Electronic Supplementary Material (ESI) for Lab on a Chip

Supplemental Information

PCR Inhibition from AOMs

Initial experiments in developing a chip integrating an AOM for DNA extraction indicated that the AOM can inhibit PCR. The extent of the inhibition and possible causes and remedies for the inhibition were investigated. The extent of PCR inhibition due to contact with the AOM was determined using λ-DNA (Promega, Madison, WI). The PCR was performed with an Eppendorf Mastercycler personal (Eppendorf, Hauppauge, NY) benchtop thermocycler in 200-μL PCR tubes (Eppendorf) with a 50-μL reaction volume. A 294-bp region of λ-DNA was amplified with forward primer 5'-GGA TTT AGT GCG CTT TCT AC-3' and reverse primer 5'-GTG TGT GAT ACG AAA CGA AG-3'. The master mix consisted of primers (200 nM each) (Invitrogen) along with Qiagen HotStarTaq (2.5 units), PCR buffer (1x), and $MgCl_2$ (2.0 mM) (Qiagen, Germantown, MD) as well as dNTPs (200 μM each) (Stratagene, Cedar Creek, TX). PCR inhibition is expected to be proportional to the AOM surface area in contact with the PCR solution. To test this, varying amounts of membrane (from the 13-mm-diameter AOMs) were broken and placed into PCR tubes. The mass of AOM added to each tube was measured, and it was expected to correlate to the surface area. A series of reactions containing masses of AOM between 0 and 5 mg were performed. Five ng of template DNA were added to half of the reactions and 25 ng were added to the other half. The solutions were thermocycled with the following program: 95°C for 15 min, 30 cycles of 95°C for 30 sec, 54°C for 30 sec, and 72°C for 45 sec, and finally 72°C for 10 min. The PCR products were then analyzed with an Agilent 2100 Bioanalyzer (Santa Clara, CA). A plot of PCR product concentration vs. mass of AOM is shown in Fig. S3A. The amplification efficiency decreased with increasing mass irrespective of the starting amount of template DNA.

The initial amount of template did not affect the concentration of PCR products, indicating other factors were the cause of PCR inhibition. The *Taq* polymerase being adsorbed to the AOM was another potential cause of decreased PCR efficiency. Adding additional *Taq* polymerase or a blocking agent, BSA, to the master mix was investigated. Qiagen recommends 2.5 units of *Taq* polymerase per reaction and this was the amount used in the above experiment shown in Fig. S3A. Adding up to 2.5 additional units, in 0.5 unit increments, was tested. Adding Blocker BSA (Thermo Scientific) to a final concentration of 1% was also tested. All reactions used 5 ng of template DNA. Each reaction, except for two positive controls, also contained ~½ of an AOM, broken into pieces (4-5 mg). The reactions were thermocycled as described above and the products were again analyzed with the Bioanalyzer. A plot of PCR product concentration vs. concentration of *Taq* polymerase is shown in Fig. S3B. Increasing the amount of *Taq* led to increased product concentration, and the addition of BSA also led to product concentrations much closer to that of the controls. To offset the potential *Taq* polymerase adsorption, 1% BSA and additional *Taq* polymerase were added to the PCR master mix for all the other experiments discussed in this paper.

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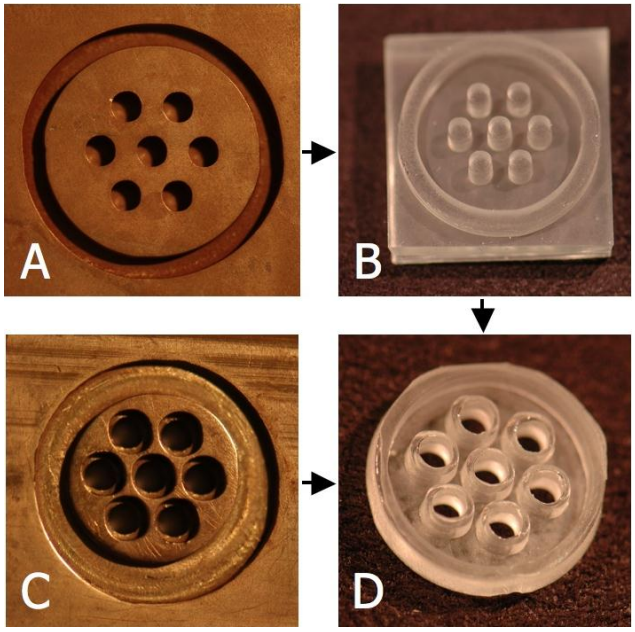

Fig. S1 Brass master (A) is used to cast PDMS half of reservoir mold (B) that is mated to a second brass master (C) to form a double-sided mold used to cast the 7-well PDMS reservoirs (D). The entire piece in B is 2.54 cm square.

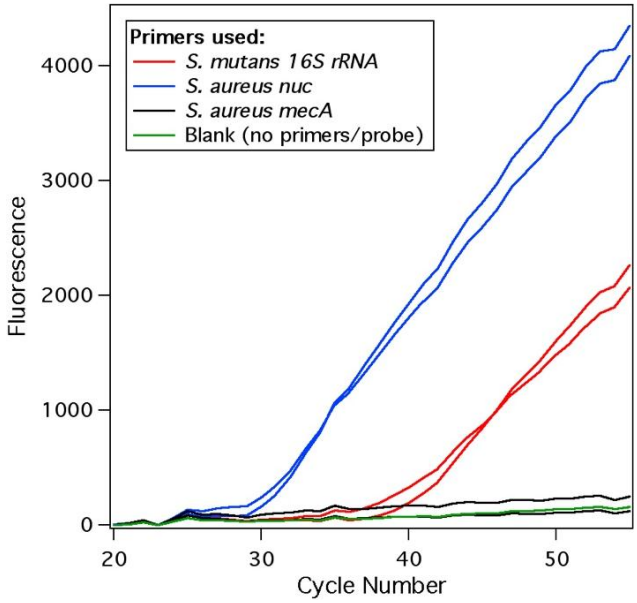

Fig. S2 Real-time amplification plot for *S. mutans 16S rRNA*, *S. aureus nuc*, and *S. aureus mecA* genes demonstrating simultaneous detection of multiple analytes (*S. mutans* and MSSA). Each well contained 1 pg of *S. mutans* gDNA and 1 pg of MSSA gDNA as the sample while the primers were varied between wells. There are two wells corresponding to each primer set and one blank well that did not contain any primers or probes.

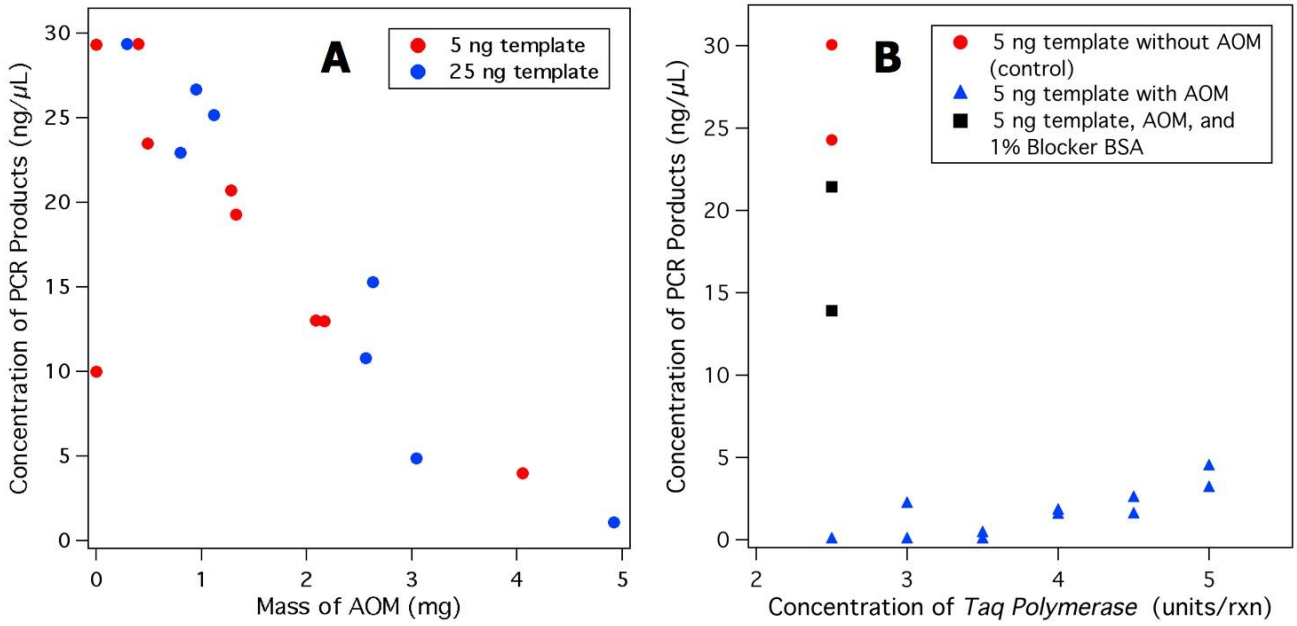

Fig. S3 (A) Plot of the concentration of PCR products vs. the mass of AOM added to the PCR solution. As the mass of AOM in the reaction mixture increases, the concentration of PCR products decreases. An outlier is seen at 0 mg AOM and 10 ng/μL PCR products. (B) Plot of the concentration of PCR products vs. the concentration of *Taq* polymerase in the reaction solution. 4-5 mg of AOM was present in all solutions except the controls, and 1% BSA was added to two solutions without additional *Taq*. The addition of BSA or extra *Taq* resulted in an increased concentration of PCR products, but the addition of BSA resulted in a much more dramatic increase.