Supplementary Material (ESI) for Lab on a Chip


# Wax-Bonding 3D Microfluidic Chips

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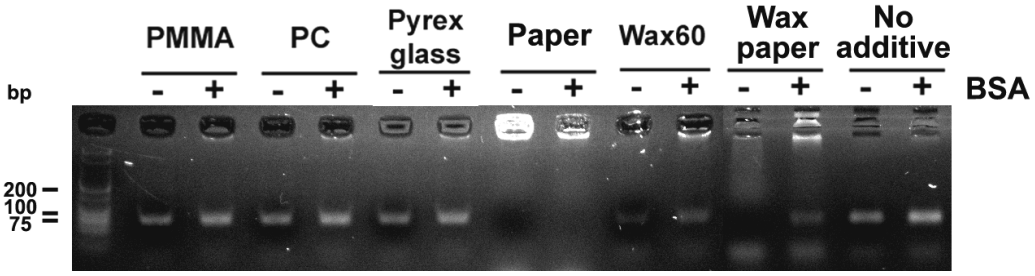


**Fig. S1** PCR biocompatibility with materials used in the study. The reaction outcome can be seen in PCR with and without BSA.

(The native picture of agarose gel electrophoresis contains few dust-like particles. However, these particles do not change the result in any way. The dark spots seen at the size of expected product are from the loading dye.)

**PCR methodology**

pEYFP-C1 vector (total size: 4.731 kbp) was purchased from Clontech. PCR amplification experiments were carried out with pEYFP-C1 plasmid containing a 589 bp CMV fragment. The oligonucleotides used in the amplification were CMV368F (ATGCGGTTTTGGCAGTACATCAATGGGCGT) and CMV409R (GGGTGGAGACTTGGAAATCCCCGTGAGTCA). The PCR consisted of the following components in their final concentrations: 0.33 $\mu$M primers (Life Technologies), 3.5 mM $MgCl_2$ (KapaBiosystems), 0.2 mM dNTP (Takara Bio Inc.), 1x FBI reaction buffer, up to 0.2 mM (0.008%) cresol red (Sigma), 1.2 M Betaine (Sigma), 2 million pEYFP-C1 template molecules, and 1.8 U/$\mu$l SpeedStar HS DNA polymerase (Takara Bio Inc.). The PCR reaction was set at 30 $\mu$l to facilitate the recovery of the PCR mix after incubation with the material. The PCR conditions were the following: 91 $^{o}$C for 20 seconds, followed by 71 $^{o}$C for 20 seconds, 35 cycles. The bench thermocycler employed was a MyGenie 96 Gradient Thermal Block (Bioneer

Corporation), which uses Peltier elements for heating/cooling and has a ramping rate of 2.5 C per second (maximum). PCR product detection was achieved by running samples in 4% Agarose gel containing SYBR Safe DNA stain (Life Technologies) and by subsequent gel imaging. A low-molecular-weight DNA ladder (NEB) was applied as a reference in estimating the sizes of the DNA fragments.

**Total reaction inhibition experiment**

Two PCR master mixes were prepared and distributed among 7 wells each. The first PCR mix was prepared without BSA; the second contained BSA at a final concentration of 2 $\mu$g/$\mu$l. The PCR mix was added to the material fragments to test the biocompatibility. The tubes were briefly vortexed to mix the material with the PCR solution. After incubation at room temperature for 30 minutes, PCR was performed on a bench thermocycler. After the PCR, the materials were removed and, for visualization, the amplification products were loaded directly onto the gel.

BSA is thought to compete with DNA polymerase for adsorption at the chip walls and, thus, to improve PCR yields. BSA also acts as a polymerase competitor in inhibitor chelation. Additionally, BSA thickens the PCR mix, facilitates primer annealing, stabilizes both the DNA and the DNA polymerase, and in so doing, acts as an osmo-protectant. We set the final BSA concentration at the 2 $\mu$g/$\mu$l.

**Chip fabrication process**

The chip is immersed in a hot-water (red ink dyed) bath (70$^{o}$C) which is placed in a vaccum chamber. After vacuating the chamber, the melt wax will be driven out from the inlet and outlet of the chip (See the red label in Fig. S2), floating to the top layer of water bath, because the melting wax has lower density than water. Repeating venting and vacuating several times to make sure all the channels are filled with. Then, take the chip out and flush it with cool water for bonding.

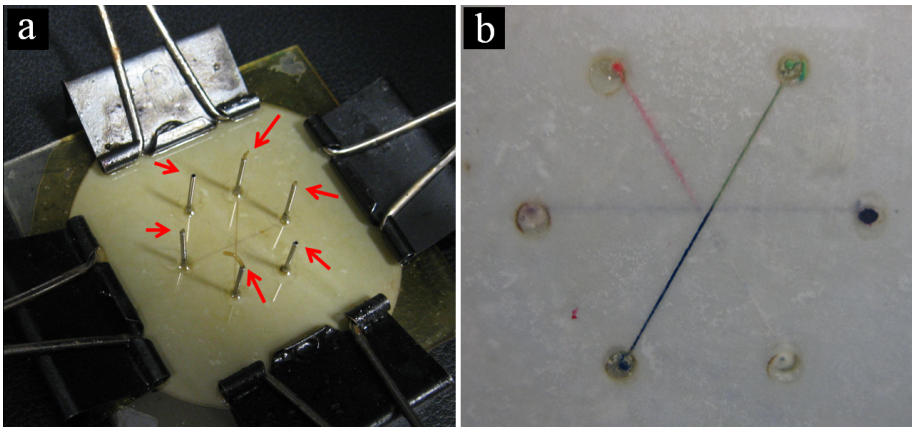


**Fig. S2** (a) Optical picture to show the process of chip fabrication. Three layers of paper were sandwiched between two glass slides, pressed together by four clips. After melting and evacuating in vacuum chamber, the melt wax will be pumped out of the channel through the inlets and outlets (As labeled by red arrow). Here the picture was taken as soon as the chip was taken out from the vacuum chamber; (b) Different colorful inks were infused into the channels to demonstrate the layer-over-layer structure.

**The bonding strength**

The bonding strength of the wax-paper bonded chip is tested using a single straight channel with an inlet and an outlet located at each end, respectively. Air was pumped in through the inlet, and the pressure was monitored using the Druck DPI 104 type digital pressure sensor (GE Druck, Leicester, UK) which is connected to the outlet. The critical bonding strength is defined when the air pressure reached a critical value, the layers bonding was broken, resulting in gas leakage.

**Table. S1** Bonding strength as measured by sandwiching wax-paper between two layers of glass or PMMA

| **Bonding Strength P=F/S** | **S=1.13×10^(-4) $m^2$** | | | **Average F** | **Critical Bonding Strength (kPa)** |
|---|---|---|---|---|---|
| **Force/N (Glass)** | 40 | 32 | 45 | 38.4 | 340 |
| **Force /N (PMMA)** | 49 | 36 | 32 | 41.8 | 370 |