Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information:

A Filter Paper-Based Microdevice for Low-Cost, Rapid, and Automated DNA Extraction and Amplification from Diverse Sample Types

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Materials and methods

STR typing from filter paper disc

DNA Typer^{TM} 15 STR system (Institute of Forensic Science, Ministry of Public Security, Beijing, China) was employed to test the quality of DNA extracted from human whole blood on the microdevice for forensic DNA typing. The STR loci in the 15-plex system are Amelogenin, D3S1358, D6S1043, D5S818, D13S317, vWA, D21S11, D8S1179, D18S51, D7S820, D16S539, CSF1PO, FGA, Penta E, and D2S1338. Two-μL human whole

blood was processed on the Figure 1A microdevice following the protocol described above. After that, a quarter of the filter paper disc was cut off with a razor blade and put into a tube for the multiplex STR amplification. Each 10 μL reaction is composed of 1 μL primer mix of the 15 primer pairs, 2 μL DNA Typer™ 15 PCR Mix, 0.6 μL hot-start polymerase, 5.4 μL DI water, and 1 μL of 50 μg/μL BSA. The thermal cycling conditions were an initial denaturation step at 95 ℃ for 11 min, followed by 29 cycles of 94 ℃ for 30 s, 59 ℃ for 3 min and 72 ℃ for 1 min, and a final extension of 60 ℃ for 30 min.

Prior to capillary electrophoresis analysis, the amplicons were diluted by mixing 1 μL of the PCR products and 10 μL of a loaing buffer composed of the Typer™ 500 internal size standard provided in the kit and Hi-Di™ formamide (Applied Biosystems, Foster City, CA) in a proportion of 1:2.5 (v/v). Capillary electrophoresis was performed on a 16-capillary ABI Prism® 3130xl Genetic Analyzer (Applied Biosystems) using the protocol developed by the Institute of Forensic Science. The STR alleles were separated at 15 kV at a run temperature of 60°C. Data were collected using the AB Data Collection Software V3.0 and analyzed using the GeneMapper® ID software V3.2 (Applied Boiosystems).

DNA sequencing from filter paper disc

To test the quality of DNA extracted on the microdevice for downstream DNA sequencing, a primer set (forward: 5'ATGCTTGCTTACCCAGACTCAG, reverse: 5′GAAGATGCTGCTTGTGTAGGTC) was designed to amplify a 520-bp fragment from the GJB2 gene for further Sanger sequencing analysis. Again, 2-μL human whole blood was processed using the Figure 1A microdevice and a quarter of the filter paper disc was cut off for the amplification of GJB2 gene following the same protocol as mentioned above. Post-PCR enzyme purification was performed by adding a 1.5 μL mixture of antarctic phosphatase and exonuclease I (New England Biolabs,

Ipswich, MA) to 4.5 μL of PCR products and incubating at 37℃ for 15 min, followed by 80 ℃ for 20 min for enzyme inactivation.

The purified PCR products were sequenced using the BigDye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's manual. Briefly, the chain-termination DNA sequencing reaction was performed in a 10-μL volume, composed of 0.5 μL each primer (10 μM), 0.6 μL 2.5X BigDye™, 2 μL 5X BigDye™ Buffer, 1 μL purified PCR product and 5.4 μL DI water. The PCR protocol was 96 ℃ for 1 min followed by 25 cycles of 96 ℃ for 10 s, 50 ℃ for 5 s, and 60 ℃ for 4 min. The Sanger sequencing products were cleaned up using the ammonium acetate-ethanol precipitation purification method, and gently resuspended in 10-μL Hi-Di™ formamide. Electrophoretic separation was performed on an ABI 3730xl DNA sequencer and data were analyzed using the Sequencing Analysis Software V5.3.1 (Applied Biosystems).

Supplementary Figures:

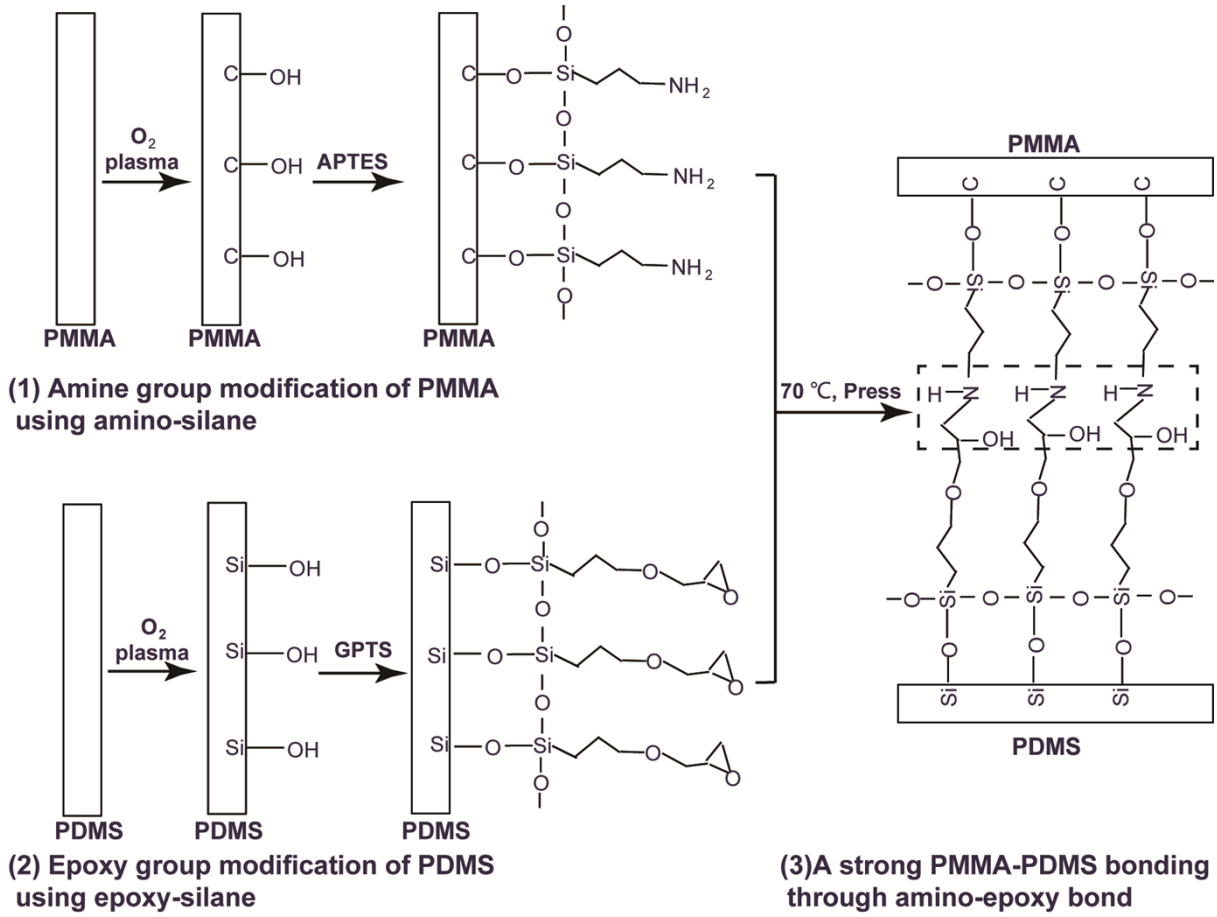

Figure 1S: Schematic of the PMMA-PDMS bonding process.

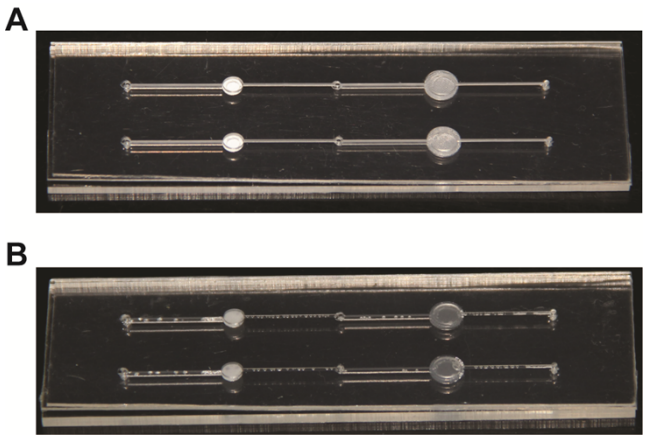

Figure 2S: Photographs of the permanently bonded microdevice (A) before and (B) after PCR. An irreversible PMMA-PDMS-PMMA bonding without bubbles was achieved using a modified protocol.