Supplementary Materials

Article

PDMS-Based Microdevices for the Capture of MicroRNA Biomarkers

Lorenzo Lunelli [1,2], Federica Barbaresco [3,†], Giorgio Scordo [3,†], Cristina Potrich [1,2,*], Lia Vanzetti [1], Simone Luigi Marasso [3,4], Matteo Cocuzza [3,4], Candido Fabrizio Pirri [3,5] and Cecilia Pederzolli [1]

[1] Fondazione Bruno Kessler, Center for Materials and Microsystems, via Sommarive 18, I-38123 Povo (Trento), Italy; lunelli@fbk.eu (L.L.); vanzetti@fbk.eu (L.V.); pederzo@fbk.eu (C.P.)

[2] CNR—Consiglio Nazionale delle Ricerche, Istituto di Biofisica, via alla Cascata 56/C, I-38123 Povo (Trento), Italy

[3] Department of Applied Science and Technology, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy; federica.barbaresco@polito.it (F.B.); giorgio.scordo@polito.it (G.S.); simone.marasso@polito.it (S.L.M.); matteo.cocuzza@infm.polito.it (M.C.); fabrizio.pirri@polito.it (C.F.P.)

[4] CNR-IMEM, Parco Area delle Scienze 37a, 43124 Parma, Italy

[5] Istituto Italiano di Tecnologia, Center for Sustainable Future Technologies @ PoliTo, Corso Trento 21, 10129 Torino, Italy

* Correspondence: cpotrich@fbk.eu; Tel.: +39-0461-314-605

† These authors contributed equally.

PCR Assay to Test PDMS Compatibility

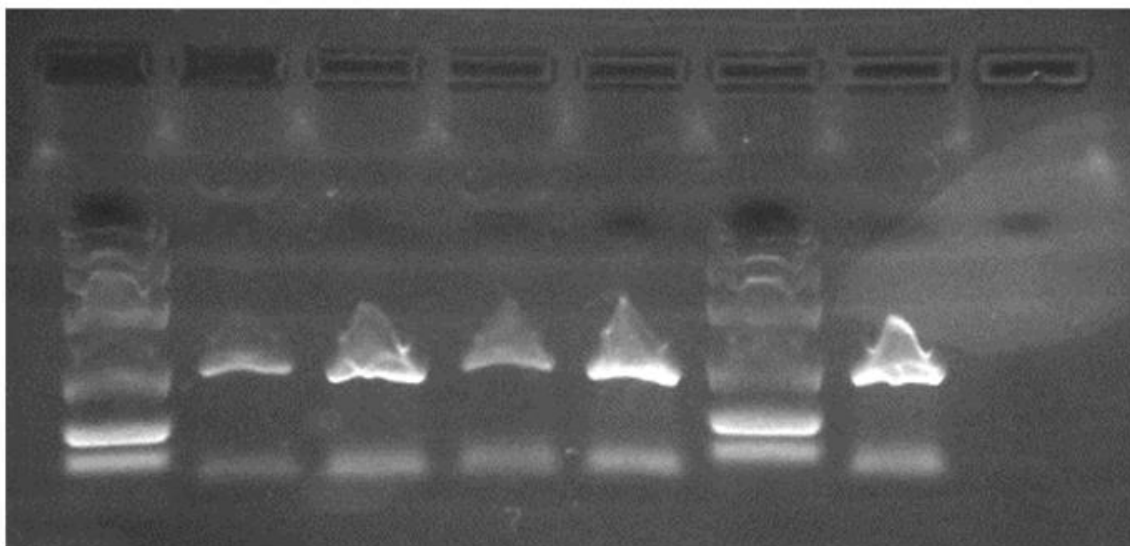

Figure S1 PCR-compatibility of spiral microdevices (lanes 2 and 3), compared to drop-shaped microdevices (lines 4 and 5) and controls (positive control: lane 7 and negative control: no template, lane 8). Lanes 1 and 6: low mass DNA ladder Invitrogen. Microdevices were cut and a piece of spiral chip (10 mg in lane 2, 3 mg in lane 3) or drop-chip (9.2 mg in lane 4 and 4.6 mg in lane 5) was submerged in PCR mix. The amplification of a fragment of the hemochromatosis gene was performed. The PCR products were loaded in a 2% agarose gel. No inhibition of PCR by thirichloromethylsilane potentially still present on microdevices channels was observed.

Material and Methods:

To test the PCR compatibility of PDMS spiral-shaped microdevices, small pieces of PDMS exposing channel surface were weighed in a PCR tube and submerged with the PCR mix to a final volume of 50 µL. Similar pieces of a drop-shaped PDMS microdevice known for its good PCR compatibility [1,2], were included in this test. The PCR mix contained 0.2 mM nucleotides, 0.5 µM of each primer, 30 ng genomic DNA and 1 unit of FastStart Taq DNA Polymerase (all reagent were purchased from ThermoFisher Scientific). The following primers were utilized: C282Yfor (5'-TGGCAAGGGTAAACAGATCC-3') and C282Yrev (5'-TACCTCCTCAGGCACTCCTC-3') to amplify a 395 bp region on hemochromatosis (HFE) gene. Primers were acquired from IDT Integrated DNA Technologies (Belgium). PCR was run on the Eppendorf thermal cycler (Mastercycler ep Gradient S) with the following thermal protocol: 5 min at 95 °C (initial DNA denaturation), 30 s at 95 °C, 30 s at 62 °C and 30 s at 72 °C (these three steps were repeated for 35 cycles), and 7 min at 72 °C (final PCR product elongation). The PCR product was analyzed on a 2% agarose gel.

Optimization of silanization time

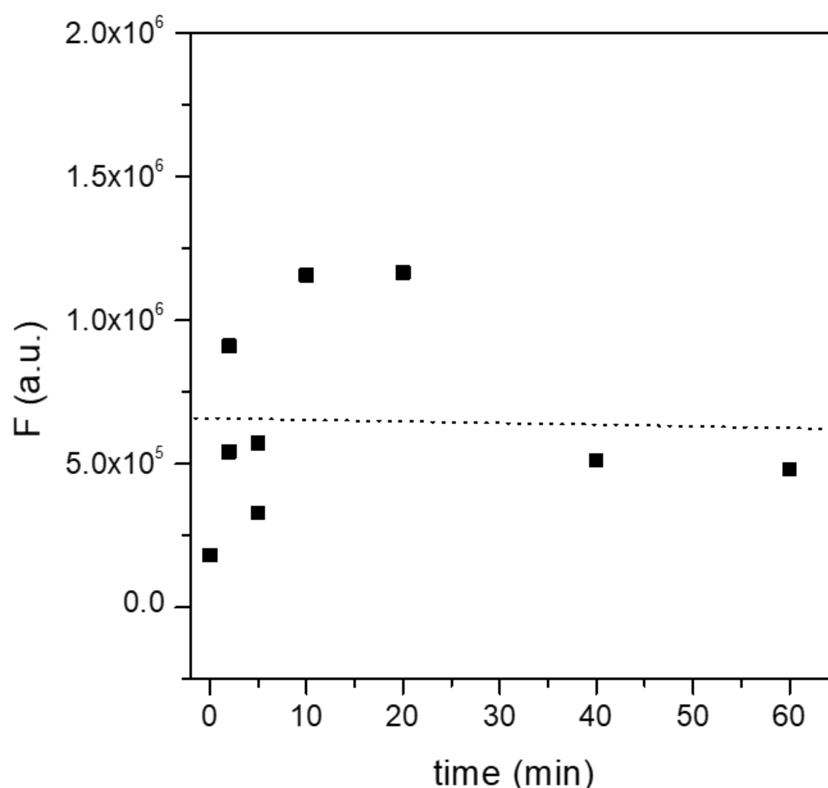

Figure S2. The microRNAs solution recovered from experiments depicted in panel (a) of **Error! Reference source not found.**5 were measured at the spectrofluorimeter, confirming 5 min of silanization as the best compromise for the silanization protocol.

Material and Methods:

The unbound microRNA collected from microdevices silanized with different times was measured with a spectrofluorimeter (FluoroMax-4, Horiba Jobin Yvon), using an excitation wavelength of 552 nm and recording the emission spectrum from 557 to 700 nm. The area between 580 and 590 nm was integrated and the obtained value is reported in Figure S2.

Fluorescent microRNAs adsorption on microdevices

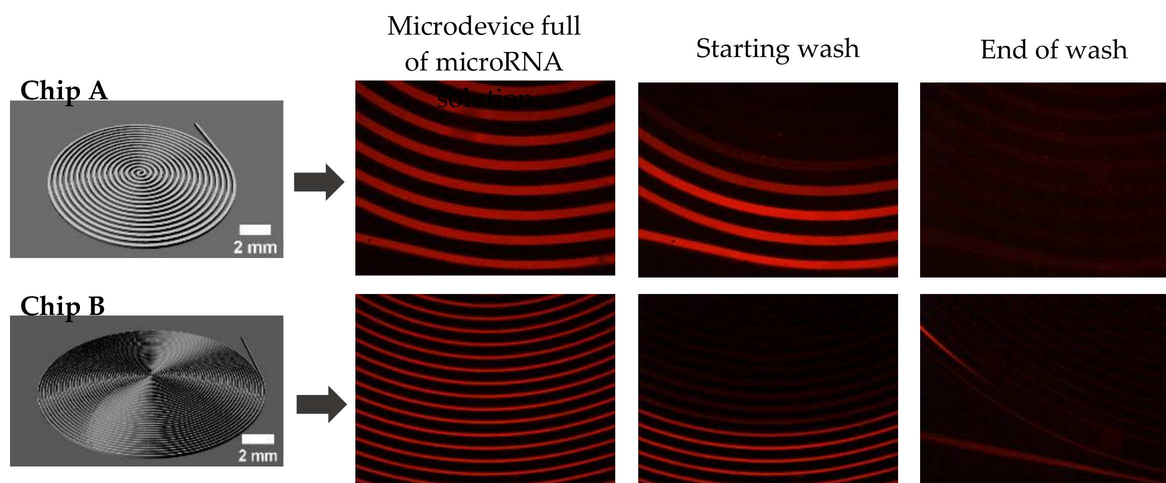

Figure S3. Imaging of insertion and washes of microRNAs in microdevices (fluorescence microscope 2.5X objective). Washing starts from the inlet hole and proceeds through the coils up to the ending straight channel (data acquisition parameters optimized for miRNA solution).

References

1. [1] C. Potrich, V. Vaghi, L. Lunelli, L. Pasquardini, G. Santini, C. Ottone, M. Quaglio, M. Cocuzza, P. CF, F. M, M. Negrini, P. Tiberio, V. De Sanctis, B. R and C. Pederzolli, "OncomiR detection in circulating body fluids: a PDMS microdevice perspective," *Lab Chip*, vol. 14, no. 20, pp. 4067-75, 2014.
2. [2] L. Pasquardini, C. Potrich, M. Quaglio, A. Lamberti, S. Guastella, L. Lunelli, M. Cocuzza, L. Vanzetti, C.F. Pirri, C. Pederzolli, Solid phase DNA extraction on PDMS and direct amplification, *Lab Chip* vol. 11, no. 23, pp 4029–4035, 2011.