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Biochemical analysis and optimization of inhibition and adsorption phenomena in glass-silicon PCR-chips

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Abstract

The use of silicon-glass chips for PCR analysis has been widely reported in the last decade, but there have been few systematic efforts to pin down the biochemical problems such systems bring forth. Here we report a systematic analysis of material-related inhibition and adsorption phenomena in silicon-glass PCR-chips. The results suggest that the previously reported inhibition of PCR by silicon-related materials stems mainly from the adsorption of Taq polymerase at chip walls due to increased surface-to-volume ratios, and not from a straight chemical action of silicon-related materials on the PCR-mix. In contrast to Taq polymerase, DNA is not adsorbed in noticeable amounts. The net effect of polymerase adsorption can be counteracted by the addition of a titrated amount of competing protein (BSA) and the ensuing reactions can be kinetically optimized in chips to yield effective amplifications in the whereabouts of 20 minutes.

Keywords: DNA-chips, PCR, adsorption, microsystems, inhibition, micro-TAS

1. Introduction

In the emergent field of DNA-chips, research is now focusing on the integration of all the analytical steps that are a necessary prerequisite for a fully independent DNA μ-TAS. Due to the special nature of the samples these systems must deal with (nucleic acids from biological origin), the need for integrating an amplification technique in these systems is self-evident, and many assays have been carried out to accommodate diverse amplification techniques to chip environments [1-4]. Even though some of these techniques, like rolling-circle (RCA) or strand-displacement amplification (SDA), present some advantages over the classical polymerase chain reaction (PCR) setup, they do not posses the broad applicability range nor the large set of standardized protocols of PCR. Therefore, and albeit some of its astringent conditions (e.g. temperature cycles that nearly reach the water boiling point) complicate chip designs, PCR is still the main target for the integration of amplification techniques in DNA μ-TAS.

Conversely, conducting PCR in a microsystem poses by itself many advantages. The main ones were first described in work with hot-air driven capillary PCR systems [5], and were demonstrated in chips some years later [6]. Conducting PCR in a microsystem provides low reagent and sample volumes, coupled with very effective heat dissipation and transfer, and leading to faster and more accurate (i.e. specific) reactions. The basic bauplan for a PCR-chip (s.c. a reservoir with access holes and some sort of sealing cover) can be implemented with almost any material that can be precisely micro-machined at the micro-scale, including glass, plastics and silicon. Nevertheless, silicon offers many substantial advantages over other technologies, since it permits the integration of thermal cycling devices or detection and control circuitry, and it is also a better heat-dissipating substrate [8]. Therefore, silicon has been the main substrate for PCR-chip development since the onset of the field, in a typical scheme that involves a micro-machined silicon chamber capped with a glass wafer, either glued [6] or anodically bonded [7-9] to the silicon substrate. However, the use of silicon poses also some drawbacks, especially in terms of PCR-friendliness issues.

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Although inhibition by silicon-related materials has already been addressed [6, 9] and the possible problems of reagent adsorption at chip walls due to an increased surface-to-volume ratio have also been hinted at in the literature [7, 8, 10], some results (especially those regarding the use of silanizing agents) are contradictory and the underlying mechanisms that govern the inhibition of PCR by silicon-related materials are still not clearly understood. Therefore, we esteem that a broad scope methodological survey of these issues, as the one described here, is of critical interest for the further development of this field.

2. Experimental

2.1. PCR-chips

PCR-chips were fabricated on 5 inches, 300 μm-thick, double-side polished <100> silicon wafers. The technological process began with the formation of a polysilicon resistor array on the back side of the chip (see Fig. 1. a-c). First, an intermediate 500 Å dry thermal oxide was grown on both sides of the wafer to mitigate the mechanical stress typical of silicon-silicon nitride interfaces, and a 1800 Å silicon nitride layer was deposited on top of it and at both sides of the wafer by low-pressure chemical vapor deposition (LPCVD). Thereafter, a non-doped 4800 Å polysilicon layer was deposited also by LPCVD on both sides of the wafer and doped with phosphorous impurities to attain an approximate 15.8 Ω/□ resistance. The resulting parasitic phospho-silicate glass (PSG) oxide layer was removed by immersion in a 5% (v/v) hydrofluoric acid (HF) solution, leading to the final tri-layer scheme depicted in Fig. 1-a. Using reactive ion etching (RIE), the front-side polysilicon layer was completely removed (see Fig. 1-b), and the back-side polysilicon layer was patterned to convey the parallel resistor grid design shown in Fig.2. Next, the whole back-side resistor structure was passivated by deposition of a 5500 Å atmospheric pressure vapor deposition (APVD) silicon oxide layer (see Fig. 1-c), and the process switched to the front side to create the PCR-chamber (Fig. 1. d-e). Using standard photolithography processes, rhomboidal (10x5 mm^2) PCR-chamber motifs (see Fig. 2) were transferred to the front side of the wafer and RIE-etched onto the exposed nitride and dry oxide layers. The dual nitride-oxide layer was then used as a window for the deep wet chemical etching (~175 μm) of the exposed front-side bulk silicon (see Fig. 1.d). Deep silicon etching was conducted by immersing the whole wafer in a 25% (v/v) tetra-methyl ammonium hydroxide (TMAH) solution at 80 ºC [11], and protecting the back side of the wafer with a dedicated methacrilate device that provided a continuous positive N_2 flow to prevent TMAH entrance. The completed PCR chamber was passivated with a homogeneous 500 Å dry thermal oxide layer that was grown on both sides of the wafer (see Fig. 1.e). The dry oxide layer provided both a PCR-friendly surface in the PCR chamber and a uniform surface for the ulterior anodic bonding of a glass cover wafer. From then on, process switched again to the back side (Fig. 1. f-k). Access holes for electrical contact with the polysilicon resistor grid were photolitographically defined and RIE-etched on the exposed oxide and nitride layers (see Fig. 1.f). A pre-metallization 5% (v/v) HF step was conducted to remove any parasitic oxide layers from the polysilicon surface and then a 1μm AlSiCu layer was deposited to fill the access holes and provide electrical contact (see Fig. 1.g). After a photolithographic process using a non-refracting photo-resist, the aluminum layer was patterned by wet chemical etching (see Fig. 1.h) in order to provide the necessary contact lanes and pads seen in Fig. 2. Thereafter, the whole electrical assembly on the back side (polysilicon resistors and aluminum contacts) was consistently passivated with a dual oxide-nitride (4000 – 11000 Å, respectively) layer to withstand deep silicon etching (see Fig.1.i). After passivation, patterns for Ø1 mm PCR-chamber access holes were photolitographically defined on the back side, and the multiple oxide-nitride passivation layer was etched in a continuous RIE-etch process (see Fig.1.j). As in the case of the front-side PCR-chamber, the exposed bulk silicon on the back side was then deep etched (~125 μm) by a single-side TMAH attack, until access holes to the PCR chamber were finally opened (see Fig.1.k). After inspection, the remaining (if any) oxide layer on top of the aluminum contact pads was removed by a short RIE etch, and the wafer was

thoroughly rinsed with 18.2 MΩ deionized water and oven-dried at 250 ºC to prepare it for the anodic bonding process. Finally, and without any further treatment, the completed device was anodically bonded on its front side to a 0.5 mm-thick SD-2 (*Hoya*) or 7740 (*Pyrex*) glass wafer at atmospheric pressure, 400 ºC and 1 kV [12] (see Fig. 1-l).

2.2. Temperature control system

PCR-chip temperature was software-controlled during operation through a 12-bit analog I/O PC-1200 data acquisition board (*National Instruments*) working at 12000 samples per second. Reference temperature readings for backup control and chip calibration were obtained from a ±1.0 ºC K-type thermocouple (*Lab Facility*), through specific cold-junction compensation circuitry (room temperature reference), and from a platinum ±0.01 ºC RTD Pt100 surface probe (*Kosmon*), by means of a custom-devised 4-wire sensing circuitry (control & calibration reference). When used for backup control, the Pt100 resistor was clamped to the chip with plastic pincers and an intermediate layer of 340 Heat-sink compound (*Dow Corning*). Calibration of the PCR-chip polysilicon resistors was accomplished by monitoring their immersion in heated mineral oil with reference sensors and using a 3rd order polynomial for interpolation of temperature from the resistance measured using a custom 4-wire sensing circuitry. Control software was developed in Labview (*National Instruments*) and consisted mainly in a fully adjustable dual PD-PID control algorithm to provide fast transients (10-15 ºC/s) and avoid integral-term overshots. The software-driven PC-1200 analog output directed a voltage-modulation driver powered by a ±12 V –10 A custom-assembled FE-17 supply (*Cebek*) and governed by a LM12CLK 80 W power op-amp (*National Semiconductor*) that stimulated directly the PCR-chip polysilicon actuator resistor grid.

2.3. Sample insertion & extraction and chip reuse

Sample insertion and extraction were typically carried out using custom designed methacrilate devices that matched tightly the shape of a pipette tip and clamped the chip access holes using toric joints. With these devices, to be described in detail elsewhere [13], sample insertion yields were typically of 100%, whilst extraction yields were brought up to the 90-95% range. Similar methacrilate devices provided airtight $Ø_i$4 mm silicone tube connections and were used for chip cleansing in order to reuse the chips. Chip cleansing was carried out using an $Ø_i$8 mm tube peristaltic pump (Watson & Marlow) that was connected to the aforementioned methacrilate devices and provided continuous rinsing with the following sequence of reagents [14]: 37% hydrochloric acid ($HCl:H_2O_2:H_2O$ – [1:1:5]), 30% ammonia ($NH_3:H_2O_2:H_2O$ – [1:1:5]), 96% ethanol, 18.2 MΩ deionized water; all reagents (*Pancreac*) were of analytical grade (ACS-ISO). After cleansing, the chips were dried with an N_2 flow and autoclaved at 2.2 Bar - 135 ºC for 15 min in a 118-LRV autoclave (*Matachana*), whilst the methacrilate devices were sterilized by 20 min UV irradiation in a FLV60 laminar airflow chamber (*EuroAire*).

2.4. PCR protocols

All control PCR amplifications were carried out on a CETUS DNA Thermal-cycler (*Perkin Elmer*). The main PCR protocol used as template a ~200 bp region of *Salmonella typhimurium* (IS200), defined by the primers 5'-ATgggggACgAAAAgAgCTTAgC-3' (sense) and 5'-CTCCAgAAgCATgTgAATATg-3' (anti-sense, both from *Tib-MolBiol*) and cloned into a PGEM®-T vector (*Promega*) that was electro-transformed into *Escherichia coli* DH5 competent cells. Prior to PCR, plasmid DNA extraction was carried out following the alkaline lysis method [15]. The PCR mix (for 25 µl) was as follows: 17 µl milliQ H_2O, 2.5 µl 10x $MgCl_2$ Taq buffer (*Roche*), 2.5 µl 10 nM dNTPs, 1.25 µl 10 µM sense primer, 1.25 µl 10 µM anti-sense primer and 0.2 µl 3.5 U/µl Expand™ High Fidelity System Taq polymerase (*Boehringer Mannheim Corp.*), while the PCR time protocol was: 95 ºC – 2 min, (95 ºC – 1 min, 61 ºC – 1 min, 72 ºC –

1 min) per 30 cycles, 72 ºC – 7 min and storage cooling at 4 ºC. Typically, 1 μl of 40-70 ng/μl template DNA was added to the PCR-mix. Chip PCR amplifications were conducted using essentially the same PCR mix as control amplifications, but with a doubled Taq concentration (0.4 μl of 3.5 U/μl per 25 μl). All PCR-chip mixes contained also a titrated amount (2.5 μg/μl, see below) of bovine serum albumin (BSA) adjuvant. Kinetic parameters were also adapted in chips for fast operation, yielding the following protocol: 95.5 ºC – 2 min, (95.5 ºC – 1 s, 61 ºC – 10 s, 72 ºC – 19 s) per 40-45 cycles and 72 ºC – 1 min.

2.5. Agarose gels and analysis of results

Agarose (3% w/v) gels were used to inspect PCR products. The gels were loaded with a loading solution containing 0.25% Bromophenol Blue (*Pancreac*) and 0.25% Xylene Cyanol (*Clonetech*) for visual inspection, and were run at 110 V – 1.0 A on electrophoresis casting-trays (*BioRad MiniSub® Cell GT*) in a 10x Tris-EDTA 0.5M / pH 8 buffer containing 0.5 μg/ml of ethidium bromide for DNA detection. Gel inspection was carried out under a UVP-TM36 transilluminator (*Mitsubishi*), and the corresponding electrophoresis bands were discriminated using a ϕX174 DNA/HaeIII ladder (*Promega*) as the size reference marker. For more detailed analyses, gel shots were taken with a DC120 camera and DNA concentrations were assessed with Digital Science 1D software (*Kodak*), using again the ϕX174 DNA/HaeIII ladder as a concentration normalizer.

2.6. Inhibition experiments

To assess inhibition from silicon related materials, SiO_2-passivated silicon, polysilicon-passivated silicon bonded to 7740 glass, SiO_2-passivated silicon bonded to 7740 glass and 7740 glass wafers were first smashed by repeatedly hammering the wafers between two cleansed methacrilate slabs. The resulting debris was filtered to obtain a fine powder ($Ø_{max}$ 250 μm) mix of the wafer constituents (e.g. SiO_2, glass and native silicon granules for a SiO_2-passivated silicon bonded to 7740 glass wafer), and small (~1 mm^2) and intact (i.e. with bonded glass still attached in silicon-glass wafer assemblies) wafer fragments were also picked out and collected from the filter top. Both items were then separately introduced in conventional eppendorf tubes, either as ground-powder (0.0025 g of powder per eppendorf) or fragments (one ~1 mm^2 fragment per eppendorf). The eppendorf tubes contained a PCR-mix with low template concentration (0.5 ng/μl). After ensuring full contact between the foreign materials and the PCR-mix, PCR was conducted normally in a thermal-cycler. To mimic experimental conditions, a ~1mm^2 eppendorf tube wall fragment was also added to positive controls. Similarly, to assess the PCR-compatibility of ARCare7759 acrylic tape (Adhesives Research) [7], peeled and unpeeled acrylic tape fragments (~1 mm^2) were inserted into eppendorf tubes (one fragment per tube) and put into contact with the PCR mix. PCR (for amplification of human p53 exon #7, mix and kinetic data not supplied) was then conducted under normal conditions in a conventional thermal-cycler.

2.7. Adsorption and BSA experiments

To detect whether adsorption was taking place at chip walls, the PCR-mix was inserted into the chips and left there for a specified amount of time at 4 ºC (to prevent reagent decay). The PCR-mix was then removed and put into eppendorf tubes, and normal PCR was carried out in a conventional thermal-cycler. With this setup, experiments were conducted to independently determine whether DNA or Taq polymerase were being adsorbed at chip walls, adding the Taq polymerase enzyme to the PCR-mix either before or after exposition to the chip walls. Taq polymerase adsorption was then counteracted by a simple increase of Taq polymerase concentration (1 μl of 3.5 U/μl per 25 μl), or by the addition of a competing protein, bovine serum albumin (BSA). In the latter case, BSA concentration was empirically titrated to achieve optimum results.

3. Results and discussion

3.1. PCR inhibition by silicon-related materials

The biocompatibility of substrate materials, and especially with regard to its influence on PCR, is a fundamental issue in the development of PCR-chips. In the case of silicon-based PCR-chips, partial inhibition of PCR amplification has been repeatedly reported [7, 9, 10] and two complementary mechanisms, straight chemical inhibition [9] and surface adsorption [7, 10] due to increased (10-fold or greater [9]) surface-to-volume ratios have been proposed to account for it. In the most thorough study of PCR inhibition by silicon related materials [9], several amplification assays were conducted with untreated and polymer-coated powdered silicon, and with bare, polymer-coated and SiO_2/Si_3N_4 passivated silicon chips. The results indicated that both native silicon and silicon nitride triggered a consistent inhibition of PCR, while silicon oxide and some polymer coatings resulted in good (albeit inconsistent in the case of polymer coatings) amplification. In the present study, similar assays have been conducted with powdered silicon and glass (see Fig. 3), confirming that the presence of silicon powder induces a strong inhibition effect on conventional PCR, while the presence of glass powder (mainly SiO_x) has small, if any, effects on amplification (a significant part of the lower yields observed in powder analyses can be attributed to handicapped sample recovery). However, the amplification assays here reported with silicon and silicon-glass fragments (see also Fig. 3) yielded markedly different results, showing no evidence of PCR inhibition by glass, silicon oxide and, most surprisingly, untreated silicon. Inhibition of PCR by chemical agents that block or otherwise destabilize Taq polymerase action has been largely described in the literature [16-17], but the common factor for all inhibiting agents is that the presence of minute quantities suffices to strongly hinder (or completely inhibit) PCR performance [16]. Consequently, should silicon be a potent chemical inhibitor of PCR, its sole contact with the PCR-mix would induce an acute inhibition of the reaction, and PCR inhibition would have been evident in fragment assays. Therefore, the lack of inhibition in the here reported fragment assays suggests that, even though silicon may present some straight chemical inhibiting activity, the main reason behind the observed inhibition of PCR by silicon-related materials is not straight chemical inhibition, but a result of the increased surface-to-volume ratios that are typical of both PCR-chip amplifications and conventional PCR assays using powdered silicon. Moreover, the presumable link with surface-to-volume ratios hints at the dominance of surface interaction processes and, most probably, at adsorption phenomena [7]. Curiously enough, support for this theory comes also from the unexpected results obtained in the only assays conducted here with a non-silicon related material: acrylic tape, a material often used in the literature for sealing access holes during operation [7, 8, 18]. Gel results in Fig. 4 show that, when peeled off, the sole contact of ARCare7759 acrylic tape with the PCR-mix induces an acute inhibition of PCR, implying that some of the gluing agents in the acrylic tape are strong chemical inhibitors of PCR, without regard to the exposed surface-to-volume ratio. The reason why this occurrence has not been previously highlighted in the literature may lie in the fact that small air bubbles tend to form at access holes [18], and they thus prevent contact between the PCR-mix and the peeled acrylic tape. Nevertheless, and in order to avoid non-reproducibility of results, access hole sealing was conducted here by clamping the chip with an inert rubber gasket [1, 9].

3.2. PCR reagents adsorption at chip walls

Although Taq polymerase adsorption has been proposed as the most probable cause of inefficiency in PCR-chips, adsorption phenomena may influence PCR by sequestering any of its active reagents (i.e. Taq polymerase or DNA in the form of template, primers or dNTPs). To discriminate between these possible and complementary processes, independent experiments were designed to test DNA and Taq polymerase adsorption in chips passivated with silicon oxide, which has been

repeatedly singled out as the most PCR-friendly silicon-related surface layer (7-9). On its own, DNA adsorption was evaluated by lowering the concentration of its most critical instance (template DNA). The results shown in Fig. 5 indicate that DNA is not adsorbed in substantial amounts at chip walls, notwithstanding the exposure time. Conversely, (see Fig. 6) exposition of Taq polymerase to chip walls reveals a marked dependence between PCR efficiency and exposure time. Although part of this dependence might be explained by ordinary decay of Taq polymerase during exposure, the experimental conditions (exposure at 4 ºC and exposure times below 5 min) strongly minimize this factor, suggesting instead the prevalence of an adsorption phenomenon.

3.3. Counteraction of Taq polymerase adsorption

Once its occurrence had been verified, Taq polymerase adsorption to chip walls could be counteracted by simple means (i.e. increasing Taq polymerase concentration) or, as suggested in the literature [7, 10, 19], by adding a protein adjuvant (s.c. BSA) that competes with Taq polymerase for active adsorption sites at chip walls. Both hypotheses were tested here, yielding relevant results. As it can be readily appreciated in Fig. 7, the addition of BSA worked far better at counteracting Taq polymerase adsorption than a simple 5-fold increase in Taq polymerase concentration. These results are significant because, even though Taq polymerase concentration can be increased beyond the 0.14 U/μl range here tested, it cannot be increased indefinitely. On the one hand, Taq polymerase is, by far, the most expensive reagent used in PCR and, on the other hand, varying its concentration in significant amounts requires the experimental titration of Mg^{++} ions that, at high concentrations, would have also a net influence on the reaction pH, thus triggering a cascade of experimental titrations for other PCR reagents (e.g. KCl). Therefore, the use of a competing and otherwise mostly inert [19-20] adjuvant like BSA confers the means for counteracting Taq polymerase adsorption in a cheap and straightforward manner.

Nevertheless, and even though BSA can be used in relatively high concentrations [20], its concentration should not be increased indefinitely, since its beneficial properties may become detrimental due to an excessive mix viscosity, and because such a substantial increase may not be required to effectively counteract Taq polymerase adsorption. Therefore, the last step in the here described chemical optimization of PCR in silicon-glass PCR-chips was the titration of the most favorable BSA concentration. Titration was initially carried out using the same experimental setup as that of the previous adsorption experiments, and the results (data not shown) suggested that its optimum concentration lied in the 1-5 μg/μl range. Once this had been established, titration experiments were conducted by directly carrying out PCR in chips, leading to a final titrated concentration of 2.5 μg/μl (experiments in chips showed a significant decrease in efficiency for 5.0 μg/μl and higher concentrations of BSA). Even when using optimized concentrations of BSA, however, chip PCR amplifications had to be conducted with a doubled amount of Taq polymerase in order to attain yields equivalent to those observed in positive controls, a fact that has been already reported in the literature [7]. The most probable explanation for this effect may reside in the very low concentrations of Taq polymerase (in contrast to other PCR-chip work [1, 6, 9]) that were used in positive controls both here (0.028 U/μl) and in [7] (0.025 U/μl). The addition of BSA counteracts Taq polymerase adsorption by competing for adsorption sites at chip walls, but this means that some polymerase will still be adsorbed in the resulting dynamic equilibrium. When working with very low Taq polymerase concentrations, the proportion of polymerase that is adsorbed in the equilibrium increases and, below a threshold, the remaining concentration of free polymerase may become excessively low to carry out PCR efficiently. Even so, the final concentration of Taq polymerase used in the PCR-chip experiments here reported remains the lowest to date (0.05 U/μl), indicating that the titration of BSA reached an optimum balance concerning the counteraction of Taq polymerase adsorption. Using these experimentally validated concentrations, PCR kinetic parameters were adapted for fast cycling in order to take advantage of the PCR-chip environment [5, 6]. The results shown in Fig. 8 are in accordance with previous reports [6, 7, 8, 18], yielding

successful (albeit not as efficient as the positive controls) PCR in 20 min and robust PCR operation in 30 min, using ~11 ºC heating and 5 ºC/s cooling rates that generated a mean cycle time of 26.6 s.

4. Conclusions

In the light of the results here described, some conclusions relevant to PCR-chips design and technology may be drawn. The first one is that silicon (and standard silicon related materials) does not seem to be a potent straight chemical inhibitor of PCR. Instead, the aforementioned results suggest that the partial PCR inhibition observed in PCR-chips is caused mainly by the increased surface-to-volume ratio these systems display, which triggers surface adsorption phenomena. It has been shown also that, in silicon oxide passivated chips, DNA adsorption at chip walls is not significant and that, conversely, effective Taq polymerase concentration decreases in a time-dependent manner due to adsorption processes. Additionally, the effectiveness of counteracting Taq polymerase adsorption by different methods has been studied, and the addition of BSA in adequate concentrations (~2.5 μg/μl) has been found to be a more efficient measure than a simpler but more expensive increase in polymerase concentration. Finally, the inhibitory effect of common-use acrylic sealing tapes has been addressed. These sealing systems have been found to induce strong inhibition effects on PCR and, thus, their use is discouraged, as they may give way to confounding reproducibility problems.

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Biographies

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Figures

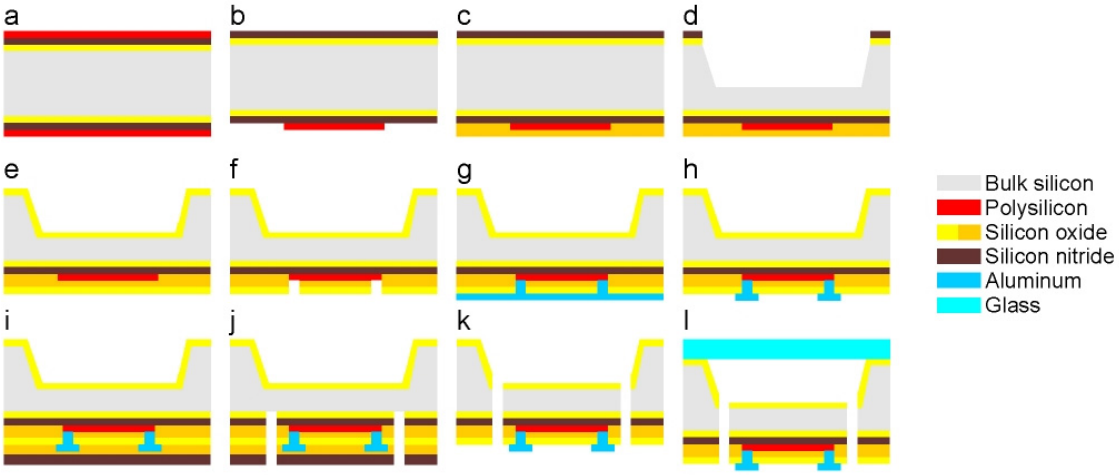

Fig. 1. Basic technological process for PCR-chips. Polysilicon resistor definition and passivation (a-c), PCR-chamber deep etch and PCR-friendly passivation (d-e), definition of contact pads (f-h), passivation (i), access holes deep etch (j-k) and anodic bonding (l).

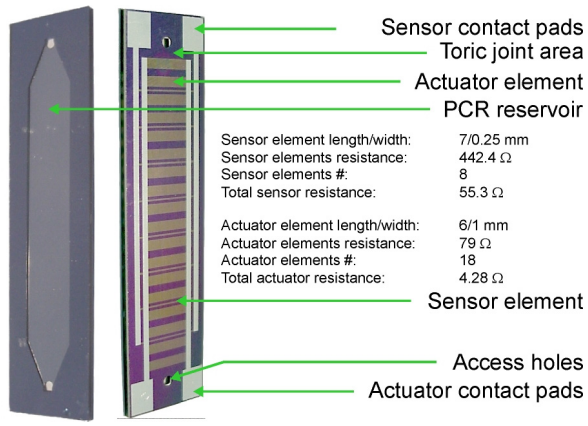

Fig. 2. Front and rear view of produced PCR-chips. The inter-digitized nature of polysilicon heater-sensor resistor grids allows homogenous heating of the PCR chamber and provides real-time average sensing while using a single polysilicon layer.

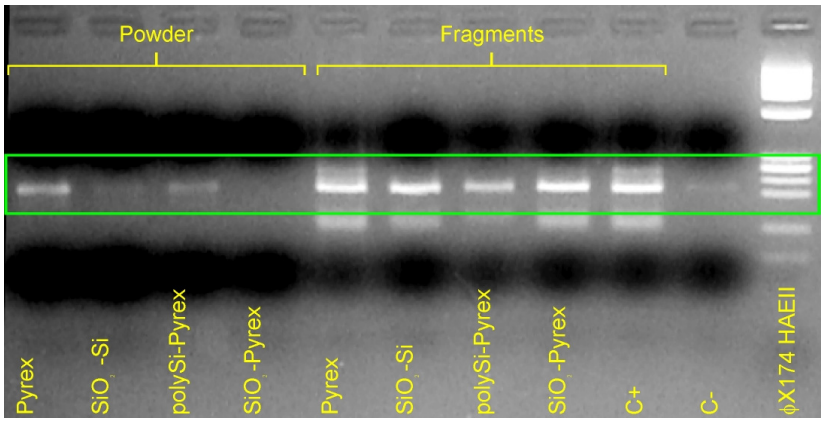

Fig. 3. Gel results for inhibition experiments. These assays were repeated thrice, and the small variations in product amount between fragment lanes were found to be non-significant. Variations in powder assays were larger, a result of handicapped sample

recovery after PCR, but the net trend in these assays, except in Pyrex powder experiments, was a marked decrease in PCR efficiency.

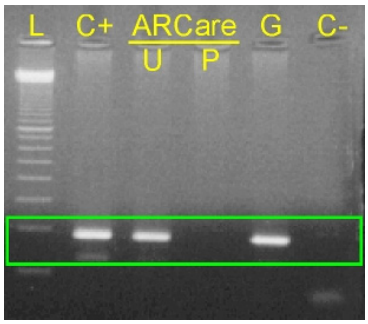

Fig. 4. Gel results for acrylic tape PCR-friendliness experiments. The amplified product is a 185 bp fragment of the human p53 exon #7. The L lane corresponds to a 100 bp incremental DNA ladder, while ARCare lanes are labeled according to tape state (U for unpeeled and P for peeled). The G lane corresponds to another putative access hole sealer, Panniker glue.

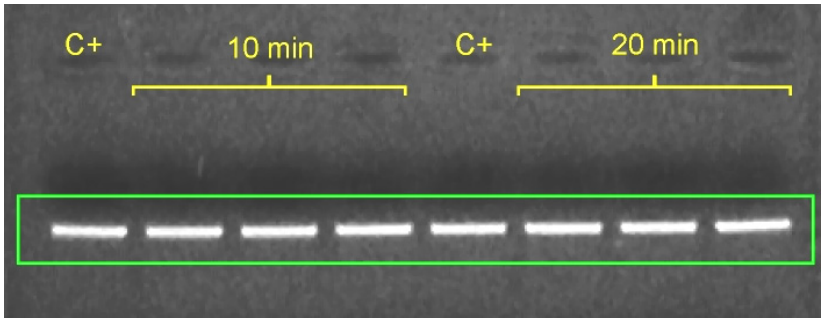

Fig. 5. Gel results for two DNA adsorption experiments using three different chips. No significant loss of efficiency can be observed, regardless of exposure time.

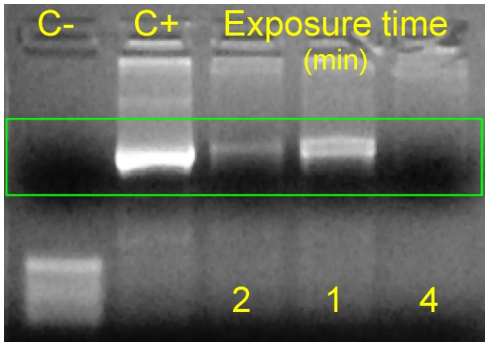

Fig. 6. Gel results for Taq polymerase adsorption experiments. PCR efficiency can be seen to decrease proportionally to exposure time, leading to an apparent complete inhibition at 4 min.

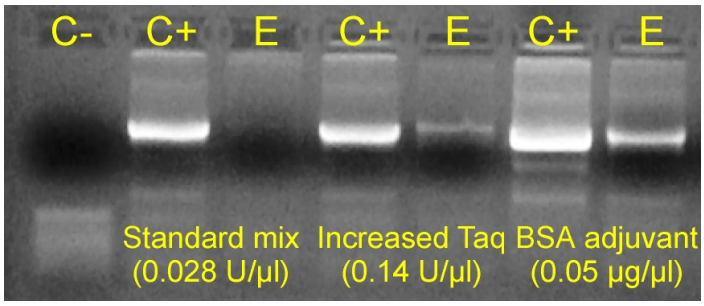

Fig. 7. Adsorption counteraction results. E lanes correspond to chip-exposed PCR-mixes. As it can be seen, the addition of 0.05 µg/µl of BSA does not have a noticeable effect on positive control outcome, but outperforms a simple increase of Taq polymerase concentration in chip-exposed mixes.

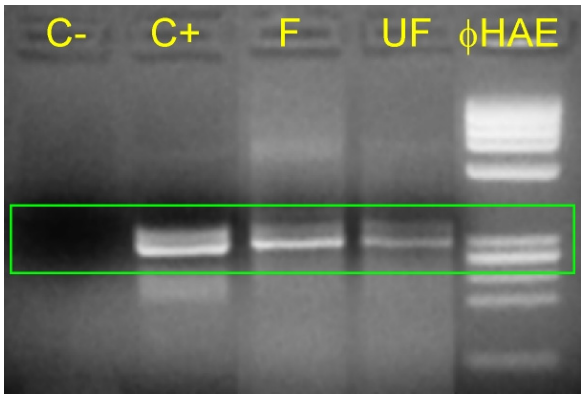

Fig. 8. Results for fast (F – 30 min) and ultra-fast (UF – 20 min) experiments. The amplified fragment here is a 310 bp section of the *Xylella fastidiosa lexA* promoter region.