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# Chip PCR. I. Surface passivation of microfabricated silicon–glass chips for PCR

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## ABSTRACT

**The microreaction volumes of PCR chips (a microfabricated silicon chip bonded to a piece of flat glass to form a PCR reaction chamber) create a relatively high surface to volume ratio that increases the significance of the surface chemistry in the polymerase chain reaction (PCR). We investigated several surface passivations in an attempt to identify 'PCR friendly' surfaces and used those surfaces to obtain amplifications comparable with those obtained in conventional PCR amplification systems using polyethylene tubes. Surface passivations by a silanization procedure followed by a coating of a selected protein or polynucleotide and the deposition of a nitride or oxide layer onto the silicon surface were investigated. Native silicon was found to be an inhibitor of PCR and amplification in an untreated PCR chip (i.e. native silicon) had a high failure rate. A silicon nitride ($Si_3N_4$) reaction surface also resulted in consistent inhibition of PCR. Passivating the PCR chip using a silanizing agent followed by a polymer treatment resulted in good amplification. However, amplification yields were inconsistent and were not always comparable with PCR in a conventional tube. An oxidized silicon ($SiO_2$) surface gave consistent amplifications comparable with reactions performed in a conventional PCR tube.**

## INTRODUCTION

As the demands of diagnostic laboratories continue to increase as a result of rising health costs, the necessity for rapid and inexpensive medical tests has become increasingly apparent (1–4). A reduction in tissue or fluid sample volumes, a reduction in test reagent volumes, high through put of samples, the reduction of contamination and an increased ease of use through automation are all necessary elements to both reduce the cost of diagnostic tests and increase the speed in obtaining results.

The implementation of microfabricated devices in the research laboratory setting has begun to address these issues in all areas of laboratory testing, from sample preparation (5) to sample reaction, separation and detection (6–9). By miniaturizing the sample preparation procedure, less of the potentially infectious specimen is necessary, translating into smaller specimens taken from patients. By miniaturizing the reaction chamber, a faster reaction (10) is possible using only nanoliter to femtoliter volumes of reagents. By miniaturizing the detection apparatus, less of the reacted sample is necessary for measurement. The combination of these three microsystems on one microfabricated device will create a single micro total analysis system (μTAS) performing the tasks of several large instruments (11,12).

Microfabricated devices are currently being used to perform a variety of chemical (13–14) and enzymatic reactions, such as the glucose oxidase reaction (15,16), ligase chain reaction (LCR) (17) and polymerase chain reaction (PCR) (18,19). These devices can also be used to directly amplify human genomic DNA from lymphocytes introduced directly into the microchambers (20). However, in order for the μTAS to compete with other systems the analytical performance of these devices must be comparable with or superior to conventional reaction systems. Surface chemistry plays an especially significant role in any reaction performed inside the microfabricated devices. The surface to volume ratio in a conventional PCR reaction tube (e.g. Perkin-Elmer MicroAmp™ Reaction Tube) is ~1.5 $mm^2/\mu l$. As the geometry and volume of the reaction chamber is altered this ratio increases ~5-fold in the case of a capillary tube and 13-fold for a PCR chip. No studies that deal specifically with the passivation (any chemical or physical treatments that render a surface inert) of surfaces in microfabricated structures and their use with PCR have been reported. In this paper we examine the effects of silicon and treated silicon surfaces on PCR and the effectiveness of silicon–glass microfabricated devices used to perform PCR. We further examine possible surface treatments to passivate the silicon–glass chips in an attempt to identify an inert surface compatible with PCR.

## MATERIALS AND METHODS

### Surface treatments of silicon powder

Silicon powder 325 mesh (Aldrich Chemical Co. Inc. Milwaukee, WI) was used to test the effect of silicon on PCR. It was treated with one of the following silanizing agents: Surfa™, AquaSil™, dimethyldichlorosilane (DMDCS), dimethylchlorosilane (DMCS), trimethylchlorosilane (TMCS) (Pierce, Rockford, IL) or Sigma-Cote™ (Sigma Chemical Co., St Louis, MO). Several hundred milligrams of the silicon powder were incubated at room temperature in an excess of silanizing agent in a test tube overnight. The excess silanizing agent was then removed and the silanized

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silicon pellets were dried overnight in an oven at 70°C. The pellets were then washed 10 times with double distilled, deionized water and again oven dried overnight. The silanized silicon powder was aliquoted into separate tubes in batches of 20 mg each and 1 ml polymer solution was added. The following polymer solutions (10 mg/ml) were prepared in 0.1 M Tris buffer, pH 8.6: poly-α-alanine, poly-L-aspartic acid, polyglycine, poly-L-leucine, poly-DL-phenylalanine, poly-DL-tryptophan, poly-L-lysine, polyvinylpyrrolidone, polyadenylic acid, polymaleimide or maleimide (Sigma Chemical Co.). The silicon was incubated with the polymer solution at room temperature overnight. The supernatant was removed and the treated silicon powder was then dried overnight in an oven at 45°C.

### PCR using treated silicon powder

The PCR reactions were performed using a Perkin-Elmer's GeneAmp® PCR Reagent Kit with AmpliTaq® DNA polymerase and run in the Perkin-Elmer GeneAmp PCR System 9600 (Norwalk, CT). A 100 µl reaction mixture contained 72 µl double distilled, deionized, autoclaved water, 10 µl 10× reaction buffer, 200 µM each dNTP, 0.3 µM each primer, 2 ng λ phage control template and 10 U AmpliTaq® DNA polymerase. The sequences of the primers were: primer 1, 5′- GATGAGGTCGTGTCCGTACAACTGG-3′; primer 2, 5′-GGTTATCGAAATCAGCCACAGCGCC-3′. The thermal conditions of the System 9600 were as follows: one cycle at 94°C for 1 min; 35 cycles at 94°C for 15 s, 60°C for 15 s and 72°C for 1 min; one cycle at 72°C for 5 min. Approximately 4.6 mg of each type of treated silicon powder was measured into separate reaction tubes. The PCR reaction mixture (28 µl) was added to each tube and then all were cycled in the System 9600 using the above conditions.

The amplified products were detected using a 2% agarose gel (N930-2774; Perkin-Elmer) in 1× TBE buffer, pH 8.3. The 1× TBE buffer contained 100 mM Tris, 90 mM boric acid and 1.0 mM EDTA (Life Technologies, Grand Island, NY). The gel was stained with 1 µg ethidium bromide (Sigma Diagnostics, St Louis, MO) per 10 ml gel. The samples were electrophoresed at 100 V for ~30 min.

### The silicon chips and thermal cycling device

Silicon chips were fabricated by the Alberta Microelectronic Center (Edmonton, Alberta, Canada) using standard photolithographic procedures (21). Each 14 × 17 mm chip was etched to a depth of 115 µm. The surface-polished Pyrex™ glass cover (14 × 17 mm; Bullen Ultrasonics Inc., Eaton, OH) was essential to guarantee good anodic bonding and to minimize non-specific adsorption. The silicon chips were soaked in a $H_2SO_4/H_2O_2$ (2:1 v/v) bath at 120°C and then washed several times with >1 l deionized, distilled water. The silicon chips were placed on an aluminum plate that was heated to 500°C on an insulated hot plate (PC-300; Corning, Corning, NY). The temperature was monitored using a surface thermometer (Hallcrest, Glenview, IL). Pyrex glass covers were placed on top of each silicon chip. The silicon and glass were anodically bonded by applying 1000 V (with a current of <1 mA) throught the aluminum plate and glass cover. A d.c. Kepco power pack (APH 1000M; Kepco Inc., Flushing, NY) was used to apply the necessary voltage.

Thermal cycling of the PCR chips was performed using a custom fabricated device from Faulkner Instruments (Pitman, NJ). The device was capable of thermal cycling four PCR chips simultaneously. This device incorporated a Peltier heater/cooler (9500/071/040; ITI Ferrotec, Chelmsford, MA) centrally located under an oxygen-free copper block (40 × 40 mm) containing a 10 kΩ thermistor (YSI 44016; Yellow Springs Instruments, Yellow Springs, OH). It was necessary to keep a constant air flow of ~40 l/min under the thermal cycling device to dissipate heat. The air flow was monitored by a flowmeter (Gilmont F-400; Gilmont Instruments Inc., Barrington, IL). The heater/cooler and thermistor were connected to a Modular Laser Diode Controller (LDC-3900; ILX Lightwave, Boceman, MT) through an RS232 interface. The Laser Diode Controller was connected to a 486 PC through a GPIB interface, where it was controlled using a virtual instrument built on LabVIEW for Windows (National Instruments, Austin, TX). The virtual instrument automated thermal cycling, giving cycle times of ~3 min.

### Surface treatments of the microfabricated PCR chips

The powdered silicon surface treatments which qualitatively showed the greatest amount of amplification were then applied to the PCR chips. The silanizing agent was pipetted into the PCR chip and allowed to incubate at room temperature for 15 min. It was then removed by applying a negative pressure to the exit port. This vacuum was applied overnight to remove all of the silanizing agent and to dry the PCR chip. The chips were then washed with 1 ml autoclaved, double distilled, deionized water. The chips were again emptied and dried by applying a vacuum to the exit port. Each PCR chip was then filled with one of the selected polymer solutions and incubated at room temperature for at least 1 h. The polymer solution was removed using a negative pressure applied to the exit port. The PCR chip was then rewashed with 0.5 ml autoclaved, double distilled, deionized water. The chip was emptied by again applying a negative pressure to the exit port of the chip.

Wafers used to fabricate the PCR chips were also treated with 1000 Å thick layers of silicon oxide ($SiO_2$) or silicon nitride ($Si_3N_4$) using standard deposition techniques (22,23) (Alberta Microelectronics Center). The silicon and glass were then anodically bonded as described above. The glass covers of these oxide- and nitride-coated PCR chips were not treated.

### PCR using the treated PCR chips

A 100 µl reaction mixture contained 74.5 µl double distilled, deionized, autoclaved water, 10 µl 10× reaction buffer, 200 µM each dNTP, 0.3 µM each primer, 1 ng template (either λ phage DNA or *Campylobacter jejuni* bacterial DNA) and 2.5 U AmpliTaq® DNA polymerase. The primers used for the amplification of λ phage DNA were as described above. The sequences for amplification of *C.jejuni* bacterial DNA were: primer 1, 5′-CTTCAGGGATGGCGATAGCA GATAG-3′; primer 2, 5′-GCACTGAACCAATGTCGGCTCTGAT-3′. Approximately 10 µl of reaction mixture were pipetted directly into the entry port of the PCR chips. The chips were then positioned on the thermal cycling device and sealed with silicon rubber gaskets. The chips were held in place by spring-activated clamps incorporated in the thermal cycling device. Thermal cycling conditions for amplifing λ phage DNA were as follows: one cycle at 94°C for 1 min; 35 cycles at 94°C for 15 s, 60°C for 15 s and 72°C for 1 min; one cycle at 72°C for 5 min. Thermal cycling conditions for amplifing *C.jejuni* bacterial DNA were as follows: one cycle at 94°C for 1 min; 35 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min; one cycle at 72°C for 5 min.

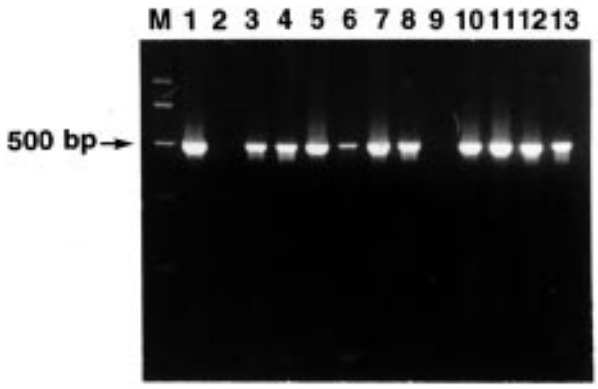


**Figure 1.** Agarose gel (1.0% in 1× TBE) electrophoresis of the PCR products amplified in the Perkin-Elmer GeneAmp™ System 9600 with silicon powder treated with SurfaSil™ in the reaction mixture. Lane M, 2.5 ng PCR Markers (G3161; Promega; Madison, WI). Lane 1, positive control containing no silicon in the reaction mixture. Lane 2, 4.6 mg untreated silicon in the reaction mixture. Lanes 3–13, 4.6 mg silicon powder treated with SurfaSil™ followed by treatment with poly-α-alanine, poly-L-aspartic acid, polyglycine, poly-L-leucine, poly-DL-phenylalanine, poly-DL-tryptophan, poly-L-lysine, polyvinylpyrrolidone, polyadenylic acid, polymaleimide or maleimide respectively. All the reaction volumes above were 28 µl.

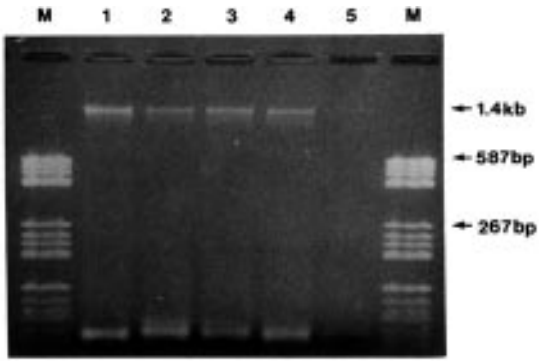


**Figure 2.** Agarose gel (1.0% in 1× TBE) electrophoresis of the PCR products amplified in PCR chips treated with a silanizing agent and polymer. Lane M, 2.5 ng pBR322 *Hae*III Digest Marker (Sigma Chemical Co.). Lane 1, positive control amplified in the GeneAmp™ System 9600. Lane 2, product amplified in an untreated PCR chip. Lane 3, product from a mixture amplified in a PCR chip treated with SurfaSil™ and polyadenylic acid. Lane 4, product from a mixture amplified in a PCR chip treated with SurfaSil™ and polyvinylpyrrolidone. Lane 5, product from a mixture amplified in a PCR chip treated with the product from a mixture amplified in a PCR chip treated with SurfaSil™ alone.

Positive controls of the PCR were run in parallel to the PCR chips in the Perkin-Elmer GeneAmp PCR System 9600 in order to ensure direct comparability with results obtained from the PCR chips. A 10 µl reaction volume was used for the positive control. Additionally, the GeneAmp PCR System 9600 was programed to mimic the thermal profile (identical ramp and hold times) of the PCR chip thermal cycler.

After cycling, the reaction mixtures were removed from the PCR chips using a custom fabricated device (Faulkner Instruments). This device clamped the PCR chips in place while a positive pressure was applied to the entry port of the chip, causing the reaction mixture to be ejected through a polyethylene tube (Clay Adams, Parsippany, NJ). The amplified mixtures were collected in polypropylene microcentrifuge tubes. Amplified products were detected using a 2% agarose gel (N930–2774; Perkin-Elmer) in a 100 mM Tris, 90 mM boric acid, 1.0 mM EDTA buffer, pH 8.3 (Life Technologies). The gel was stained with 1 µg ethidium bromide (Sigma Diagnostics) per 10 ml gel. The samples were run at 120 V for ~30 min.

## RESULTS AND DISCUSSION

### Influence of passivation treatments on powdered silicon

Initial tests using silicon powder indicated that untreated silicon is an inhibitor of PCR (Fig. 1, lane 2). In Figure 1, lane 1 represents the positive control, where no silicon was present in the reaction mixture, and lane 2 represents the reaction that included ~4.6 mg powdered silicon in the reaction mixture. Subsequent lanes (lanes 3–13) show signals from reactions conducted with silicon powder coated with the silanizing agent SurfaSil™ followed by coating with polymer solution. PCR reactions conducted with silicon powder coated with the silanizing agent SigmaCote™ followed by polymer solution showed poorer amplification compared with the use of SurfaSil™ alone (data not shown).

The silicon surface treatment was the only variable from tube to tube. The different surfaces had a variety of effects on the reaction. Some surfaces completely inhibited the reaction, such as SurfaSil™ followed by a coating of poly-L-lysine (Fig. 1, lane 9) and others showed amplification that was comparable with the positive control, such as SurfaSil™ followed by a coating of polyadenylic acid (Fig. 1, lane 11). In general the silicon powder coated with SurfaSil™ followed by a polymer coating produced stronger amplification signals than reactions run with silicon powder coated with SigmaCote™ and a polymer coating.

### Influence of passivation treatments on PCR chips

*Use of silanization and polymer coatings.* Surface treatments identified as 'PCR friendly' were then applied to the PCR chips under the assumption that PCR would react to surface treatments in the same manner on silicon chips as they did on silicon powder. We selected the following surface treatments: SurfaSil™ followed by treatments with poly-α-alanine, polyadenylic acid or polyvinylpyrrolidone; SigmaCote™ followed by treatments with polyglycine, poly-L-leucine or polyadenylic acid. The yields of products from reactions run in the treated PCR chips paralleled the yields from reactions run with treated silicon powder in reaction tubes in the System 9600 (data not shown). For example, where silicon powder coated with SurfaSil™ and polyadenylic acid gave higher yields of amplified product than silicon powder coated with SurfaSil™ and polyglycine, PCR chips coated with SurfaSil™ and polyadenylic acid gave higher yields of amplified product than PCR chips coated with SurfaSil™ and polyglycine. The same was true in selecting a silanizing agent for the PCR chips. PCR chips treated with SurfaSil™ and a polymer gave much stronger amplification signals than those treated with SigmaCote™.

Figure 2 shows results from four PCR chips cycled together. The results indicate that an untreated PCR chip (native silicon) (Fig. 2, lane 2, and Fig. 3, lane 2) displays some degree of inhibition of Taq DNA polymerase. PCR chips coated with 'PCR friendly' reagents such as SurfaSil™ followed by polyadenylic acid or polyvinylpyrrolidone (Fig. 2, lanes 3 and 4 respectively) produce amplifications that are equivalent to those run in conventional polyethylene tubes (Fig. 2, lane 1). Lane 5 displays

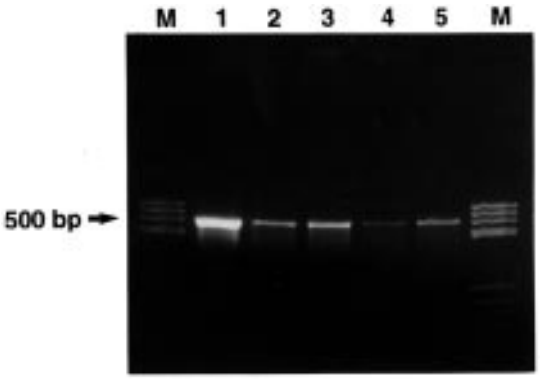


**Figure 3.** Agarose gel (1.0% in 1× TBE) electrophoresis of the PCR products amplified in PCR chips treated with an oxide or nitride layer. Lane M, 2.5 ng of pBR322 *Hae*III Digest Marker (Sigma Chemical Co.). Lane 1, positive control amplified in the GeneAmp™ System 9600. Lane 2, product amplified in an untreated PCR chip. Lane 3, product from a mixture amplified in a PCR chip with a 1000 Å layer of thermally deposited silicon dioxide ($SiO_2$). Lane 4, product from a mixture amplified in a PCR chip treated with a 1000 Å layer of silicon nitride ($Si_3N_4$). Lane 5, product from a mixture amplified in a PCR chip with a 1000 Å layer of silicon dioxide ($SiO_2$) produced utilizing CVD.

the results from an amplification in a PCR chip treated with SurfaSil™ without any polymer treatment. Although signals like this were common, amplifications using silane-treated PCR chips were inconsistent, giving yields that were not quantitatively uniform and variable from run to run. However, occassional signals were obtained that were even greater than those obtained from the conventional system. These irregular results were most likely due to a lack of uniformity in the treatments of the silicon chips. There were often visible variations in the surfaces of these chips, most likely the result of the manual methods used to coat them. It was also possible that the surface treatments may have degraded during thermal cycling. As a result, we investigated procedures that were standardized and more easily quality controlled.

*Use of silicon oxide and silicon nitride depositions.* Silicon oxide ($SiO_2$) and silicon nitride ($Si_3N_4$) deposition on the silicon surfaces of the PCR chips produced uniform surfaces that were accurate to within 10 Å, as determined by the Alberta Microelectronics Center. This uniform type of surface is ideal for mass produced PCR chips, where surface interactions are critical to reaction efficiency. By ensuring identical surfaces from wafer to wafer, identical PCR reactions would be guaranteed from chip to chip.

Results from the $SiO_2$- and $Si_3N_4$-coated PCR chips are shown in Figure 3. Lane 1 shows the positive control run in a polyethylene tube (10 µl reaction volume). Lane 2 shows the same amplification mixture run in an untreated silicon PCR chip. Lanes 3 and 5 show the same pre-amplification mixture run in PCR chips with a 1000 Å layer of thermal oxide and chemical vapor deposited (CVD) oxide ($SiO_2$) respectively. Lane 4 shows the same pre-amplification mixture run in an PCR chip with a 1000 Å layer of thermal nitride ($Si_3N_4$). Native silicon and the $Si_3N_4$-coated PCR chip showed marked inhibition. The thermal oxide-coated PCR chips produced the highest yields among the four surfaces. These results, shown in Figure 3, were consistent and reproducible from chip to chip and from run to run, implying uniform surface coatings and uniform heating and cooling in the thermal cycling device. Further measures have been taken to improve yields from the PCR chips. It has been possible to obtain yields comparable with conventional PCR tubes by using a thicker oxide layer (20). Yields may be further improved by examining the thermal profile of the PCR chips more closely and by optimizing conditions within the PCR chips. These issues are currently under investigation.

These experiments have shown that silicon is an inhibitor of PCR. Silicon and glass may be treated with a silanizing agent to passivate the surface for use with PCR, however, these passivation methods are time consuming and have not been standardized or optimized. As a result, the use of these surfaces with PCR produces variable results and, in the long-term, would not be cost or time efficient for mass production. Amplifications run in PCR chips treated with a 1000 Å thick oxide layer consistently resulted in amplification signals that were comparable with those obtained in MicroAmp™ reaction tubes using conventional PCR thermal cycling devices. Deposition of oxide surfaces is a standardized industry procedure that is reproducible and inexpensive and can be accomplished in a mass production setting.

The microfabricated silicon devices are effective for PCR and may be manufactured quickly and inexpensively, but have not been rigorously optimized. Current developments are directed toward the examination of thermal transfer in the PCR chips and the interaction of silicon with individual PCR reagents. Related studies on microfabricated sample preparation and detection systems are also being conducted. The discovery of an inert, 'PCR friendly' surface for microfabricated silicon devices is one important step towards the construction of an integrated, inexpensive, automated microfabricated PCR analysis system.

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