Minimizing the Surface Effect on PCR in PDMS-Glass Chips by Dynamic Passivation

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Abstract

Dynamic passivation of surfaces of PDMS-glass PCR chips using PEG or PVP was achieved in a conventional thermocycler. The effects of molecular weight of the polymers and the polymer concentration in PCR mixture on surface passivation were investigated. Efficiency of the dynamic passivation follows the order: PVP10000 > PVP55000, PEG8000> PEG10000> PEG400. 0.4% and 0.025% (w/v) are the suitable final polymer concentrations in PCR mixture. The competitive adsorption is the principal activity while the effect of polymers on *Taq* polymerase is a minor function during the PCR after passivation.

Keywords: Dynamic passivation; PDMS-glass; PCR; Chip; Polymer

1. Introduction

PDMS has become a popular material for microreactor fabrication. One of the easiest techniques to make microfluidic chips is fusing a patterned PDMS layer onto a glass wafer, which could be used for PCR or some other applications [1-4]. However, both PDMS and glass as microchip substrates are less than ideal for PCR because of the proclivity of Taq polymerase to be adsorbed to the chip surface. So far plenty of studies have been done on chip surface passivation to prevent the adsorption of polymerase onto the microreactor surface, especially on silicon-glass chip surface, but much less has been studied on PDMS-glass chips.

The commonly used protocol to passivate glass or silicon surface is static passivation, using Bovine serum albumin (BSA) adsorption, oxide, silanization or covalent polymer to coat and passivate the surface [5]. One of the most effective polymers used to prevent protein adsorption onto glass or silicon surface is PEG

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(polyethylene glycol), which is either tethered to aminated glass or silicon surface for static passivation [6], or mixed into the PCR reaction mixture for dynamic passivation[7-8]. PVP40 was also reported as a good additive, in dynamic passivation, which was even more active than PEG8000 when it was applied to passivate silicon dioxide-glass chips[8-9].

The well-documented process for passivation of PDMS reactors is also based on coating BSA, or covalently coating a chemical polymer, such as 2-methacryloyloxyethyl phosphorylcholine (MPC based polymer with a silane coupler) [10], to coat the PDMS surface followed by washing and curing. These coating methods are static and time consuming, and there is a risk that these coatings would partially lift off the surface during the high temperature reaction.

Compared to static passivation, dynamic passivation is easy to process without additional steps. PEG is a well-known biocompatible polymer, and PVP has been recognized as a PCR enhancer [11]. Moreover, since PEG has been used as a liquid substrate to immobilize enzymes for some other enzymatic reactions to improve the enzyme's activity and stability [12], the interaction between polymerase and the polymers may also have a potential benefit for the PCR amplification.

In this study, we dynamically passivated the PDMS-glass micro-PCR reactors using PEG and PVP of different molecular weights. The effects of molecular weight of the polymers and the concentration of polymer in PCR mixture on the surface passivation were investigated as well. Meanwhile, we also studied the possible effect of polymers on polymerase in PCR.

2. Experimental

2.1. Polymers

PEG 400, PEG8000, PEG10000, PVP10000 and PVP55000 were purchased from Sigma and used without further purification.

2.2. PDMS-Glass chip fabrication

We fabricated a master wafer with the desired microstructures at the solid-state electronics laboratory. The fabrication procedure of the master wafer began with spin-coating a layer of photoresist (PR 1813, Hoechst Celanese) at 4000 rpm for 30 sec on a silicon wafer. The wafer was soft-baked at 90 °C in an oven for 30 min and then exposed to UV radiation (404.7 nm, 10 mJ/cm^2, 20 sec) to define the desired microstructure. After removal of the activated photoresist by developer solution (MF 319, Hoeschst Celanese) for 60 sec, the wafer was hard-baked at 110 °C for 30 min. The microstructure pattern was transferred to the silicon wafer by a deep reactive ion etching. The photoresist was then removed

by a resist stripper (PRS 2000, JT Baker Inc.). To fabricate the PDMS chip, a mixture of PDMS prepolymer and its curing agent (Sylgard 184, Dow Corning) in a ratio of 10:1 was mixed and then degassed in vacuum for 30 min. The mixture was poured into the master and cured at 65 C for 1 hr. After curing, the PDMS replica was peeled off from the master wafer. The PDMS replica and glass slide were treated by oxygen plasma in the March Etcher (PX-1000, March Instrument Co., CA, USA*)* for 30s. Each PDMS-glass chip consists of 12 reaction chambers. For each chamber, the ratio of surface area/ volume is 21.6mm^2/ul, about 14.4 times that is in a MicroAmp reaction tube.

2.3. Pre-passivation of the PDMS-glass chip

For experiments testing the passivation effects of polymers on PCR in chips, the chips were treated in two ways: pre-passivation and in-situ passivation.

The pre-passivation, in which 10 of 12 chambers were fully filled with polymer solutions (10% w/v in water), every two chambers of which containing same polymer solution; the rest two chambers on the same chip were filled with nucleonase free water as a control. After 30min, an airflow (0.1kg) was used to blow the liquid out off the chambers for 30sec. In this case, no polymer was added directly into the PCR mixture. Each chip gained about 1mg (± 5% for 10 batches) weight after the treatment. In both cases, each chamber was filled with 5μL PCR mixture. The chips were sealed with Epoxy Gel (Devcon, 5minute Epoxy Gel; USA) and placed on the plate block in a thermal cycler (Hybaid PCR Express; UK) for PCR.

2.4. In-situ passivation of the PDMS-glass chip

Another treatment on the PCR chip was using only nucleonase free water to wash the chambers, and then drain the water out before injecting the PCR mixture by using an airflow (0.1kg) for 30sec; the polymer solutions (10% w/v in water, final concentration in PCR mixture was 0.4% w/v) were added to the PCR mixture individually. The rest procedures were as same as described in 2.3.

2.5. PCR using E. Coli genomic DNA as template

PCR mixture contained 200 μM dNTP, 2.5 mM $MgCl_2$, 0.25 U Taq polymerase (Promega), PCR Buffer (1x in final solution), 1μM each primer, and 100 ng *E. Coli* genomic DNA (Extracted from *E. Coli* O157:H7 strain). Primers for *E. Coli* Dihydrofolate Reductase (DHFR) gene, a 601-bp PCR target, are the forward primer 5'-GATTACAAACGTTTGTAATCCCATTGTAATGCGGCGAGTCC-3' and the reverse primer 5'-AACTAATTAGAATTCTAATTATGCAATAGCTGTGAGAGTTCTGC-3'. For experiments testing the concentration dependence of PCR, the DNA amplification was carried out in GeneAmp reaction tubes; polymers with certain final concentrations (0%, 0.025%, 0.4%, 1%) were also added into the PCR

mixture, or mixed with polymerase prior to adding the PCR mixture to the GeneAmp reaction tubes. For experiments testing the passivation effects of polymers on PCR in chips, the chips were treated as described in section 2.3 and 2.4. The amplification procedure was: 2 min at 94 ^{o}C, followed by 35 cycles of 94 ^{o}C for 30 sec, 55 ^{o}C for 30 sec, 72 ^{o}C for 40 sec, and finally heated at 72 ^{o}C for 5 min. All of the PCR amplifications in tubes were run in parallel. All experiments were made in triplicate at least.

3. Results and discussion

3.1. PCR in GeneAmp reaction tubes with polymers in the reaction mixture

Both PEG and PVP are hydrophilic polymers, which can induce a changing of enzyme's activity by affecting the water structure surrounding the protein molecules and therefore affecting the protein's formation. The interaction between PEG or PVP with Taq polymerase has not been reported, but as frequently used polymers, the interactions of PEG and PVP with some proteins (such as BSA) are well studied [13,14]. Farruggia et al found an equilibrium constant value of about $10^{2}\ M^{-1}$ for BSA in-low molecular weight PEG complex formation, which suggests a very weak interaction [15]. They also reported [16] that in the presence of all the PEG tested (PEG 600-8000), significant decrease in protein relative surface hydrophobicity was observed, which suggests that PEG presence modifies the protein's surface area exposure to the solvent; but this effect had no obvious trend towards molecular weight of PEG.

Most researchers believe that PEG or PVP passivates surfaces by competitive adsorption with proteins onto the surfaces in case of dynamic passivation. We wanted to know if there is another effect of these polymers on the polymerase in PCR. PEG or PVP are known to form loose and weak complexes with proteins, this may improve the hydrophilicity of the protein, and enhance the enzyme's physical and thermal stability. Thus the polymers may then influence the properties of the polymerase depending on their molecular weight, structure and concentrations. To further study these questions, experiments were run in GeneAmp reaction tubes and PDMS-glass chips respectively.

Figure 1 shows the effect of assayed polymers on PCR reactions in GeneAmp reaction tubes, in which the polymers were either added into the reaction mixtures after the addition of polymerase, or, mixed with polymerase first and then added into the reaction mixtures (sample 7 and 8).

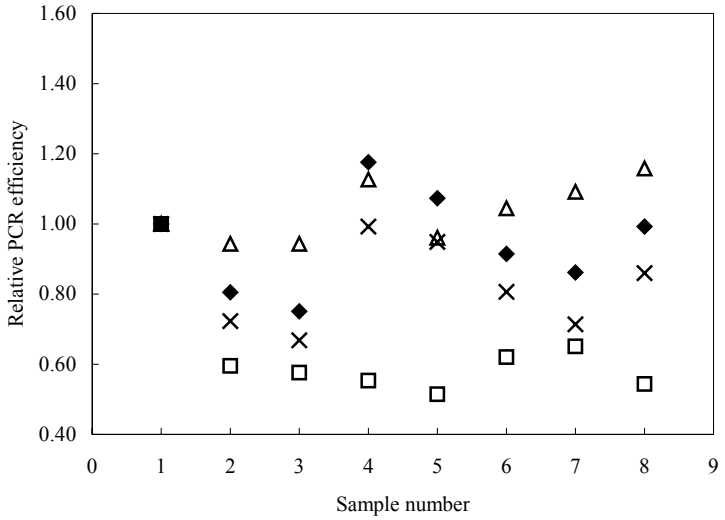

Figure 1. Polymer concentration and molecular weight dependence on PCR reactions in GeneAmp reaction tubes
Final polymer concentrations in PCR mixtures are: ◆ 0.025%, △ 0.4%, x 0.1% , □1% .

Sample 1: PCR mixture not containing polymer.

Sample2-6: Polymer (in order of PVP10000, PVP55000, PEG400, PEG8000, PEG10000) was added into PCR mixture after the addition of polymerase respectively.

Samples 7-8: PVP10000, PEG10000 was mixed with polymerase first and then added into the reaction mixture respectively.

Figure 1 shows that 0.4% and 0.025% are the suitable final polymer concentrations in PCR mixture. As the concentration reaches 1%, PCR inhibition was observed in the presence of all the polymers tested. Among assayed polymers, PEG400 and PEG8000 are the best dynamic passivation materials. More interesting, at all tested concentrations, sample 7 gave a higher yield than sample 2, so did sample 8 to sample 6; which means to a degree, both PEG and PVP can affect the property of Taq polymerase; Furthermore, sample 8 gave a higher yield than sample 7, showing that with the same molecular weight, PEG is more efficient than PVP. This difference may be explained by the positive charge of PVP, which may form complex with DNA or dNTP due to charge-charge interaction and therefore have a negative effect on PCR.

The results obtained at 1% polymer concentration maybe due to polymer-protein or polymer-DNA complex formation, which might prevent polymerase moving to the DNA chain, and thereafter inhibit PCR.

At lower concentrations, in which shorter PEG (PEG 400 and PEG8000) appeared to enhance PCR efficiency better, the improvement of hydrophilic environment of polymerase may become dominant; with the same weight concentration, the numbers of repeating units in all samples containing PEG are

the same, as a result the interaction between longer PEG and the enzyme is weaker than that between shorter PEG and the enzyme.

3.2. Effect of polymers on the chip passivation

PEG is well known for its extraordinary ability to prevent protein adsorption. Modifying surfaces with PEG to improve blood compatibility and to minimize protein adhesion has been well documented [17,18].

For passivation of silicon or glass surface, the effect of molecular weight and density of PEG on passivation has been studied by X-ray photoelectron spectrometry spectroscopy (XPS) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry in the surface mode (surface-MALDI-ToF-MS) [14].

On PEG tethered asymmetric membranes, BSA adsorption amount depends on the molecular weight of PEG[4]. The order of passivation efficiency is correlated as: PEG400>PEG600>PEG1000>PEG200. No similar trends in dynamic passivation with PEG on glass or other surfaces have been reported.

So far, in all the documented dynamic passivation experiments, polymers were mixed with PCR solution prior to loading the reaction mixture into chips. Since native PDMS or glass surface is negatively charged, PVP is positively charged, hence if PVP was adsorbed onto the surfaces before loading the PCR mixture, which may facilitate preventing polymerase adsorbed onto the PDMS or glass surface. So we speculated that rather than adding the polymer into the PCR mixture, but forcing the surface adsorbing PVP prior to loading the PCR mixture, in theory it may give better passivation than the competitive adsorption in the presence of DNA and polymerase.

Figure 2 shows the result of effect of polymers on PCR efficiency in PDMS-glass chips with the two different methods of adding the polymers.

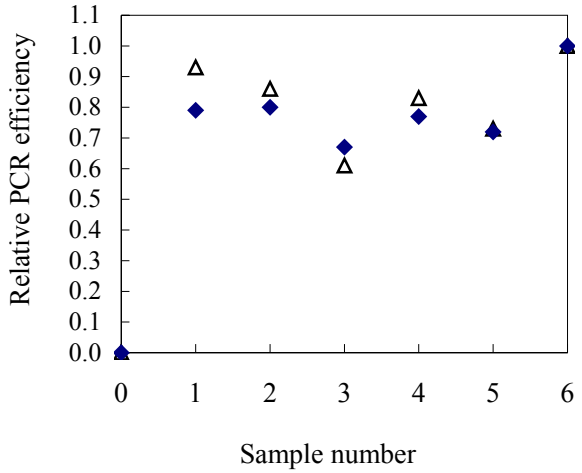

Figure 2. Effect of Polymer on PCR efficiency in PDMS-glass chip
△, polymer solutions (10% w/v) were not mixed in PCR mixtures but injected into chip chambers and drained before loading PCR mixtures; ◆Polymers' final concentration was 0.4%; polymer solutions were mixed in PCR mixtures.
Sample 0: PCR were run in chips without passivation. Sample 1-5: PCR mixture containing PVP10000, PVP55000, PEG400, PEG8000 and PEG10000 respectively; Reactions were run in chips. Sample 6: PCR were run in tubes without polymer.

Figure 2 shows that when PVP 10000 or PVP 55000 solutions were introduced into the PCR chambers before injecting the PCR mixture, the surface effect of PDMS and glass material on PCR was almost completely erased; and the efficiency of passivation follows the order: PVP10000 > PVP55000, PEG8000> PEG10000> PEG400. In case of the "in-situ" dynamic passivation (polymer solutions were added into PCR mixtures directly), the order of passivation efficiency of PEG and PVP is almost the same with what in pre-PCR passivasion: PVP10000, PVP55000 > PEG8000> PEG10000> PEG400. There were no PVR products without polymer passivation.

In the case of pre- PCR passivation, the polymers were possibly trapped by the porous PDMS surface and the glass surface. Moreover, in the presence of PVP, either glass or PDMS adsorbed PVP due to charge-charge interaction. This may be the reason for that pre-PCR passivation has higher passivation efficiency in the presence of PVP. Unlike what was observed in tube PCR, PVP is more active than PEG in passivation of PDMS-glass chip. With PEG 400(sample number 3), an opposite effect on the passivation efficiency was observed. This may be due to the smaller molecular weight of PEG400, which would allow it untrapped from the surface but diffused into the solution.

The phenomena that pre-PCR passivation has higher passivation

efficiency indicates that competitive adsorption is the principal activity while the effect of polymers on polymerase is a minor factor during the PCR after passivation.

4. Conclusion

An efficient dynamic passivation on PDMS-glass PCR chip can be achieved using polymers. PVP10000, PVP55000, and PEG8000 are good materials for dynamic passivation on PDMS/glass surfaces for PCR. Efficiency of the dynamic passivation follows the order: PVP10000 > PVP55000, PEG8000> PEG10000> PEG400. 0.4% and 0.025% (w/v) are the suitable final polymer concentrations in PCR mixture. Both PEG and PVP have a weak effect on Taq polymerase. Competitive adsorption is the principal activity while the effect of polymers on polymerase is a minor factor during the PCR after passivation.

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Reference

[1] Yu X, D. Zhang, T. Li, L. Hao and X. Li. Sensors and Actuators A: Physical. 108(2003):103-107.
[2] Hong, J.W., T. Fujii, M. Seki, T. Yamamoto, and I. Endo. Electrophoresis 22(2001):328-333.
[3] Hong, J.W., T. Fujii, M. Seki, T. Yamamoto and I. Endo. (2000) p. 407-410. In A. Dittmar and D. Beebe(Eds.), 1st Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine and Biology. Proceedings. IEEE. Piscataway, NJ.
[4] Martin, R.S., A.J. Gawron, and S.M. Lunte. Anal. Chem. 72 (2000): 3196-3202.
[5] Xu Z.K., F.Q. Nie, C. Qu, L.S. Wan, J. W. and K. Yao. Biomaterials 26(2005):589-598.
[6] Kricka L.J., P.Wilding. Anal Bioanal Chem 377(2003):820-825.
[7] Giordano B.C., E.R. Copeland and J.P. Landers. . Electrophoresis 22(2001):334–340.
[8] Lou X. J., P. Nicholas J; W. Peter; F. Paolo, K. Larry J. BioTechniques 36(2004):248-252.
[9] Yang J., Y. Liu, C. B. Rauch, R. L. Stevens, R. H. Liu, R. Lenigk and P. Grodzinski. Lab Chip, 2(2001): 179–187
[10] Fukuba T., T. Yamamoto, T. Naganuma and T. Fujii. Chemical Engineering Journal. 101(2004): 151-156.
[11] Bickley,J. Hopkins,D. in: Saunders, G., Parkes, H.,(Eds.), Analytical Molecular Biology-Quality and Validation,81-102, Thomas Graham House,

UK,1999
[12] Maruyama T., H. Yamamura, T. Kotani, N. Kamiya and M. Goto. Org.Biomol.Chem., 2(2004):1239-1244.
[13] Kingshott, P., H. Thissen, and H.J. Griesser. Biomaterials. 23(2002):2043–2056.
[14] Kingshott, P., S. McArthur, H. Thissen, D.G.Castner, and H.J. Griesser. Biomaterials. 23(2002):4775-4785.
[15] Farruggia B., B. Nerli, H. Di Nucci, R. Rigatuso, G. Picó. Int. J.Biol. Macromol. 26(1999):23-33.
[16] Farruggia B., B. Nerli, and G. Picó. Journal of Chromatography B. 798(2003):25–33.
[17] Irvine D.J., Mayes A.M., Satija S.K., Barker J.G., et al. 1998. J Biomed Mater Res. 40:498.
[18] Nicholas J. P., X.J. Lou, P. Fortina, L.J. Kricka, and P. Wilding. Biomedical Microdevices 6(2004):75-80.