Material Biocompatibility for PCR Microfluidic Chips

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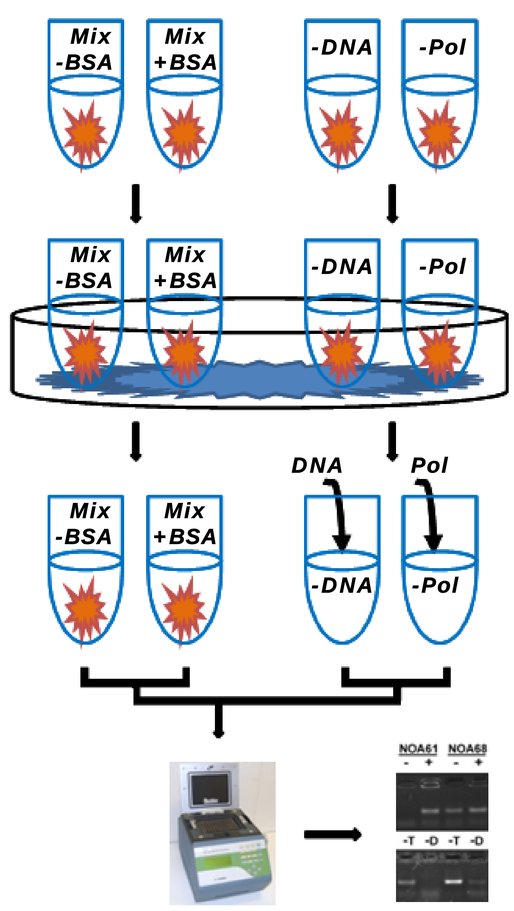
Introduction

Biological molecules suspended in fluid and transported through microfluidics channels interact with the channel-wall material. The interaction is even stronger in high surface-area-to-volume ratio (SAV) microfluidics channels and may lead to adsorption and inhibition of biomolecules. In the present study, we employed polymerase chain reaction (PCR), one of the most frequently used enzymatic reactions in microfluidics, as a model in an investigation of the biocompatibility of various materials used in microfluidics fabrication.

The PCR is a technique to amplify the target DNA. Our PCR consisted of 35 cycles, with temperature transitions between 91 °C and 71 °C, each step for 20 seconds. The PCR consisted of the following components:

- 0.75 µM primers
- 200 000 plasmid DNA molecules (template)
- 0.2 mM dNTP
- 3.5 mM $MgCl_2$
- 2 µg/µl BSA (for the reaction mix with BSA)
- 1.2 M Betaine (additive for dynamic passivation)
- 0.2 mM cresol red (for color)
- 1x reaction buffer
- 0.025 U/µl SpeedStar Hot Start DNA polymerase

The biocompatibility assay



Step 1
- Add PCR mix to the material
  "Mix" - PCR mix with all components
  "-DNA" - PCR mix without template DNA
  "-Pol" - PCR mix without polymerase

Step 2
- Incubate on ice for 30 min
- Leave fragmented material for "Mix"
- Remove material for "–DNA" and "–Pol"

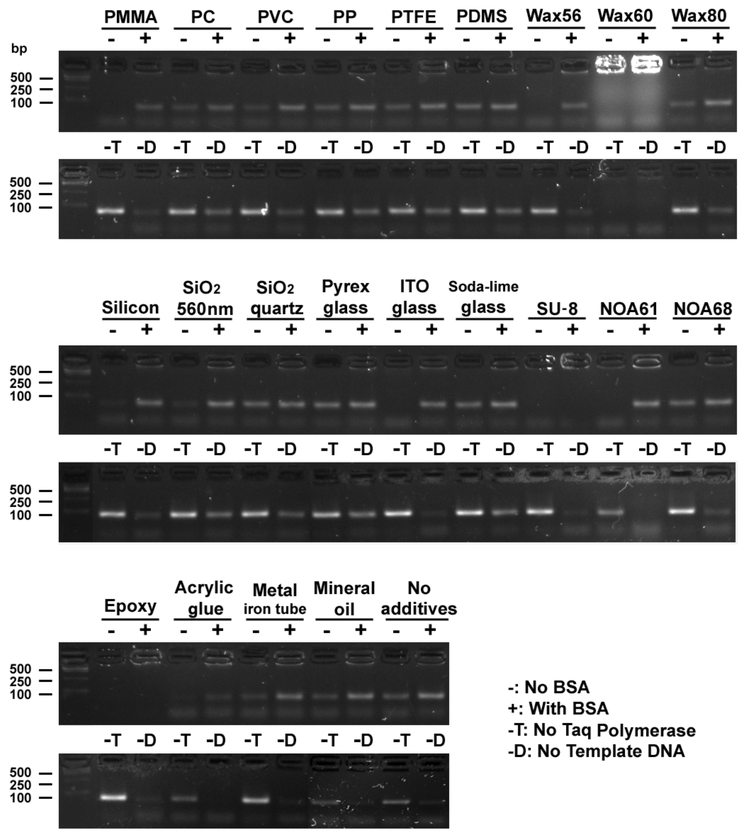
Step3
- Do not add anything for the "Mix"
- Add template DNA for "-DNA"
- Add SpeedStar polymerase for "-Pol"

Step 4
- Perform PCR cycling
- Run agarose gel

BSA - bovine serum albumin

Results

Agarose gel after PCR of 71 bp DNA fragment



Tabular summary

A. PCR inhibition through various materials
B. Comparison of PCR mix without BSA or containing BSA
C. PCR inhibition through material interaction with template DNA (no polymerase test)
D. PCR inhibition through material interaction with DNA polymerase (no DNA test)

PCR product yield is indicated by the star (x):.
- *(-) means no product is observed*
- *(xxxxx) indicate no PCR inhibition*

PMMA - polymethyl-methacrylate, PC - polycarbonate,
PVC - polyvinyl chloride, PP - polypropylene,
PTFE - polytetrafluoroethylene, PDMS-polydimethylsiloxane,
Wax 56 °C - white wax: paraffin from Nacalai Tesque,
Wax 60 °C - yellow wax: shiftwax from Nikka Seiko
Wax 80 °C - black wax: wax W from Apiezon
ITO - indium tin oxide glass,
SU-8 - cured SU-8 epoxy-based negative photoresist,
NOA61 and 68 - Norland Optical Adhesives 61 and 68.

Material	Signal - BSA	Signal + BSA	Signal - DNA	Signal - Pol
PMMA	-	xxxx	xxxxx	xx
PC	xx	xxxxx	xxxxx	xxxx
PVC	x	xxxxx	xxxxx	xxx
PP	xxxxx	xxxxx	xxxxx	xxxxx
PTFE	xxxxx	xxxxx	xxxxx	xxxxx
PDMS	xxxxx	xxxxx	xxxxx	xxxxx
Wax 55 °C	-	xxxxx	xxxxx	x
Wax 60 °C	-	-	-	-
Wax 80 °C	xxx	xxxxx	xxxxx	xxxxx
Silicon	-	xxxxx	xxxxx	xx
SiO_2 560 nm	-	xxxxx	xxxxx	xxxxx
SiO_2 quartz	xxxxx	xxxxx	xxxxx	xxxx
Pyrex glass	xxxxx	xxxxx	xxxxx	xxxxx
ITO glass	-	xxxxx	xxxxx	x
Soda-lime glass	xxxxx	xxxxx	xxxxx	xxxx
SU-8	-	-	xxxxx	x
NOA61	-	xxxxx	xxx	-
NOA68	xxxx	xxxxx	xxxxx	xxxx
Epoxy glue	-	-	xxxxx	x
Acrylic glue	-	x	xxx	-
Metal tubes	x	xxxxx	xxxxx	x
Mineral oil	xxxx	xxxxx	xxxxx	x
No additives	xxxxx	xxxxx	xxxxx	x

Observations

PCR mix inhibition experiments
- Either no signal or only a weak signal was obtained in PCR with PMMA, PVC, 56 °C and 60 °C wax, silicon, 560 nm SiO_2, ITO glass, SU-8, NOA61, epoxy and acrylic glues, and metal tubes (it indicates inhibition).
- However, if BSA was included in the PCR mix, a strong signal could be obtained for the PMMA, PVC, 56 °C wax, silicon, 560 nm SiO_2, ITO glass, NOA61 and metal tubes. That indicates 60 °C wax, SU-8, the epoxy and acrylic glues were PCR-inhibitory with or without additive BSA.
- Inclusion of BSA in the PCR reaction can significantly improve materials' surface biocompatibility, which improves, in turn, reaction performance and yield outcomes.
- Most bio-friendly materials exhibit similar signals regardless of the inclusion or not of BSA in the PCR mix; these are PP, PTFE, PDMS, 80 °C wax, SiO_2 quartz, pyrex glass, soda-lime glass, NOA68 and mineral oil.

Template DNA inhibition
- Total adsorption of template DNA observed for the 60 °C wax

DNA polymerase inhibition
- PP, PTFE, PDMS, 80 °C wax, 560 nm SiO_2, Pyrex, and soda-lime glass do not have any strong effects on polymerase.
- Slight DNA polymerase inhibition was observed with PC, PVC, SiO_2 quartz and NOA68.
- A very strong or total inhibition was observed with PMMA, 56 °C wax and 60 °C wax, ITO glass, SU-8, NOA61, epoxy and the acrylic glues

Achieving successful PCR

- Select biocompatible components (there are at least 12 crystalline forms of SiO_2; wax do not form a chemically homogenous group)
- Perform passive (static) or/ and active (dynamic) surface passivation
- Use adjuvants, additives or co-solvents (like BSA)
- Include mineral oil – then material interaction with walls are reduced
- Avoid using metal in enzymatic reactions
- Perform Two-temperature PCR with optimized component concentrations
- Increased template DNA and DNA polymerase concentrations leads to higher product yield

Conclusions

As part of the current miniaturization trend, biological reactions and processes are being adapted to microfluidics devices. And as PCR is the primary method employed in DNA amplification, its miniaturization is central to efforts to develop portable devices for diagnostics and testing purposes. A problem, however, is the PCR-inhibitory effect due to interaction between PCR reagents and the surrounding environment, which effect is increased in high-SAV microfluidics. Here, we introduced a simple test for assessing the biocompatibility of materials in PCR. Our test, instead of using microfluidic devices, can be easily conducted in common PCR tubes using a standard bench thermocycler to identify materials that inhibit DNA polymerase or DNA itself.