Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Identification of Methicillin-resistant *Staphylococcus aureus* using an integrated and modular microfluidic system

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Reagents and materials. Genomic DNA samples including MSSA (ATCC 25923D-5), MRSA (ATCC BAA-1556D-5), MS-CNS (ATCC 12228D-5) and MR-CNS (ATCC 35984D-5) were all purchased from American Type Culture Collection (Manassas, VA). PC and PMMA sheets used as the fluidic substrates were purchased from Good Fellow (Berwyn, PA). Chemicals used for the PMMA surface modification and hybridization assays included 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS) and 20X SSC buffer (3 M sodium chloride, 0.3 M sodium citrate, pH 7.0), which were purchased from Sigma-Aldrich (St. Louis, MO). A 10% sodium dodecyl sulfate (SDS) stock solution, which was used for post-hybridization washing, was received from Ambion (Austin, TX). Disodium hydrogen phosphate (Na_2HPO_4), sodium phosphate (Na_3PO_4), and 2-propanol were obtained from Sigma-Aldrich (St. Louis, MO). Oligonucleotide primers and probes were obtained from Integrated DNA Technologies (Coralville, IA). Their sequences and melting temperatures (T_m) are listed in Table S1. All solutions were prepared in nuclease free water purchased from Invitrogen Corporation (Carlsbad, CA).

Fabrication of the fluidic cartridge. The modular fluidic cartridge, which follows a design concept similar to our previously published work,[1, 2] consisted of one module interconnected to a motherboard (see Figure 1A); the motherboard, which was made from PC, was used for two thermal reactions, PCR and LDR, while the module fabricated in PMMA contained DNA probes spotted on an air-embedded waveguide that used evanescent excitation for fluorescence readout. The PMMA module was interconnected to the PC motherboard using short pieces of Tefzel™ tubing (OD = 1/16”, ID = 250 µm, Upchurch) inserted between conically-shaped holes placed on the backsides of both the motherboard and module to provide a leak-free interconnection.

The PC motherboard was replicated using double-sided hot embossing from two brass molding tools mounted opposite to each other on a JENOPTIK HEX02 hot embossing machine (Jena, Germany). Brass mold masters were used to create microstructures on the PC substrate and contained microchannels for PCR and LDR on the frontside of the substrate and thermal isolation grooves[3] and conical interconnectors on the backside. The mold masters were fabricated using high-precision micromilling.[4] The layout of the PC motherboard is depicted in Figure 1A. The dual-depth serpentine channel, which consisted of 32 cycles for PCR and 15 cycles for LDR, was 80 µm in width and 80/240 µm in depth; the dual depth was designed to prolong the residence time in the extension/ligation zone by increasing the channel cross-section causing a net decrease in the linear flow rate in these zones when operated at a constant volume flow rate. Fluid access channels (DNA sample and LDR mixture) were 200 µm

in width and 240 µm in depth. The PC motherboard was assembled by thermal fusion bonding the substrate and a PC cover plate (250 µm thick) together by placing in a convection oven at 150°C for 20 min.

The PMMA module was assembled from two hot-embossed PMMA parts; one consisted of a 100 µm deep microchannel for the universal microarray on the frontside and a 1.2 mm x 10 mm air-embedded planar waveguide with an integrated prism on the backside (see Figure 1B). Located on the backside were also positioned conical ports for fluidic connections to the PC motherboard. The air-embedded waveguide was double-sided hot embossed into the PMMA substrate via micro-replication from two brass molding tools. Twenty-four (4 x 6) waveguide modules could be replicated on one PMMA wafer in a single embossing step.

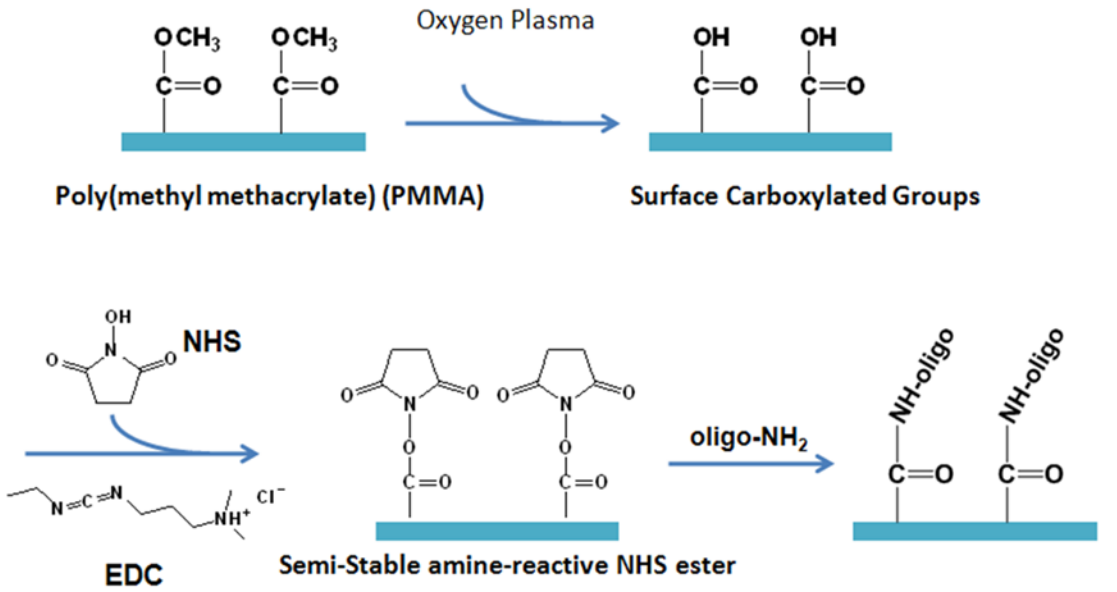
PMMA surface modification for amine modified oligonucleotide zip-code probe attachment was carried out using a previously published protocol,[5-7] which is schematically illustrated in Scheme S1. Briefly, surface carboxylic acids were generated by placing PMMA substrates in the vacuum chamber of a Technics Series 8000 micro-reactive ion etcher (Surplus Process Equipment Corp., Santa Clara, CA) for 1 min using a 200 mTorr oxygen pressure and a 50 W radio frequency. Then, the PMMA substrates were incubated in a coupling buffer containing 50 mg/mL EDC and 5 mg/mL NHS in MES (2-(N-morpholino)ethanesulfonic acid) buffer (pH 6.0) for 10 min to form succinimidyl ester intermediates, which can react with amine-terminated oligonucleotide probes (3'-end) to form a stable amide bond. The 3'-amino-modified oligonucleotide zip-code probes (see Table S1 for sequences) were dissolved in 200 mM Na_2HPO_4/Na_3PO_4 buffer (pH 9.0) to a final concentration of 50 µM and were dispensed on the activated PMMA surface using a Perkin-Elmer Piezorray® non-contact microarray instrument (Downers Grove, IL). Dispensing volumes per spot were 330 ±30 pL and the size of the spots were ~150 µm in diameter. After incubation in a humidified chamber at room temperature for 4 h, the spotted PMMA substrates were washed with 0.1% SDS to remove non-specifically adsorbed oligonucleotides and stored at 4°C until used for the measurements. Following spotting of the DNA probes onto the waveguide, the waveguide and the interconnection layer were assembled together using a thermal annealing method at 107°C for 20 min.

Table S1. Sequences of oligonucleotides used in the PCR/LDR/universal array zip-code hybridization assay.

PCR product size (bp)	Oligos	Sequence (5'→3')	T_m (°C)[e]
148	*mecA*-forward	TGGTATGTGGAAGTTAGATTGG	51.2
	mecA-reverse	ATATGCTGTTCCTGTATTGGC	52.8
	mecA-com	p[a]ATTCCTGGAATAATGACGCTA–Cy5[b]	51.2
	cZip1-*mecA*	[c]**GCTGAGGTCGATGCTGAGGTCGCA**ATGTATGCTTTGGTCTTTCTGC	69.2
	Zip-code 1	TGCGACCTCAGCATCGACCTCAGC–spacer-NH_2[d]	64.9
98	*spa*-forward	CATTACTTATATCTGGTGGCG	50.6
	spa-reverse	GTTAGGCATATTTAAGACTTG	46.5
	spa-com	p[a]TTGCGCAGCATTTGCAG–Cy5[b]	54.9
	cZip21-*spa*	[c]**GGTCAGGTTACCGCTGCGATCGCA**TTTTGTTGAGCTTCATCGTG	69.0
	Zip-code 21	TGCGATCGCAGCGGTAACCTGACC–spacer-NH_2[d]	65.3
161	SG16S-forward	TGGAGCATGTGGTTTAATTCGA	54.7
	SG16S-reverse	TGCGGGACTTAACCCAACA	56.8
	SG16S-com	p[a]TTGGTAAGGTTCTTCGCG–Cy5[b]	52.5
	cZip25-SG16S	[c]**GGTCTACCTACCCGCACGATGGTC**GAGTTGTCAAAGGATGTCAAGAT	68.8
	Zip-code 25	GACCATCGTGCGGGTAGGTAGACC–spacer-NH_2[d]	62.7
172	*femA*-foward	CAACTCGATGCAAATCAGCAA	54.1
	femA-reverse	GAACCGCATAGCTCCCTGC	58.6
	femA-com	p[a]ATAATTAATCCGTTTGAAGTAGTTT–Cy5[b]	49.0
	cZip15-*femA*	[c]**CGCATACCAGGTCGCATACCGGTC**CCATCTCTGCTGGCTTCTTT	71.0
	Zip-code 15	GACCGGTATGCGACCTGGTATGCG–spacer-NH_2[d]	63.5

176	PVL-forward	ACACACTATGGCAATAGTTATTT	50.2
	PVL-reverse	AAAGCAATGCAATTGATGTA	48.4
	PVL-com	p[a]GAGTTTTCCAGTTCACTTCATATT–Cy5[b]	51.3
	cZip5-PVL	[c]**GCTGTACCCGATCGCAAGGTGGTC**TTATGTCCTTTCACTTTAATTTCAT	66.5
	Zip-code 5	GACCACCTTGCGATCGGGTACAGC–spacer-NH_2[d]	63.7

[a] p: phosphorylated.
[b] Cy5: λ_{ex} = 649 nm, λ_{em} = 670 nm.
[c] The bold sequences are complementary to the sequences of zip-code probes.
[d] spacer-NH_2: $(CH_2CH_2O)_6PO_4$-NH_2.
[e] Conditions: oligo concentration, 1 μM; Na^+ concentration, 50 mM.



Scheme S1. The procedure used for PMMA surface modification and oligonucleotide probe immobilization. The plasma-modification process for the DNA microarray involved three steps; (1) oxygen plasma exposure of the polymer surface; (2) EDC/NHS treatment to facilitate the formation of the succinimidyl ester intermediate; and (3) carbodiimide coupling of amine-terminated oligonucleotide probes to the activated PMMA surface (via an amide bond).

Contact angle measurements. Water contact angle measurements were used to probe the effect of oxygen plasma treatment on the PMMA surface. Substrates (2.54 cm x 1.27 cm x 0.25 cm) were cut from a commercial PMMA sheet. After oxygen plasma treatment, contact angle values of the PMMA surfaces were measured using a VCA 2000 contact angle system equipped with a CCD camera (VCA, Billerica, MA) based on the sessile drop method. Approximately 2 µL of deionized water was placed on the PMMA surface using a syringe and the contact angle of the water droplet was measured immediately using the software provided by the manufacturer. The measurements were repeated at least three times at separate positions on each sample surface. Data points represent the mean of three measurements with the error bars showing the standard deviation.

X-ray photoelectron spectroscopy. PMMA sheets (1 cm x 1 cm x 0.25 cm) treated under the chamber pressure range of 50–300 mTorr were analyzed with an Axis 165 X-ray photoelectron spectrometer (Kratos Analytical) using a monochromatized X-ray source (Al Kα 1486.6 eV) with a power of 150 W. The binding energy scale was calibrated to $Au4f_{7/2b}$= 84.0 eV. The base pressure was 2 x 10^{-10} Torr and the operating pressure was 2 x 10^{-9} Torr. The photoelectron

takeoff angle was 90° (with respect to the sample surface). Survey and high-resolution spectra were obtained using pass energies of 160 and 20 eV, respectively. The neutralizer was turned on during the analysis to compensate for any possible charge effects on the insulating polymer surfaces. Core level binding energies for C1s and O1s were determined by referencing the methyl carbons to 285.0 eV and carbonyl oxygens to 532.2 eV.[8] Curve fitting of the high-resolution spectra of the pristine and oxygen plasma-modified PMMA was performed using asymmetric 70% Gaussian and 30% Lorentzian component profiles after subtraction of the baseline using a linear background.

Supporting Results

Characterization of oxygen plasma-modified PMMA surfaces. In our protocol, PMMA sheets were subjected to an oxygen plasma treatment for the generation of surface carboxylic acid groups that allowed covalent attachment of amine-terminated oligonucleotide zip-code probes to these surface groups through carbodiimide coupling.[1, 2, 7] The modification process is believed to involve the creation of free radicals on the polymer surface and the subsequent coupling of these free radicals with active species from the oxygen plasma environment.[9] Oxygen plasma treatment of the PMMA sheets resulted in the surfaces becoming hydrophilic, which can be monitored using sessile water contact angle measurements; lower water contact angles can be indicative of a higher surface load of carboxylic acid groups.[10] Changes in the water-contact angles of the oxygen plasma treated PMMA surfaces under varied chamber pressures are summarized in Table S2. As can be seen from this data, there was a rapid decrease in the contact angle of the samples treated in the pressure range from 50 to 200 mTorr. This decrease in the contact angle could be attributed to the incorporation of polar functional groups, which caused the plasma-irradiated PMMA surfaces to become more hydrophilic.[9, 11] When the chamber pressure was further increased from 200 to 300 mTorr, the contact angle actually increased from 53.7° to 65.3°. This was most likely due to decreased activated oxygen species in the plasma associated with high rates of recombination and collisional quenching at this high pressure.[12] The higher the pressure, the shorter the mean free path an active species can travel before experiencing collisional quenching and thus, lowering the levels of surface generated carboxylic acid functional groups on the PMMA surface.

In order to better understand the effects of plasma treatment on the PMMA surfaces, the chemical structure of the plasma-modified PMMA surfaces treated at different chamber pressures was analyzed using X-ray photoelectron spectroscopy (XPS). The C1s spectra were resolved into three or four characteristic peaks, which were identified according to reported

chemical shifts.[8] The peaks at binding energies (BEs) of 285.0, 286.7, 287.9 and 289.0 eV corresponded to the groups, C–C, C–O, C=O and O=C–O, respectively. The ratios of the peak area under various chamber pressures are given in Table S2. It can be seen that C=O groups was present for all of the modified surfaces. In addition, the C=O group rapidly increased from 2.31% at a pressure of 50 mTorr to 12.96% at a pressure of 200 mTorr and then showed a much lower rate of increase to 14.71% between 200 – 300 mTorr. The C–O group relative intensity increased from 14.06% (pristine PMMA) to 21.97% at a pressure of 50 mTorr and then, deceased to 17.54% at 300 mTorr. For pristine PMMA with a 14.12% relative surface content of the O=C–O group, its composition increased to 18.75% at a pressure of 200 mTorr, but then decreased to 16.90% at 300 mTorr. Thus, in the pressure range of 50 to 200 mTorr, the formation of hydroxyl or peroxyl (C–O), carbonyl (C=O) and carboxylic (O=C–O) groups could contribute to the observed decrease in the water contact angles. In the case of 300 mTorr chamber pressure, the increase of the C=O group and decrease in C–O and O=C–O groups could account for the increase in the contact angle because the C=O group is less hydrophilic compared to C–O and O=C–O groups. Prediction of the predominant effect resulting in increased contact angle is difficult due to the experimental uncertainties and overlap of native PMMA peaks with oxidized species in the core-level spectra for XPS.

Table S2. Chemical compositions of the PMMA substrates before and after oxygen plasma treatment as deduced by XPS.

Pressure (mTorr)	C 1s (Atomic conc., %)	O 1s (Atomic conc., %)	O/C	Water contact angle (°)
0	74.03	25.97	0.35	74.9±0.6
50	65.71	34.29	0.52	57.8±0.9
100	66.06	33.94	0.51	57.9±1.0
200	63.39	36.61	0.58	53.7±0.6
300	63.15	36.85	0.58	65.3±0.7

Pressure (mTorr)	C–C (%)	C–O (%)	C=O (%)	O=C–O (%)
0	71.83	14.06	0	14.12
50	57.91	21.97	2.31	17.81
100	59.29	16.59	6.61	17.51
200	49.58	18.71	12.96	18.75
300	50.85	17.54	14.71	16.90

The optimal chamber pressure of 200 mTorr was further confirmed by the immobilization of fluorescent dye (Cy5) labeled oligonucleotide probes to plasma treated surfaces. Figure S1

shows the fluorescence intensities of PMMA surfaces as a function of the chamber pressure. The fluorescence intensity markedly increased when the pressure was increased from 50 to 200 mTorr. However, when the pressure was further increased to 300 mTorr, the fluorescence intensity decreased. Therefore, for the remaining studies of this paper a chamber pressure of 200 mTorr was selected.

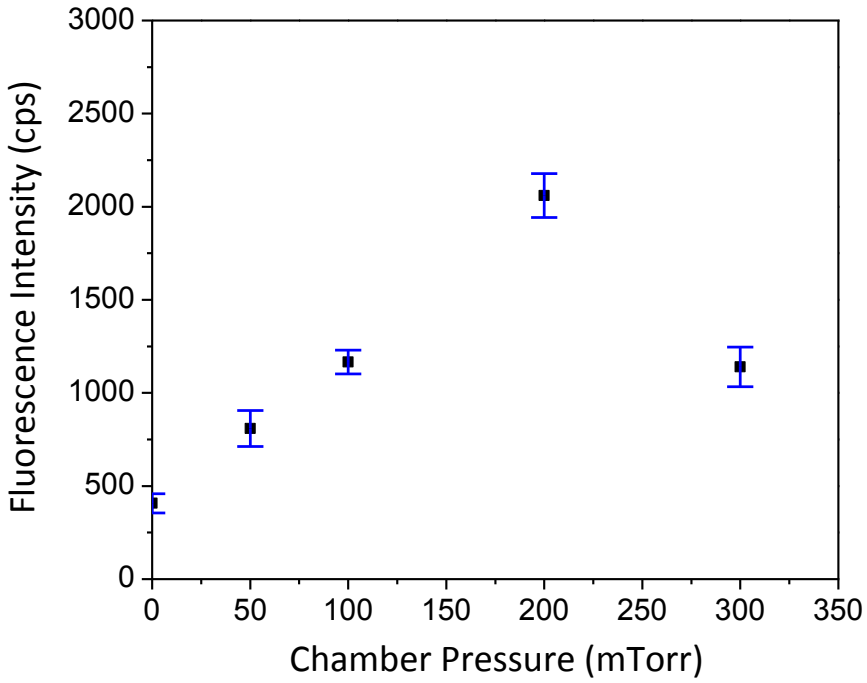


Figure S1. Effects of the oxygen plasma chamber pressure on the probe density covalently tethered to PMMA surfaces. PMMA slides were oxygen plasma-irradiated under various pressures ranging from 50 to 300 mTorr. The slides were activated with EDC/NHS, spotted with fluorescent dye-labeled oligonucleotide probes in 200 mM phosphate solution and allowed to incubate for 1 h. The data points represent the mean of three measurements with the error bars showing the standard deviation.

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