Supplemanary Material (ESI) for lab on a chip

Wax-Based Microfluidic Chip and Its Application in One Step 3D Bonding

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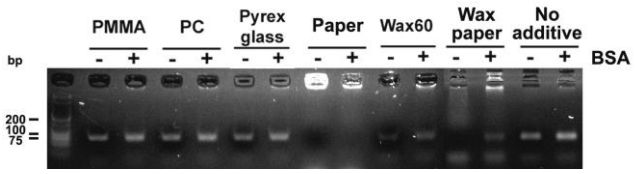

Fig. S1 PCR biocomapatibility with materials used in the study. The reaction outcome can be seen in PCR with and without BSA.

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 μM 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/μl SpeedStar HS DNA polymerase (Takara Bio Inc.). The PCR reaction was set at 30 μl 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 μg/μl. 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 μg/μl.