Supporting information of

Plasma nanotextured polymeric lab-on-a-chip for highly efficient bacteria capture and lysis

K. Tsougeni [a], G. Papadakis [b], M. Gianneli [b], A. Grammoustianou [b], B. Dupuy [d], P. N. Petrou[e], S. E. Kakabakos[e], A. Tserepi [a], E. Gizeli [b, c] E. Gogolides[a*]

[a] *Institute of Nanoscience and Nanotechnology, NCSR Demokritos, 15310 Aghia Paraskevi, Attiki, Greece*

[b] *Institute of Molecular Biology & Biotechnology, FO.R.T.H, 100 N. Plastira str, 70013 Heraklion, Greece*

[c] *Dept. of Biology, Univ. of Crete, Vassilika Vouton, Heraklion, Greece*

[d] *Laboratoire Pathogenèse des Bactéries Anaérobies, Institut Pasteur, Paris, France*

[e] *Institute of Nuclear & Radiological Sciences & Technology, Energy & Safety, NCSR Demokritos, 15310, Aghia Paraskevi, Greece*

Table S1. Bacterial strains and plasmids used in this study

Strains and plasmids	Genotype/relevant characteristics
Salmonella 6910	WT ATCC14028
E. coli TG1	WT *E. Coli* K12

Plasmids	Genotype/relevant characteristics
pFPV25,1	GFP, $Ampi^R$
pMW211	RFP, $Ampi^R$

$Ampi^R$ refers to ampicillin resistance.

Direct detection of Salmonella-GFP cells on plasma nanotextured polymers: Bacteria sample preparation

In Fig. S1 a schematic for bacteria sample preparation is shown. Photos of Lysogeny Broth (LB) medium 1) before cultivation, 2) after overnight cultivation at

37 °C in LB. 3) After first centrifugation (4000 rpm 4 min). The following steps include removal of LB supernatant, addition of PBS and centrifugation (2 repetitions). 5) OD measurement at 600 nm. The following standard was used: for OD=0.2 (linear region), $C=3.2*10^8$ cells/mL. Serial dilutions down to 10^2 cells/mL. 5) Perform plating on petri dishes with LB-agar (3 replicates) for both initial concentrations and chip effluents.

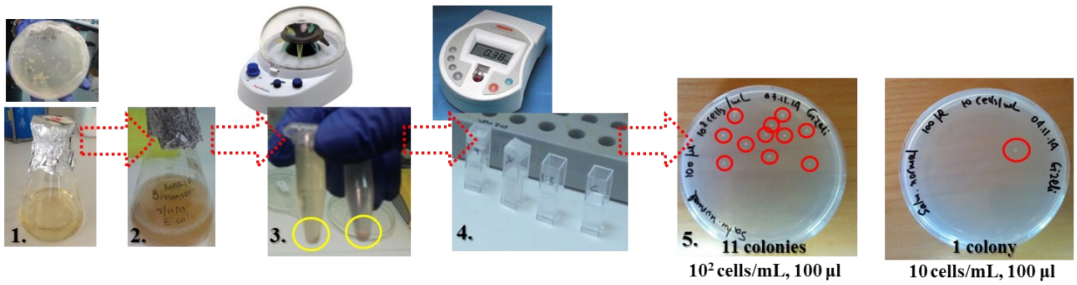


Fig. S1 Schematic of bacteria sample preparation.

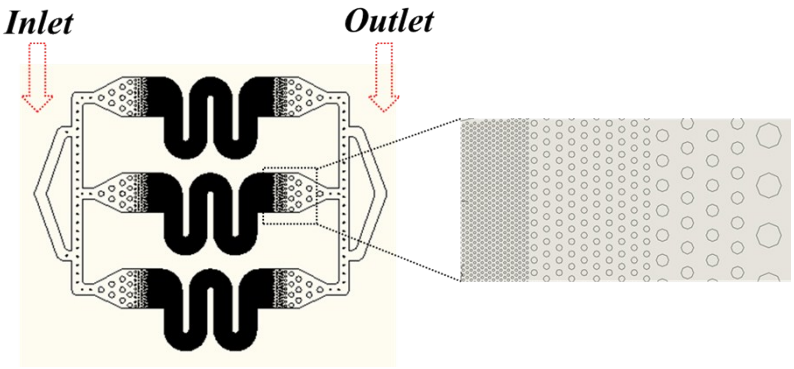
Table S2. The primer pairs used and the corresponding fragment sizes produced by PCR

Target gene	Primer pair	PCR fragment size (bp)
Salmonella *purE*	Forward: GACACCTCAAAAGCAGCGT Reverse: AGACGGCGATACCCAGCGG	635
Salmonella *ttrR*	Forward: GGATGATGATACGGCGGTC Reverse: CTTCCGCAATTTCACGGTTC	455
E. coli *ompG*	Forward: GTGATGTGTGCGGGAATGG Reverse: CAAATTTATACATCGACAACCAACC	313

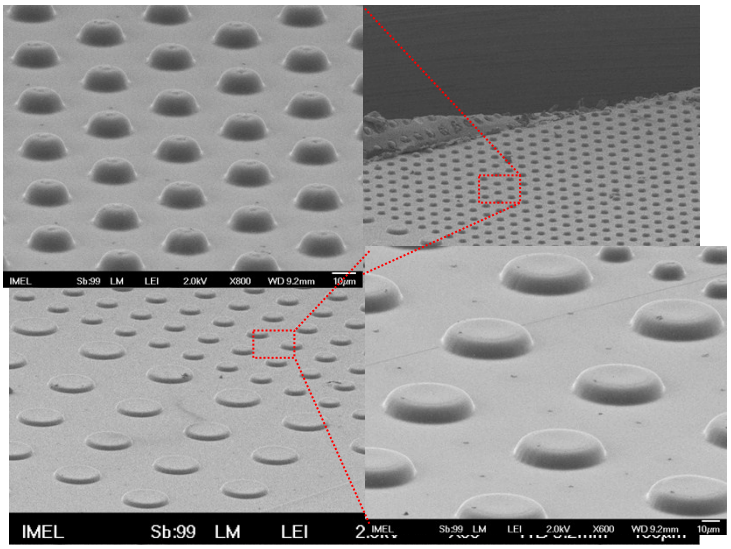
Fabrication of bacteria capture module

Fig. S2 (a, b) shows the layout of the chip design with 3 parallel microchannels and illustrates SEM images of the PMMA bacteria capture module

after lithography with a thin inorganic–organic hybrid photoresist (ORMOCER – Ormocomp). The etching rate of Ormocomp photoresist is 25 nm/min after an initial 120 nm thickness loss during the first minute (see K. Tsougeni et al., Photolithography and Plasma Processing of Polymeric Lab on Chip for Wetting and Fouling Control and Cell Patterning, Microelectr. Eng., 2014, 124 (0), 47-52).



(a)



(b)

Fig. S2 (a) Layout of the chip design with 3 parallel microchannels. (b) The microfluidic channels of PMMA after lithography with ORMOCER photoresist. The Si containing polymer layer (~ 6 µm) was exposed through a photo-mask using UV broadband light 365-nm, and then the soluble unexposed part was removed (developed) by means of MIBK (methyl-isobutyl-ketone) and IPA (iso-propyl-alcohol).

Table S3. Total capture module area and volume of capture module

Total Capture Module Area
1. Area $_{\text{empty chip}}$ = 668.6 mm^2
2. Area $_{\text{post projection}}$ = 162.4 mm^2
3. Area $_{\text{free chip}}$ = 506.2 mm^2
4. Area $_{\text{post sidewall}}$ = 491.8 mm^2
5. Area $_{\text{ALL CHIP}}$ = 998 mm^2
Volume of Capture Module
Volume $_{\text{empty chip}}$ = 16.7 µl
Volume of posts = 4 µl
Volume of wells = 7.1 µl
VOLUME $_{\text{FREE}}$ = 19.8 µl

Direct counting of fluorescence images

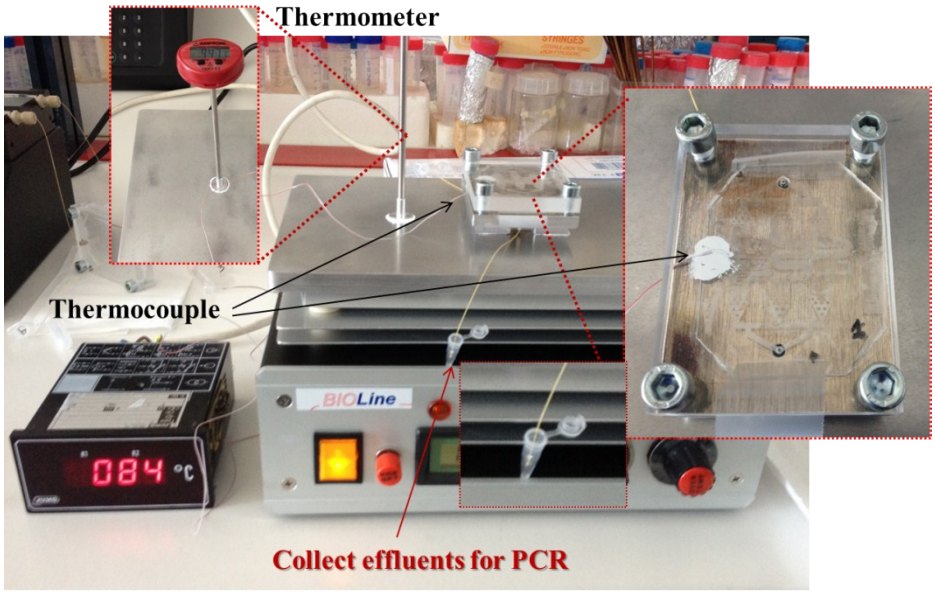
Table S4. Counting of Data

Antibody	**Initial Concentration (cells/mL)**	**Total InjectedNumber of Cells in chip**	**Total captured cells in chip**	**Capture efficiency (%)**
Polyclonal: AbD Serotec	10^8	10^7	3610234	36
	10^7	10^6	508929	51
	10^6	10^5	75686	76
	10^5	10^4	9002	90
	10^4	10^3	1213	100
Polyclonal: KPL	10^8	10^7	2704840	27
	10^7	10^6	441924	44
	10^6	10^5	62480	62
	10^5	10^4	8858	88
	10^4	10^3	1730	100
	$5*10^2$	$5*10^1$	1294	100
Monoclonal: MyBioSource	10^8	10^7	853697	9
	10^7	10^6	188848	18
	10^6	10^5	39401	39
	10^5	10^4		

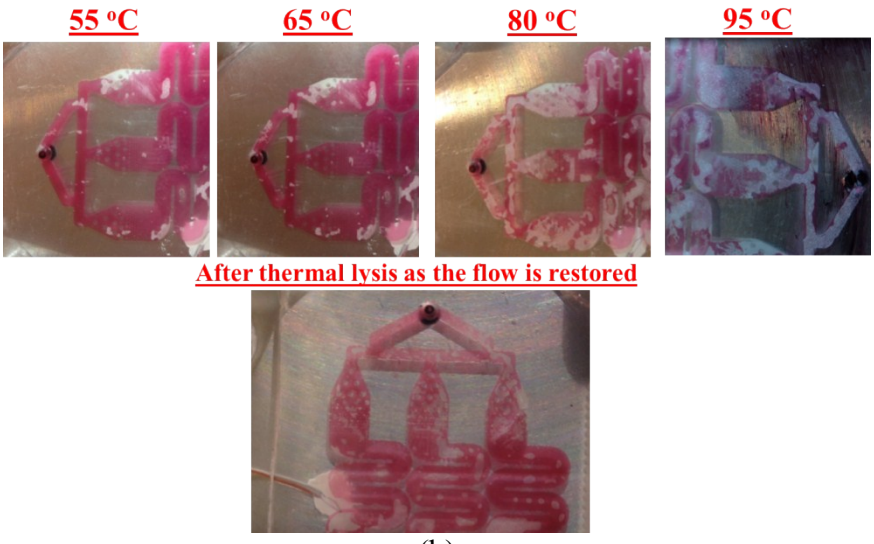
	10^4	10^3	1741	100

Thermal lysis on chip performed on a hotplate: Experimental set-up

Fig. S3 (a) illustrates the experimental setup that used for bacteria thermal lysis on chip. Thermal lysis was performed on a hotplate. A thermocouple and a thermometer were used to control the temperature inside the microchannel. The device holder and the tube where the effluents were collected are shown in enlargement. Fig. S3 (b) shows snapshots during heating of chip. The vapor formed at 95 °C but no leakage was observed after flashing the chip to collect the lysis effluents.



(a)



(b)

Fig. S3 (a) The experimental set up for thermal lysis on chip. (b) Snapshots during heating of chip.

Thermal lysis on chip followed by conventional off chip PCR and agarose gel electrophoresis of PCR products: How much effluent to collect

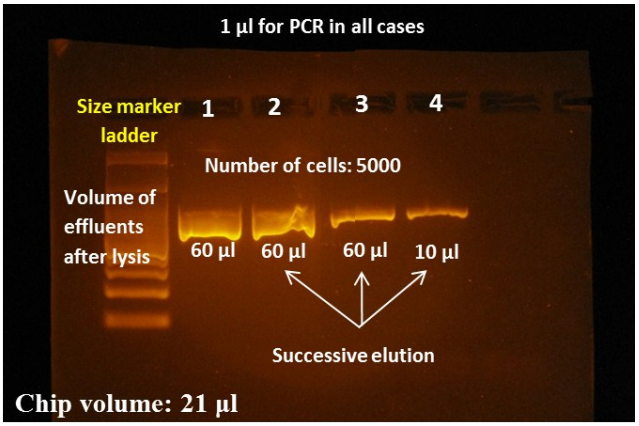


Fig. S4 Agarose gel electrophoresis of salmonella DNA (derived from 5000 cells) after thermal lysis on chip and off chip PCR. Lane 1 corresponds to 60 µl effluent. Lanes 2, 3, 4 correspond to successive elutions. The total volume extracted from chip was 190µl.

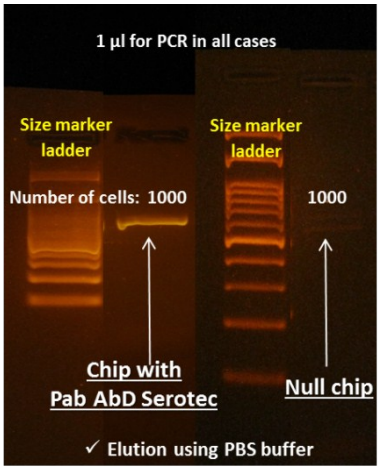


Fig. S5 Comparison experiment between a null chip containing mouse gamma globulin rather than AbD Serotec anti-LPS antibody and a chip with the specific antibody of AbD Serotec. 1000 cells were injected in both cases.

Table S5. Specificity of *S.* Typhimurium versus *E. coli* on PMMA cell capture microchips

Cell [mixture (1:1)]	Initial Concentration (cells/mL)	Injected Volume (µl)	Total Injected Number of Cells in chip	Injected cells/mm^2	Captured Cells/mm^2	Selectivity
Monoclonal antibody MyBioSource						
Salmonella (green)	1,00E+08	20	2,00E+06	3,16E+03	2,77E+02	492:1
***E. coli* (red)**	1,00E+08	20	2,00E+06	3,16E+03	5,63E-01	
Polyclonal antibody AbD Serotec						
Salmonella (green)	1,00E+08	20	2,00E+06	3,23E+03	3,92E+02	500:1
***E. coli* (red)**	1,00E+08	20	2,00E+06	3,23E+03	7,88E-01	
Polyclonal antibody KPL						
Salmonella (green)	1,00E+08	20	2,00E+06	3,23E+03	5,38E+02	330:1
***E. coli* (red)**	1,00E+08	20	2,00E+06	3,23E+03	1,63E+00	