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

A Continuous Flow PCR Array Microfluidic Chip Applied for Simultaneous Amplification of Target Genes of Periodontal Pathogens

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Experimental section

Preparation of the PCR solution

To reduce the absorption of Taq-polymerase, Bovine Serum Albumin (BSA) was added into the PCR solution(Cady et al. 2005). 50 μL PCR reagent consisted of 2.0 μL DNA template, 4.0 μL dNTP mixture (2.5 mM), 5.0 μL 10× Fast Buffer I, 1.0 μL forward and backward primer (10 μM). 4.0 μL PVP (0.085 mM), 0.25 μL SpeedSTAR HS DNA Polymerase (5 U μL^{-1}) (Takara, Dalian, China), 10.0 μL BSA solution with a final concentration of 0.2 μg/μL, and 20.25 μL ultra-pure water. For comparison, the sample was amplified in T100 thermal cycler (Bio-Rad, USA). The PCR consisted of 40 cycles of 95°C (10 sec) and 64°C (30 sec) after 95°C (2 min). For negative control, the DNA template was not added into the PCR solution.

Capillary electrophoresis system

All the PCR products were validated in a self-built capillary electrophoresis (CE) system, which has been detailed described in Ref(Li et al. 2019; Li et al. 2021). In brief, it was composed of a confocal optical system, a MODEL 610E high voltage power supply (TREK, USA), and a photomultiplier (PMT) (R928, Hamamatsu photonics, Japan). The confocal optical system was based on a BX51 epi-illumination microscope (Olympus, Japan). The light centered at 490 nm was achieved from a mercury lamp. Then it passed through the mixture of DNA and SYBR Green I in the capillary. The fluorescence from the mixture was collected by a PMT. NI-USB-6212 card (National Instrument, USA) and a self-built LabVIEW software were used for input signal generation and output signal detection. The total length and the effective length of the capillary (Ruifeng Chromatography Ltd., Handan, China) were 15 cm and 12 cm, respectively. The samples were electrokinetically introduced into the capillary. The capillary was cleaned by deionized water after each run. All the separations were performed in dark house at room temperature.

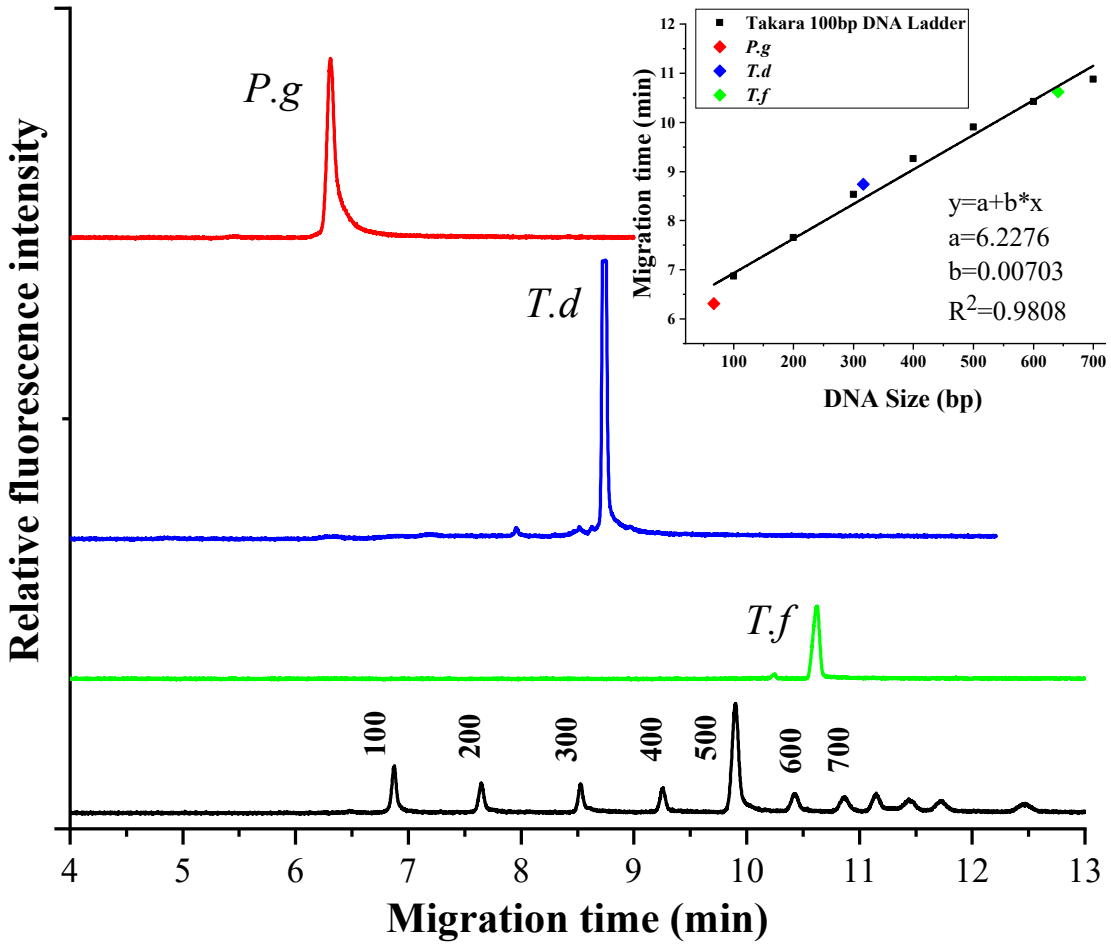

Fig.S1 The electropherogram of periodontal pathogens after they were amplified by the T100 Thermal cycler. Electrophoretic conditions: 0.5% HEC (1300k) in 0.5× TBE solution; electric field strength: 100 V/cm; sample injection (1500 V, 2.0 s); total length and effective length of the capillary: 15.0 cm/12.0 cm.

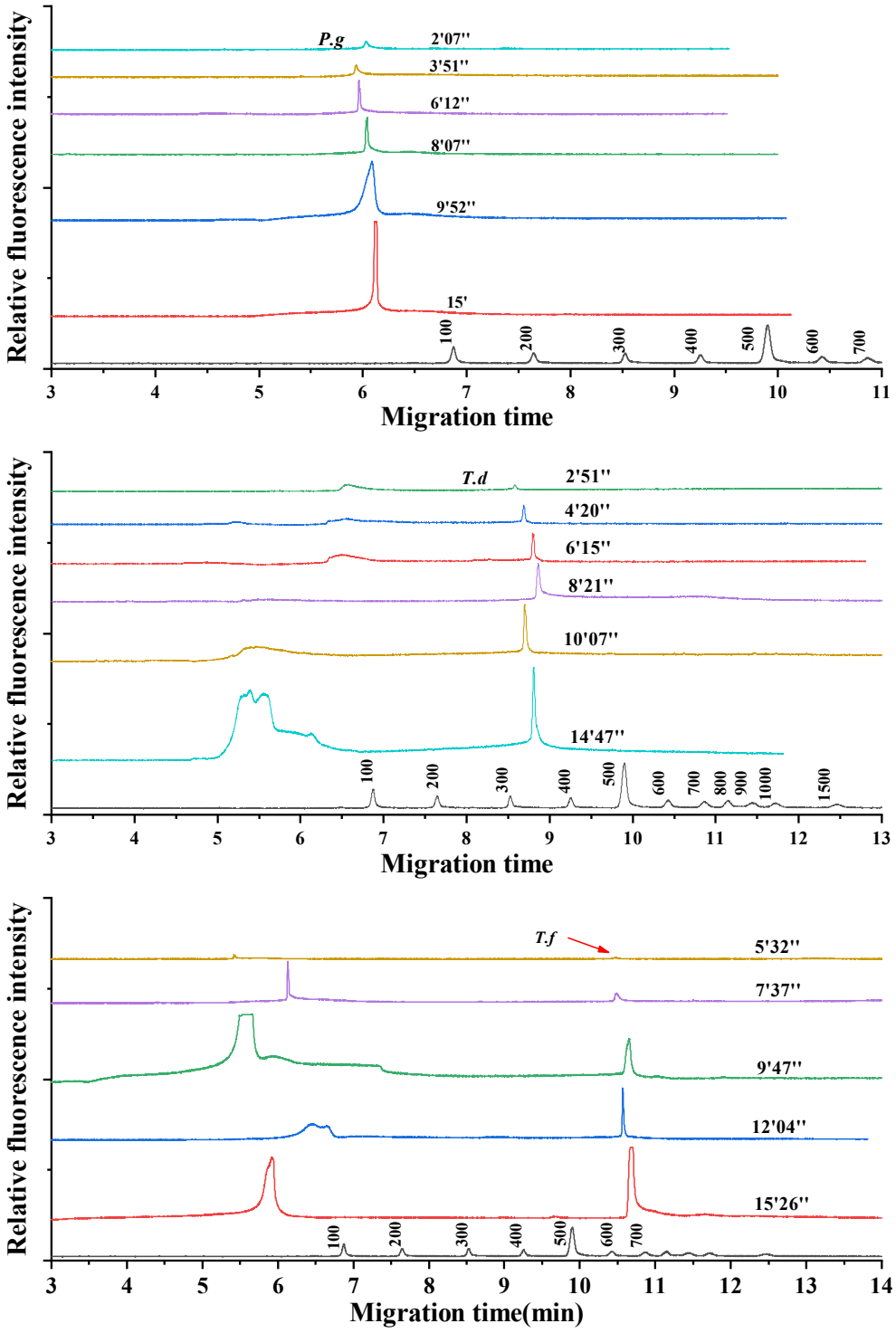

Fig.S2 The electropherogram of PCR products of *P.g T.d T.f* after they were amplified in the CF-PCR array microfluidic chip with different running time. Electrophoretic conditions: 0.5% HEC (1300k) in 0.5× TBE solution; electric field strength: 100 V/cm; sample injection (1500 V, 2.0 s); total length and effective length of the capillary: 15.0 cm/12.0 cm.

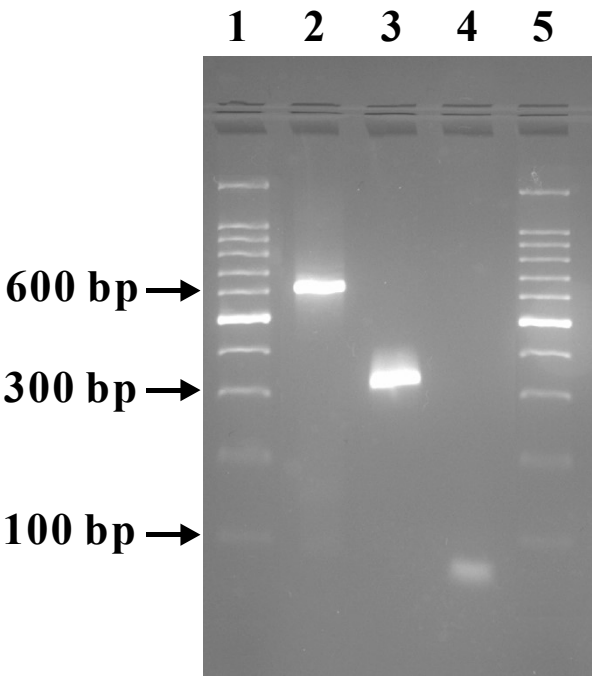

Fig.S3 Slab gel electrophoresis of 100 bp DNA Ladder(Takara, Japan)(lane 1 and lane 5) and CF-PCR products of *P.g*(lane4), *T.d*(lane 3), and *T.f*(lane 2). Electrophoretic conditions: 3.0% agarose(Solarbio, USA), 4 V/cm(Bio-Rad PowerPac Basic, USA).

Table S1 The physical parameters of materials in simulation.

Material	Density (kg/m^3)	Specific Heat (J/ kg·K)	Thermal Conductivity (W/ m·K)	Viscosity (kg/ m·s)
Aluminum	2719	871	202.4	--
Silicon dioxide	2200	966	270	--
Water	998.2	4182	0.6	0.001003
PDMS	1000	1.46×10^{-3}	0.15	--

Table S2 Detailed information about the time consumption and the peak height.

Time consumption	No.1	No.2	No.3	Average	RE
Part I	8.38	8.50	8.24	8.37	0.130
Part II	8.50	8.17	8.33	8.33	0.165
Part III	8.20	8.26	8.75	8.40	0.302
Peak height	No.1	No.2	No.3	Average	RE
Part I	2.69643	2.63483	2.58943	2.64023	0.053704004
Part II	2.55012	2.20392	2.62772	2.460586667	0.225640806
Part III	1.95141	2.2039	3.01042	2.388576667	0.553131674

The unit for the time consumption is min. RE denotes relative error. Average means average value. No.1-No.3 means three times.