2006; Liu et al. 2007) and medical applications (Easley et al. 2006; Hashimoto et al. 2007). DNA purification and PCR-mediated amplification are a requirement for most genetic analysis. A crucial role in these biotechnological platforms is played by PCR, which amplifies minute amounts of DNA to measurable quantities with a broad applicability range and a large set of standardized protocols. PCR in microdevices has been extensively studied (Hashimoto et al. 2006; Chang et al. 2006; Zhang et al. 2006), pushing amplification performance to the detection of individual DNA molecules (Dettloff et al.

Proceedings of the XIX Congress of the Italian Society of Pure and Applied Biophysics (SIBPA), Rome, September 2008.

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Table 1 Materials used as planar samples and their main properties

Third and last columns refer to the treatments applied to the wafer

Sample	Description	Etching	Oxidation
TG-SO	Thermally grown silicon oxide	No	Yes
PECVD-SO	Plasma-enhanced chemical vapor deposition-SO	No	Yes
RIE-SO	Reactive-ion-etched silicon oxide	Yes	Yes
Pyrex	Pyrex	No	No

2008). Likewise, great progress has been made in aspects of on-chip micromachining (fabrication, bonding, and sealing), choice of substrate materials, surface chemistry, and reaction vessel architecture (Wang et al. 2006a; Gheorghe et al. 2006; Walsh et al. 2006; Frey et al. 2007). Furthermore, point-of-care testing is emerging as a new frontier in medicine, particularly because it can help in medical decision-making. The salient features of a point-of-care device include the following: short time to results (<15 min), ease of use (including sample preparation and fully automated sample processing), portability (small form factor, ideally handheld), small sample volumes (<30 μL requirement allows use of finger stick rather than venous sample), multifunctionality (capable of combining different types of tests required along the path of care), and low cost (Sista et al. 2008). This approach permits good results to be obtained in terms of microdevice performance, but the basic knowledge about how the microdevice works at a molecular level is often lacking. Indeed, most of the published literature is focused on fabrication of functional devices, while only a few studies are dedicated to characterizing the influence of surface materials on PCR reagents (Christensen et al. 2007). It is well known that inhibition effects on PCR are correlated to the structural materials and to the increased surface-to-volume ratio (Shoffner et al. 1996; Erill et al. 2003), but studies on this topic often utilized materials such as particulates (Wang et al. 2006b; Shoffner et al. 1996), preventing the immediate transfer of the obtained results to microdevices, or leading to contradictory conclusions. Herein, we focus our analysis on the direct determination of the extent of adsorption of key PCR reagents on materials commonly utilized for microdevice fabrication, i.e., silicon oxide and Pyrex. After chemical and morphological characterization of the sample surface properties, fluorescence microscopy measurements were conducted to observe the adsorption of Taq DNA polymerase or nucleic acids on the different materials. Moreover, microdevices built from these materials confirmed that contact with PCR mix has deep effects on the performance of PCR carried out on the same solutions recovered from the microdevice. Duration of contact and surface composition of materials utilized in microchip fabrication were the main factors affecting PCR efficiency, but surprisingly we found that both the source of enzyme and the fabrication processes deeply influenced microdevice performances. Finally, we established the best conditions to directly perform PCR in a silicon/Pyrex microdevice, without any conditioning or passivation of microdevice surfaces or any increment in Taq polymerase concentration.

Materials and methods

Materials

Planar substrates

Planar substrates, cut into 1 × 1 cm^2 pieces, were utilized to assess material effects on PCR components (Table 1). Silicon oxide (SO) films were grown on silicon wafer by thermal oxidation (hereafter TG-SO substrates) or were deposited by means of plasma-assisted deposition (PECVD-SO substrates). To evaluate the effects induced on surfaces by the processes used for channel formation (etching), some samples were treated by applying first a reactive ion etch (RIE) process to the whole sample and then growing a silicon oxide layer (160 nm deep) by thermal oxidation (RIE-SO substrates).

Microdevices

Three differently fabricated microdevices were employed for PCR experiments, i.e., CS, BS, and BR, whose characteristics are described in Table 2. Basically, a TG-SO substrate was bound to a Pyrex cover, while three different methods of microchannel fabrication were tested, utilizing the same channel geometry (500 μm width, 100 μm depth,

Table 2 Characteristics of microchips utilized to incubate PCR mixtures

Sample	Bottom	Cover	Channels	Channels oxidation
CS	TG-SO	Pyrex	On cover, sand blasting	No
BS	TG-SO	Pyrex	On bottom, sand blasting	Yes
BR	RIE-SO	Pyrex	On bottom, RIE process	Yes

The reaction chamber volume was 25 μL to facilitate the recovery of PCR mix after incubation and its use in standard PCR tubes. All microchips had a serpentine-like shape with total channel length of 33 cm

33 cm total length). In particular, the channels were fabricated via sand-blasting in the Pyrex cover (CS microdevices), via sand-blasting in the silicon oxide bottom (BS microdevices) or via RIE process on the silicon oxide bottom (BR microdevices). Channels fabricated on silicon were then coated by a 100- to 160-nm-thick silicon oxide film. All chips were sealed by anodic bonding of the silicon substrate with the Pyrex cover carrying two holes produced by sand-blasting for fluid inlet and outlet. Finally, each wafer was diced into 12 microdevices, each with a serpentine-like shape and 25 μL volume.

Enzymes

Taq DNA polymerases from different suppliers were utilized and their PCR efficiency in microdevices was compared with their performance in standard macro conditions. The utilized enzymes were: AmpliTaq Gold (Applied Biosystems), Taq polymerase (Biodiversity, Italy), NovaTaq (Novagen, Germany), and FastStart Taq (Roche Diagnostics GmbH, Germany). All the enzymes were utilized with the buffer supplied by the manufacturer.

Surface analysis

X-ray photoelectron spectroscopy (XPS) measurements were performed using a Scienta ESCA 200 instrument equipped with a hemispherical analyzer and a monochromatic Al K_α (1,486.6 eV) X-ray source. The emission angle between the analyzer axis and the sample surface was 15° and 90°, corresponding to a sampling depth of approximately 2–3 nm and 10 nm, respectively. For each sample Si 2p, O 1s, and C 1s core lines were recorded at a pass energy of 150 eV, with an energy resolution of 0.4 eV, using a low electron energy flood gun to compensate sample charging. The quantification, reported as relative elemental percentage, is made using the integrated area of the deconvoluted core lines, after Shirley background subtraction (Shirley 1972), and using atomic sensitivity factors, as suggested by the manufacturer. This procedure gives a semiquantitative analysis allowing surface chemistry comparison among different samples. Materials were analyzed without any surface treatments or after cleaning with Piranha solution (80% H_2SO_4:20% H_2O_2).

Atomic force microscopy (AFM) measurements were performed using a Solver PRO instrument (NT-MDT, Russia) using scanning-by-sample piezo tubes with 10 and 50 μm scanning fields and 2 and 4 μm *Z* range. The data were taken in air in semicontact mode, using cantilevers with nominal spring constant of 4.5 N/m and frequency resonance of 150 kHz (NSC35, μMasch). The acquired images were plane-fitted and line-by-line leveled using dedicated software (SPIP version 4.2.6.0, Image Metrology A/S, Denmark); the same software was also used for surface characterization and roughness determination.

Fluorescence analysis

Planar samples, cut into 1 × 1 cm^2 pieces, of the materials described in Table 1 were washed with water and then incubated with 10 units of Taq DNA polymerases from different producers, preactivated when required, in 500 μL appropriate buffer, for 20 or 60 min at room temperature. After three washes with water, samples were incubated with an anti-Taq polymerase antibody (Novagen Taq Antibody) at 4 μg/mL concentration in its buffer for 20 min at room temperature, followed by two washes with Dulbecco's phosphate-buffered saline (DPBS; Sigma). The primary antibody binding efficacy was checked by means of Western blotting experiments and was found to be optimal for all Taq polymerases employed. Samples were incubated with 20 μg/mL fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Anti-Mouse Polyvalent Immunoglobulins (G,A,M)–FITC antibody produced in goat, Sigma) for 20 min at room temperature. After six washes with DPBS, wet samples were mounted on a microscope slide and fluorescence images were taken using a Leica DMLA microscope (Leica Microsystems, Germany), equipped with a mercury lamp and fluorescence filter L5. All samples were observed with a 40× objective, acquiring multiple pictures with a cooled charge-coupled device (CCD) detector (Olympus), each covering an area of about 300 × 225 μm^2.

Biocompatibility test

To assess the compatibility of the serpentine microstructures described above (Section "Materials") with PCR, we chose a volume of 25 μL so that the filling solution could be utilized, after recovery, in standard polyethylene PCR tubes. Microchips were washed prior to use with ultrapure water and filled with the PCR mix containing a hot-start Taq polymerase preactivated at 95°C for 10 min when required. The basic PCR mix contained 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 0.5 μM of each primer HFE H63D for and rev, 5% trealose, 50 ng purified genomic DNA (Wizard® Genomic DNA Purification Kit, Promega Corporation), and 1.25 or 6.25 units of Taq polymerase in 25 μL total volume. The amplified region is part of the hemochromatosis gene (*HFE*), and specifically is related to the H63D mutation involved in the disease (Jouanolle et al. 1997; Beutler et al. 1996). The primer sequences are: ACATGGTTAAGGCCTGTTGC (HFE H63D_F) and GCCACATCTGGCTTGAAATT (HFE

H63D_R). The composition of the mix was modified as needed. In particular, for passivation experiments, microchips were treated with 2 mg/mL bovine serum albumin (BSA; Sigma) for 20 min before incubation with the mix supplemented with 0.1 mg/mL BSA. After the incubation time at room temperature (15 or 60 min), the PCR mix was transferred into a PCR tube and the reaction was performed in standard conditions by using an Applied Biosystems 9700 thermal cycler. The protocol used included the following steps: 1 cycle at 95°C for 10 min, 35 cycles at 95°C for 20 s, 62°C for 20 s, and 72°C for 10 s, and 1 cycle at 72°C for 1 min. At the end of PCR, 9 μL product was mixed with 1 μL loading buffer (10× BlueJuice gel loading buffer, Invitrogen) and loaded onto a 2% agarose gel containing ethidium bromide to check for the presence of the corrected band (208 bp). The intensity of the band was quantified in a Gel-Logic 1500 detection system (Kodak, Eastman Kodak Company) using Kodak Molecular Imaging software (version 4.0) after normalization with respect to the positive control present in each gel to have a comparison of the PCR efficiency in different conditions.

Results and discussion

Surface analysis

XPS analysis of the pure materials (Table 1) indicated the presence of all elements expected, with carbon content that increased from the relatively low values found on PECVD-SO (5.8%) to reach values of over 20% for Pyrex. Table 3 presents a summary of the surface elemental compositions. From a chemical point of view, we can conclude that the main characteristic that differentiates these samples is carbon content, while silicon and oxygen content were similar in all samples, for both 15° and 90° (not shown) measurements. The carbon content halved from a sampling depth of 2 nm (15° emission angle) to 10 nm (90° emission angle) in all samples except for PECVD-SO, which had the lowest carbon content also at 15°. Taken together these results suggest that carbon is present in the structure of materials and not only at the surfaces.

Table 3 Elemental composition (atomic percentage) determined by XPS for the substrates used in microdevices production, obtained from the integrated area of the deconvoluted core lines at 15°

Sample	Si 2p (%)	O 1s (%)	C 1s (%)
TG-SO	40.9	50.6	9.3
PECVD-SO	39.5	54.7	5.8
RIE-SO	36.1	47.8	16.1
Pyrex	33.8	42.3	23.9

The standard error does not exceed 1–2% of the reported value

Table 4 Roughness of planar samples determined with AFM measurements using a scan area of 1 μm^2

Sample	S_a^1 (nm)
TG-SO	0.10 ± 0.01
PECVD-SO	4.1 ± 0.5
Pyrex	1.2 ± 0.3
RIE-SO	9.3 ± 3.5

Morphological analyses by AFM on areas of 1 μm^2 of planar samples demonstrated that the silicon oxide materials studied have different roughness, in the order: TG-SO < PECVD-SO < RIE-SO, with TG-SO being the flattest substrate (Table 4). Pyrex falls in between, with roughness higher than TG-SO but negligible when compared with PECVD-SO or RIE-SO. Roughness analyses along the channels of the two kinds of sand-blasted chips (CS and BS, Table 2 and Section "Materials"), gave very similar results, i.e., roughness of the order of 1–3 nm at short scale (250 nm), while at longer distances (tens of microns) the features (typical lateral dimension 2–4 μm) produced by the sand-blasting process cause a huge increase of roughness, that reaches values of the order of a few microns. These data permit to conclude that the two sand-blasted surfaces are very similar from a morphological point of view.

Taq polymerase and DNA adsorption

A first biological characterization of these samples was performed by assessing the adsorption of key components of the PCR mixture, such as nucleic acids (primers, template) and Taq polymerase, on their surfaces. Fluorescence microscopy revealed no or negligible adsorption of nucleic acids on all the examined materials (data not shown), while we found direct evidence that Taq enzymes were adsorbed on these materials. These results are in agreement with previous literature that claimed Taq surface adsorption as the main reason for PCR inhibition in microdevices (Erill et al. 2003; Christensen et al. 2007). Published results were obtained indirectly, i.e., through the evaluation of the efficiency of PCR. Our immunofluorescence experiments indicate that Taq DNA polymerase is adsorbed on all the studied surfaces, but to an extent that is dependent on both sample type and the source of the enzyme. In particular, PECVD-SO was found to be the material most favorable to the adsorption of NovaTaq (Figs. 1, 2), while Pyrex and specially RIE-SO were less prone to aspecifically adsorb this enzyme. In principle, these materials could be considered to be very similar, but as demonstrated by Fig. 1a,b and by the images of Fig. 2, their ability to adsorb the enzyme involved in PCR differs greatly. Surface chemical analysis by XPS, however, revealed that, probably as

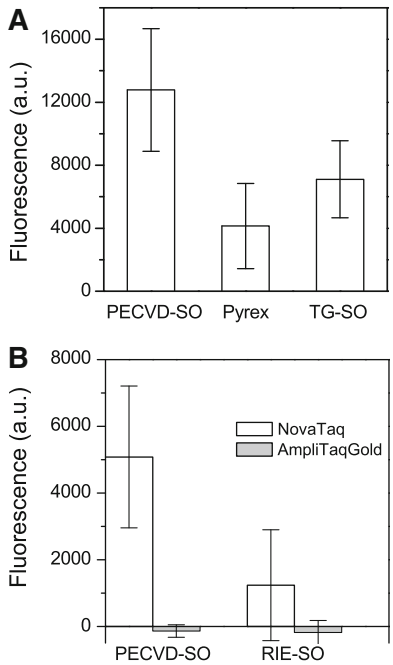

Fig. 1 Taq DNA polymerase adsorption on the materials studied. **a** 20-min incubation of NovaTaq on planar samples. **b** 60-min incubation of NovaTaq (*white bars*) or AmpliTaqGold (*gray bars*) on two planar substrates. Immunofluorescence was recorded by an optical microscope as described in "Materials and methods". Data were corrected for the possible aspecific adhesion of the secondary antibody

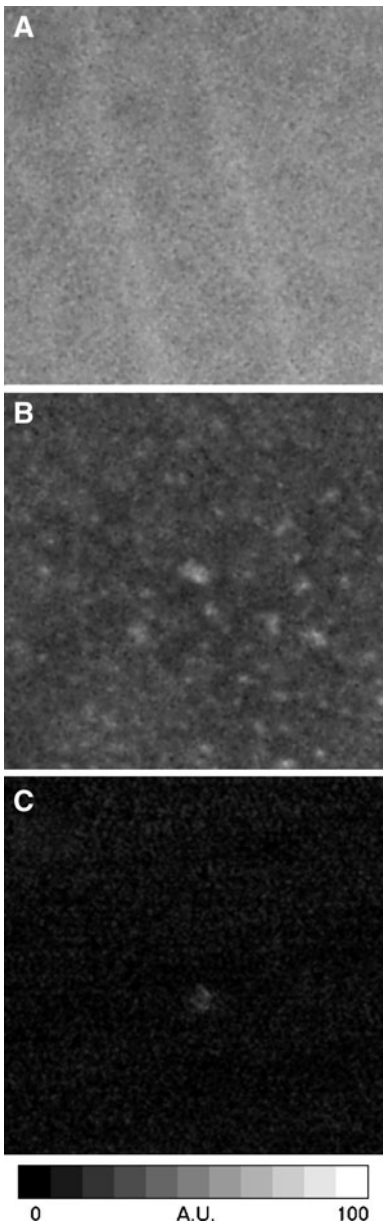

Fig. 2 Examples of surface distribution of Taq DNA polymerase on planar substrates, observed by fluorescence microscopy after immunostaining: **a** NovaTaq on PECVD-SO, **b** NovaTaq on Pyrex, and **c** AmpliTaqGold on RIE-SO. Images show a 30 × 30 μm^2 representative area of the acquired images. No correction for aspecific secondary antibody adhesion was applied to these images. The same *gray scale* is utilized for the images (fluorescence intensity, arbitrary units)

consequence of the different sample treatment methodologies, the main difference among these materials is their carbon content. While in principle carbon should be present in negligible amounts on these materials, XPS data show that a substantial fraction of the surface is instead constituted of carbon for RIE-SO and Pyrex samples. Cleaning of these surfaces with Piranha solution was not able to decrease the carbon content substantially (data not shown), leading to the conclusion that this carbon layer is relatively tightly bound to the surface. This finding is reinforced by the fact that carbon is present in a nonnegligible amount also 10 nm inside the sample, as evidenced by XPS data at 90°. We can assume then, for the NovaTaq enzyme, that a different carbon content on the surface can strongly influence the enzyme adsorption on the substrate. Moreover, different Taq polymerases are adsorbed to very different extents on the same material, as shown by the different amounts of adsorption of NovaTaq and AmpliTaq Gold (especially on PECVD-SO) in Fig. 1b. These data correlate well with the PCR performances of different commercial DNA polymerases, when the PCR mix was previously brought in contact with microdevices, as discussed below (Section "PCR compatibility"). We also found that the surface roughness of the various materials studied was not the main factor involved in Taq polymerase adsorption, at least when examined at submicron lateral scale. In fact, AFM measurements (Table 4) showed that RIE-SO has a more irregular surface than PECVD-SO, but examining the same materials at short-range scale, their roughness resulted very similar, around 1–2 nm. So, at a lateral scale that matches protein dimension, these two materials result similar, while the same enzyme (i.e., NovaTaq; Fig. 1) is adsorbed much more by PECVD-SO than by RIE-SO,

indicating that other factors such as chemical differences can play a major role in Taq adsorption.

The importance of the surface chemical composition on Taq adsorption is confirmed through similar immunofluorescence experiments on materials whose surface is modified through successive steps. Firstly, the surface charge is modified by the introduction of amine groups by means of aminosilanes, as described by Fiorilli et al. (2008). On silanized Pyrex and TG-SO an increase of the adsorbed enzyme is obtained, reaching levels typical of untreated PECVD-SO, while on this last material the adhesion remained substantially unchanged, even after silanization (data not shown). Adding a further modification step to the treatment of materials, i.e., covalently binding an amine-reactive polyethylene glycol (methoxypolyethylene glycol 5000 propionic acid *N*-succinimidyl ester; Fluka), we obtained a marked reduction of NovaTaq adsorption (with respect to the untreated materials), especially on PEG-PECVD-SO and PEG-TG-SO surfaces, as shown by immunofluorescence analysis (data not shown). In fact, it is well known (Erill et al. 2003; Wang et al. 2006b; Zhang et al. 2006) that passivation of the surfaces can improve or even completely restore PCR efficiency. So we obtained modulation of Taq adsorption by the introduction of specific chemical groups on the examined surfaces. Moreover, fluorescence measurements were also performed on DNA to asses if other important components of the PCR mix were captured by material surfaces. The incubation of genomic DNA used as template in PCR or oligonucleotides used as primers resulted in negligible adhesion of DNA on substrates (data not shown), confirming directly that enzyme adsorption is the most important phenomenon involved in PCR inhibition.

PCR compatibility

The next step was to perform biological tests based on the incubation of the complete PCR mix in the serpentine microstructures made of the same materials tested for surface properties and ability to adsorb PCR reagents. Figure 3 compares the PCR yield of different solutions brought into contact with our microdevices under various conditions. The use of differently fabricated microdevices, even though based on similar materials (Table 2), greatly influences the overall PCR response, as shown in Fig. 3. In fact, as well as parameters whose effect is expected, such as the adverse influence of increased incubation time or the positive effects following surface passivation treatments and increasing enzyme concentration (Christensen et al. 2007; Zhang et al. 2006), other parameters were found to play an important role. Such parameters are of primary importance and need to be carefully considered when interpreting the effect of materials on PCR performed in different laboratories; for example, the amplification yield depends on the activation state of Taq polymerase when the enzyme is brought into contact with the microsystem, being strongly reduced when the hot-start enzyme is preactivated, i.e., when its condition during PCR is simulated (Fig. 3a). Interestingly, by increasing the incubation time to 60 min the PCR response is dramatically reduced to zero (Fig. 3b), suggesting that the kinetics of reagents sequestration by microchip surfaces is relatively slow and is incomplete

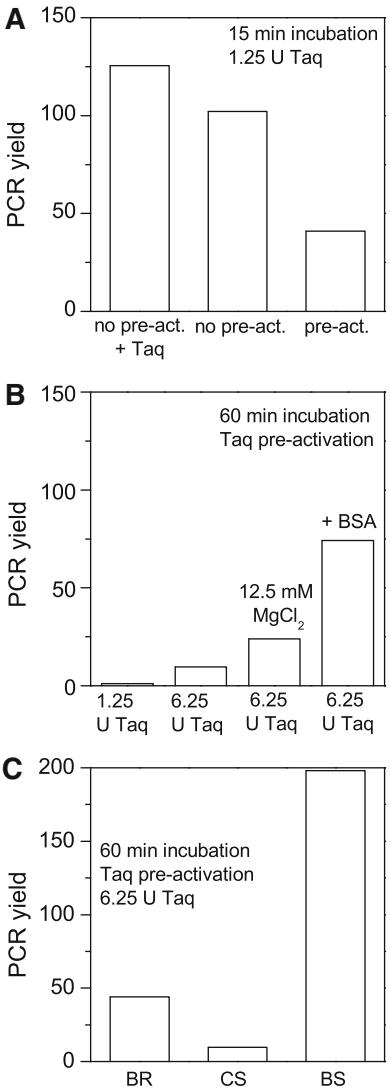

Fig. 3 PCR yield as a function of Taq polymerase preactivation or addition of 1.25 U fresh enzyme (**a**), increment of reagents and passivation (**b**), and microchip type (**c**). PCR mix was incubated in the microchip and then transferred to a PCR polyethylene tube for standard amplification, followed by gel electrophoresis. Yield is calculated from bands intensity of agarose gel, referred to 100% for the positive control, i.e., a standard reaction (1.25 U Taq) performed in a PCR tube kept at room temperature for the same time as the mix in the microchip. All experiments were performed by using Taq polymerase from Biodiversity. The microchips used in **a** and **b** were CS

after 15 min. When Taq polymerase and/or $MgCl_2$ concentration are increased, the PCR yield improves but is not completely restored. Also, a passivation step by BSA (or PEG, data not shown) enhances PCR performances, even if this treatment is insufficient to permit complete recovery of PCR yield. Finally, differently fabricated microsystems are found to affect PCR to different extent (Fig. 3c). In fact, microchips made utilizing the same materials (TG-SO and Pyrex) show very different results: a PCR yield $\sim$10% is obtained for CS microdevices while a much better value ($\sim$200%) is typical when employing BS chips. On both kind of microdevices the channels are obtained utilizing a sand-blasting process, the main difference lying in the material that hosts the channels (Pyrex in CS chips versus silicon in BS chips; Table 2). As reported in Section "Surface analysis" only small differences are found in terms of roughness at both large and small scales. Furthermore, in BR chips, the channel surface is oxidized before chip bonding, while CS chips are not further treated after sand-blasting. In fact, XPS analyses demonstrated presence of contaminants in planar samples treated in the same way as CS chips, such as zinc, calcium, and sodium, which are attributed to the sand-blasted treatment. These elements strongly inhibit the amplification reaction, possibly because they can compete with magnesium ions (Rådström et al. 2004; Wilson 1997). It is also reported (Shoffner et al. 1996; Felbel et al. 2004) that silicon oxidation could act as a passivation step helping the PCR process, and our data seems to confirm this hypothesis. However, more steps involved in the fabrication process need to be taken into account. In fact, PCR yield related to BR chips, bearing the same oxidation treatment, is reduced compared with BS microdevices, even though a significant increment is produced in comparison with CS chips (Fig. 3c). We can conclude that it is extremely important to control every step carefully, from device fabrication to accurate choice of reagents to obtain the best PCR performances.

In addition, the selection of PCR reagents is important because, for example, different Taq DNA polymerases are adsorbed to a different extent on the material surface, as discussed above. In Fig. 4 three commercial Taq polymerases are compared. As shown, all Taqs had similar performances in conventional PCR amplification systems using polyethylene tubes (25 µL reaction volume; lanes 2, 5, and 8), but their efficiency varies when PCR is directly performed in 25 µL BR microchips (lanes 1, 4, and 7, Fig. 4). In particular, AmpliTaqGold and FastStart Taq have similar efficiency (comparison between lanes 4 and 7), while NovaTaq gives a lower yield (lane 1). A correlation between PCR performances and Taq polymerase absorption is clearly shown. In fact, NovaTaq is adsorbed by RIE-SO and Pyrex more than other commercial

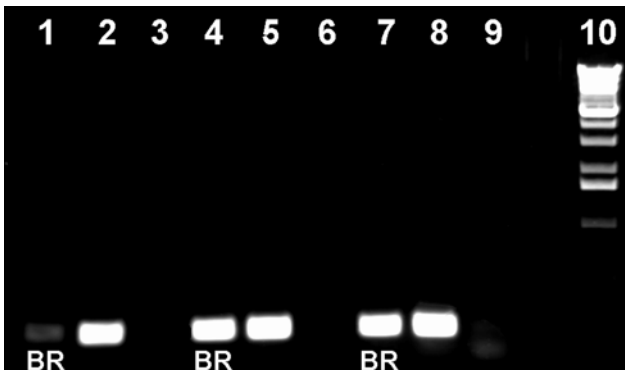

Fig. 4 Agarose gel electrophoresis of PCR products (208 bp) obtained in microdevices or in standard tubes by using different Taq DNA polymerases. *Lane 1* microdevice BR + NovaTaq, *lanes 2 and 3* positive and negative control in tubes + NovaTaq, *lane 4* microdevice BR + AmpliTaqGold, *lanes 5 and 6* positive and negative control in tubes + AmpliTaqGold, *lane 7* microdevice BR + FastStart Taq, *lanes 8 and 9* positive and negative control in tubes + FastStart Taq, *lane 10* 1-kb marker Invitrogen

enzymes (Figs. 1, 2) and gives worse yield in terms of amplified product. Moreover, when CS microchips were used to perform the amplification directly, no PCR products were found, as predicted from the low PCR yield resulting from experiments with PCR mix incubation (Fig. 3c).

Conclusions

Modern microfluidic platforms integrate DNA purification, target amplification by PCR, and DNA detection in a single device. Combination of these processes into a single tool minimizes sample loss and contamination problem while reducing analysis time and costs. In this context, miniaturization of the PCR is an important part of the research and development of MEMS, because PCR is a technique central to most biotechnological applications, such as pathogen surveillance in food, diagnosis of inherited diseases or infections, and even forensic science. However, DNA amplification is a crucial challenge for designing a performing PCR device due to the negative interaction between PCR reagents and the surrounding environment, i.e., the materials encapsulating the PCR mix. The PCR inhibition effect is particularly important because of the increased surface-to-volume ratio in microsystems, which enhances the interaction between the surfaces and reagents in the PCR mixture. In this study we have demonstrated directly that the PCR inhibition effect often observed in microsystems is due to Taq polymerase adsorption on surfaces rather than DNA adsorption phenomena. This process varies with the material, the enzyme, and the surface chemistry. It can be greatly influenced by the microchip fabrication methodology used. Enzyme concentration,

presence of cofactors such as Mg^{2+} or adjuvating agents such as BSA could help restore, at least partially, PCR performance, but correct choice of the enzyme and suitable fabrication processes also have to be considered. All these contributing factors have in fact to be taken into account when a performing microdevice is designed and realized. Here we have shown that functional silicon and Pyrex microchips for PCR can be realized without the necessity for passivation treatment or increment in standard Taq polymerase concentration, when appropriated reagents are utilized in combination with a proper fabrication process. In particular, we have demonstrated that the use of hot-start enzymes of good quality and an oxidizing step after microchannels production are crucial requirements.

The application of these results to fabrication of new integrated chips (performing the detection of single-gene mutations starting from blood DNA purification) is currently under realization (paper in preparation).

Acknowledgments This work was accomplished in the framework of Laboratory of Miniaturized Electrobiochemical Technologies for Analysis and Research (LATEMAR), Center of Excellence funded by Italian Ministry for Education, University and Research (MIUR) grants-FIRB 2003-2004-for public/private structures involved in research fields characterized by strategic value. We also are grateful to Dr. O. Rossotto (Olivetti I-Jet S.p.A., Italy) and to Dr. M. Cocuzza (Physics Department at Politecnico of Turin and Chi-Lab, Materials and Microsystems Laboratory – LATEMAR Unit, Italy) for the provision of planar samples and microdevices, and to Dr. I. Vallini (Biodiversity S.p.A., Italy) for supplying the Taq polymerase from Biodiversity.

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