On-Chip Cell Lysis by Antibacterial Non-leaching Reusable Quaternary Ammonium Monolithic Column

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Bacterial culture

Kocuria rosea (ATCC 186), Pseudomonas putida (ATCC 12633), and Escherichia coli (ATCC 35218) were cultured in nutrient broth (NB), while Micrococcus luteus (Schroeter) (ATCC 4698) cells were cultured in trypticase soy broth (TSB). The Kocuria rosea (ATCC 186), Pseudomonas putida (ATCC 12633) cells in the cultured media were placed in a shaking incubator at 26 °C (37 and 30 °C for Escherichia coli (ATCC 35218) and Micrococcus luteus (Schroeter) (ATCC 4698), respectively) and were shaken at 180 rpm for 18-36 hours. The bacterial cultures were grown until their mid-late exponential phase was reached. The concentration of the bacterial cultures was quantified by the plate-count technique, by performing serial dilution plating on solid agar media (NB agar for ATCC 186, ATCC 12633 and ATCC 35218, and trypticase soy agar for ATCC 4698) and incubation overnight at 26 °C for ATCC 186 and ATCC 12633 (37 and 30 °C for ATCC 35218 and ATCC 4698, respectively). The Pseudomonas putida (ATCC 12633) and Escherichia coli (ATCC 35218), Micrococcus luteus (ATCC 4698) and Kocuria rosea (ATCC 186) counts were on average 1.6×10^5, 1.5×10^5, 1.8×10^5 and 1.9×10^5 colony forming units (CFUs) per milliliter of culture. These concentrations were used in all the experiments done in this work unless otherwise mentioned.

PCR reagents and experimental setup

The structure of Micrococcus luteus (ATCC 4698), Kocuria rosea (ATCC 186), Pseudomonas putida (ATCC 12633), and Escherichia coli (ATCC 35218) primers used in the PCR reaction is provided in Table S1. The PCR tubes were first preheated and incubated at 95°C for 3 min for initial denaturation, and then the PCR thermal cycler was programed to run for 30 cycles at 95°C for 30 sec, 55°C (6°C gradient) for 70 sec, 72°C for 70 sec, and 72°C for 10 min for the final extension step.

Table S1: Primers used in the PCR experiments

Bacterial strain	Primer direction	Primer sequence
ATCC 12633	Forward	GTTGCCAATGACGAAACCTAC
	Reverse	CGTAGCCGTGTCTACTGATTTA
ATCC 35218	Forward	CTTATGGCGGCGTGTTATCT
	Reverse	CTCCGGTACGTGCGTAATTT
ATCC 4698	Forward	GTCAGAGAGTTCTGGCGTAATC
	Reverse	CAGTTGATGCCAGACGAGATAG
ATCC 186	Forward	ACGATCGTCGAGATGGAGAA
	Reverse	GTGGTTGGTGTTGTCGTAGA

PPM characterization

The chemical structure of the three PPM columns investigated in this work is provided in Figure S1. It can be seen that the cross-linked PPM networks have positive charges scattered along the polymer structure, due to the presence of DADMAC.

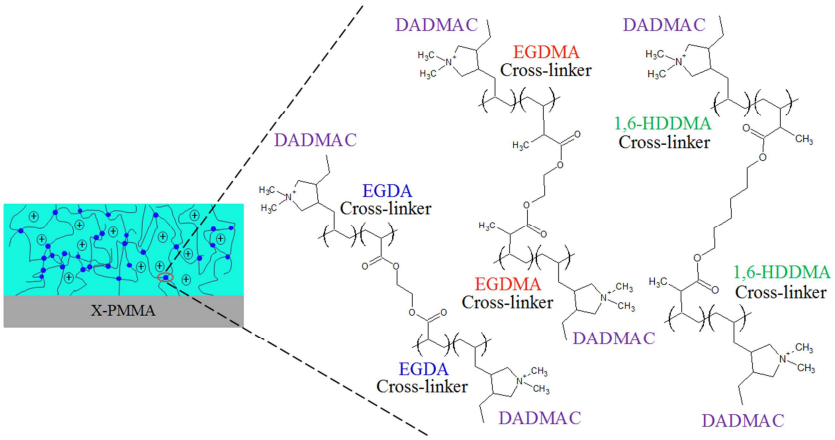

Figure S1: Structure of cross-linked poly(DADMAC-*co*-EDGA), poly(DADMAC-*co*-1,6-HDDMA), and poly(DADMAC-*co*-EGDMA) networks.

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra for the DADMAC monomer (in powder form), poly(DADMAC-*co*-EGDMA), poly(DADMAC-*co*-EGDA), and poly(DADMAC-*co*-1,6-HDDMA) were obtained on a Nicolet 4700 FTIR spectrometer as shown in Figure S2. The band at 3470 cm^{-1} is attributed to the N–H stretching in the unsaturated primary amine groups of DADMAC.[1] The peak at 1741 cm^{-1}, corresponding to the C=O stretching from EGDA, EGDMA and 1,6-HDDMA[2] is absent in the DADMAC spectrum. The absorption at 1644 cm^{-1} represents the C–N stretching vibration in secondary amine group, followed by the NH bend. The band at 1482.5 cm^{-1} indicates the presence of a long carbon chain with a high degree of regularity for the backbone structure.[3]

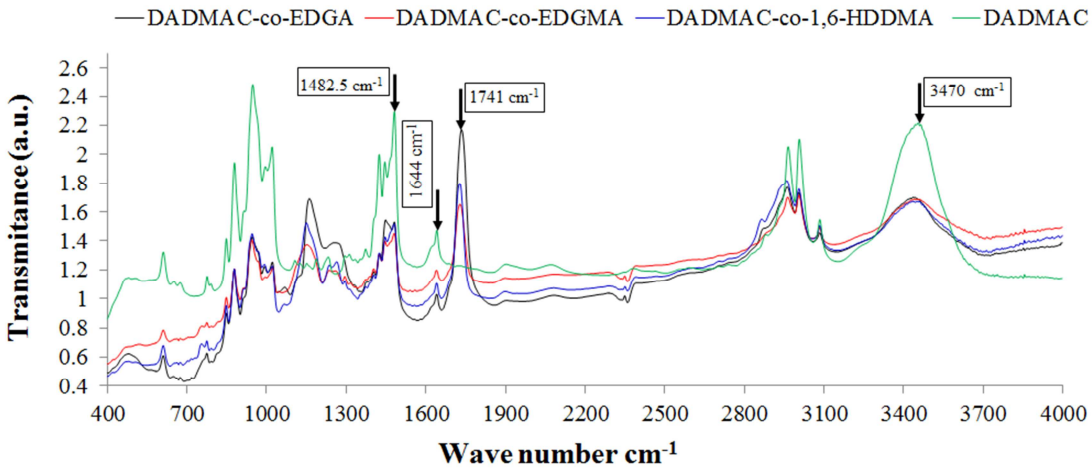

Figure S2: ATR-FTIR spectra for DADMAC (powder), poly(DADMAC-*co*-EDGA), poly(DADMAC-*co*-1,6-HDDMA), and poly(DADMAC-*co*-EGDMA).

Antibacterial activity

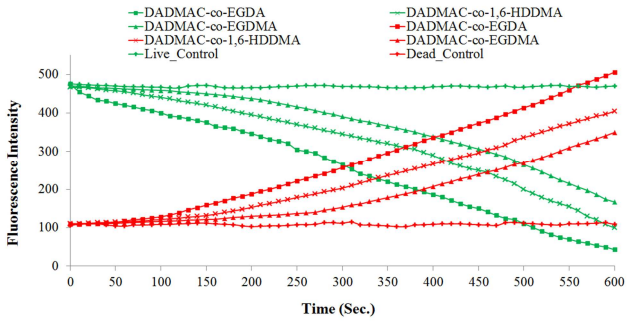

Figure S3: Green (live) and red (dead) stain intensity variation with the contact time for poly(DADMAC-*co*-EGDA), poly(DADMAC-*co*-1,6-HDDMA), and poly(DADMAC-*co*-EGDMA) networks.

Cell Lysis Efficiency

Table S2: Standard deviation (σ) on the measurements presented in Figure 9, repeated 3 times.

PPM	ATCC 12633	ATCC 35218	ATCC 4698	ATCC 186
Poly(DADMAC-*co*-EGDA)	275.3	270.7	337.7	210.6
Poly(DADMAC-*co*-1,6-HDDMA)	398.7	139.8	473.4	348
Poly(DADMAC-*co*-EGDMA)	230.5	473.8	199.6	309.7

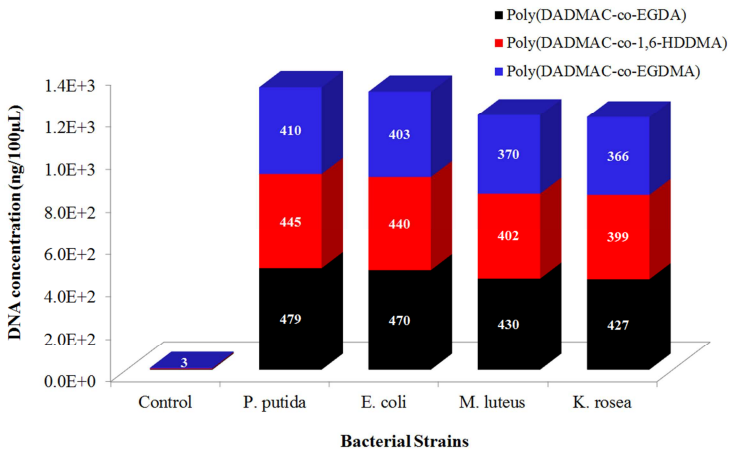

Figure S4: Absolute concentration of DNA present in unlysed cells (control) and in the crude cell lysates collected at the outlet of the three PPM columns.

Table S3: Standard deviation (σ) on the measurements presented in Figure 10, repeated 3 times.

PPM	ATCC 12633	ATCC 35218	ATCC 4698	ATCC 186
Poly(DADMAC-co-EGDA)	4.3	1.8	3.3	2.6
Poly(DADMAC-co-1,6-HDDMA)	2.9	3.5	1.7	2.2
Poly(DADMAC-co-EGDMA)	3.2	2.1	4.5	3.9

Reusability of the microchips

The florescence intensity of EtBr mixed with the PBS recovered from the microfluidic channel in the back-flush cycle showed insignificant DNA carryover, reaching only 0.6% of the maximum EtBr intensity reported, as shown in Table S4 and plotted with the control sample in Figure S5.

Table S4: DNA carryover after each backwash cycle

Cycle number	Fluorescence intensity	Cycle number	Fluorescence intensity
1	16457	24	17061
2	16860	25	18933
3	16451	26	17781
4	16993	27	17554
5	16375	28	17999
6	16700	29	18091
7	15793	30	17715
8	16991	31	18003
9	18964	32	18804
10	18970	33	18811
11	17378	34	19012
12	17556	35	18020
13	17809	36	17549
14	17985	37	17578
15	18001	38	17791
16	18129	39	18099
17	17701	40	18365
18	18730	41	18807
19	18000	42	19262
20	19113	43	19578
21	19567	44	19793
22	19996	45	19985
23	16672		

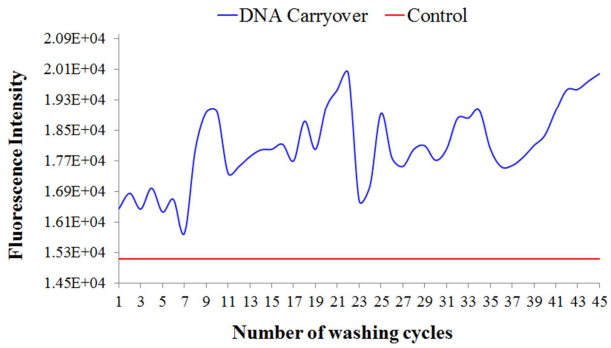

Figure S5: DNA carryover collected in wash cycles (PBS) after each PPM use and control sample.

References

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