Supplementary Material (ESI) for Chemical Communications

A New, Simple, Approach to Confer Permanent Antimicrobial Properties to Hydroxylated Surfaces by Surface Functionalization

*Othman Bouloussa, Francis Rondelez and Vincent Semetey**

Institut Curie, CNRS UMR 168, 26 rue d'Ulm, 75248 Paris Cedex 05, France

*Corresponding Author

Email Address: vincent.semetey@curie.fr

General Methods. All chemicals were purchased from Sigma-Aldrich. NMR experiments were carried out on a Bruker Avance 300 MHz. UV Analysis were performed using Perkin-Elmer, Lambda 800. FT-IR spectra were obtained using a Nicolet, Magna IR-550. The UV quartz plates (45 × 12.5 × 1.25 mm) were purchased from Hellma. Contact angles were measured with a digidrop from GBX (Romans sur Isère, France). An ellipsometer (Sentech SE 500) operating at a 70° incidence angle was used to measure the polycationic polymer layer thickness on the silicon wafer substrates. XPS measurements were performed with a Surface Science Instruments (SSI) M-Probe Spectrometer. The samples were irradiated with monochromatic Al K_α X-rays (1486.6 eV) using a spot size of 150 μm and 40 W power. For each sample, two survey spectra were recorded with pass energy of 150 eV, from which the surface chemical compositions were determined. In addition, one set of high-resolution spectra (C_{1s}, O_{1s}, N_{1s}) was recorded with pass energy of 25 eV, from which the chemical states were determined. Charge compensation for these electrically-insulating specimens was achieved using a beam of *ca*. 4 to 9 eV electrons at a flood gun current of *ca*. 0.1 mA with an electrically grounded 90% transmission nickel mesh screen positioned *ca*. 1 mm above the sample surface. Atomic Force Microscopy (AFM) studies were carried out with a Nanoscope III from Digital Instruments. The images were acquired in tapping mode under ambient conditions with standard silicon cantilevers and a resonance frequency around.

I. Synthesis and characterization of copolymer 2

Polymer synthesis. The starting material, poly-(vinylbenzylchloride) **1** (1g, Mn = 55000, Ip = 1.82), was dissolved dry tetrahydrofuran (30 mL). Then *N,N'*-dimethylaminopropyltrimethoxysilane (287 μL, 1.31 mmoles) was added in a 1% stoichiometric ratio and the mixture was stirred under reflux during 6 hours at 50°C. *N,N*-

dimethylbutylamine (3.30 mL, 23.6 mmol) was added to the precedent solution and 30 mL of ethanol was added to the solution after 1 h of stirring (to avoid polymer precipitation). The reaction was allowed to stir for 12 h at 50°C. The solution was concentrated and the resulting product was dissolved in 50 mL of ethanol. Then *N*,*N'*-dimethylbutylamine (3.30 mL, 23.6 mmol) was added to this ethanol solution in order to complete the conversion of the chloride groups into quaternary ammonium. After stirring for 12 h at 50°C, the copolymer **2** was precipitated in diethyl ether, dried under vacuum, dissolved in water and lyophilised (1.65g, 95%). ^{1}H NMR (300 MHz, D_2O) δ 7.7-6.2 (m, H arom, 40H), 4.73-4.00 (m, -CH_2- benzyl, 20H), 3.55-2.55 (m, -OCH_3, N^+-CH_3, N^+-CH_2, 89H), 2.19-1.08 (m, CH, -CH_2-, 68H), 1.05-0.50 (m, -CH_3, -CH_2-Si, 29H); FTIR-ATR (diamond) : 3357, 3018, 2958, 2770, 2917, 2873, 1380-1490 cm^{-1}; UV-vis (in water) : 260 nm (ε = 481 $M^{-1}.cm^{-1}$), 215 nm (ε = 6234 $M^{-1}.cm^{-1}$), 190 nm (ε = 316782 $M^{-1}.cm^{-1}$).

II. Surface chemistry and characterization

Cleaning. Quartz and glass slides were cleaned by using a mixture solution of H_2SO_4/H_2O_2 (70/30). The slides were then rinsed in de-ionized water and dried. Paper and fabrics of cotton were just washed with water before treatment.

Surface Functionalization. Due to its cationic charges, the copolymer **2** could be readily dissolved in water at least up to a concentration of 10 mg/mL. In the case of cotton fabrics, 1 × 1 cm^2 pieces were cut and impregnated with a 1 mg/mL polymer solution. The cotton was then put in an oven for drying during 1 hour at 120°C. Solid substrates like glass, quartz slides, ceramics, oxidized silicone or Si/SiO_2 wafers were immersed in a polymer solution (1 mg/mL). The solution was then evaporated to dryness in an oven at 120°C. For all substrates, the unbound copolymer was then removed by extensive washings with distillated water.

Density of quaternary ammonium groups.

Method A. Density of the quaternary ammonium groups on the surface can be monitored directly by measuring the absorbance at 260 nm of a quartz slide functionalized with copolymer **2**. The surface density can be calculated with an assumption that the averaged extinction coefficient in solvents can be applied for the immobilized copolymer. The observed extinction coefficient at 260 nm is 4.81 × 10^5 cm^2/mol in water. Because the polymer is present on both sides of the substrates, the absorbance measured, A, is divided by two and transformed into a unit of N^+/cm^2, taking the surface density, D, to be :

$$D = A/(2\varepsilon_{260}) = 0.0076/(2 \times 4.81 \times 10^5) = 4.7 \times 10^{15}\ N^+/cm^2$$

Method B. The surface density of quaternary ammonium groups could be measured by a colorimetric method based on fluorescent complexation and UV-vis spectroscopy as described by Tiller et al.[1] Quartz slides (45 × 12.5 × 1.25 mm) were immersed in a solution of fluorescein sodium salt (1% in distilled water) for 10 minutes. Due to their negative charges, the fluorescent markers bind strongly to the cationic sites and the unreacted molecules can then be removed by exhaustive washing with distilled water. The bound fluorescein molecules were then exchanged by immersing the modified samples in a small volume (2 mL) of a solution of monovalent salt (hexadecyltrimethyl ammonium chloride, $C_{16}H_{36}N^+Cl^-$, 98%, Fluka, 0.5% in distilled water), and sonicated for 45 min. After adding 0.3 mL of saturated sodium bicarbonate solution, the absorbance of the resulting solution was measured between

175 and 600 nm, and the concentration of fluorescein was calculated, taking a value of 67852 $M^{-1}.cm^{-1}$ for the extinction coefficient corresponding to λ_{max} = 501 nm, the absorbance obtained at 501 nm being corrected by subtraction from the baseline. The density of cationic groups was then derived from this concentration, assuming a stoichiometric ratio of 1:1.

Method	Density
A	$4.7 \times 10^{15}\ N^{+}/cm^{2}$
B	$1.2 \times 10^{15}\ N^{+}/cm^{2}$

The lower density obtained by the method B compared to the method A could be explained by the steric hindrance, indicating that densities obtained by method B should be corrected by a factor 4 (Tiller *et al* have found a factor 7).[1]

Microcontact printing of copolymer 2. We first fabricated a master with the desired patterns on silicon wafers using classical photolithographic techniques.[2] The two components of the polydimethylsiloxane (Sylgard 184, Dow Corning Inc.), PDMS for short, were mixed in a ratio of 10:1 by volume and were then cast on the master. After heating to 60°C for 8 hours, the PDMS stamp was removed from the master.

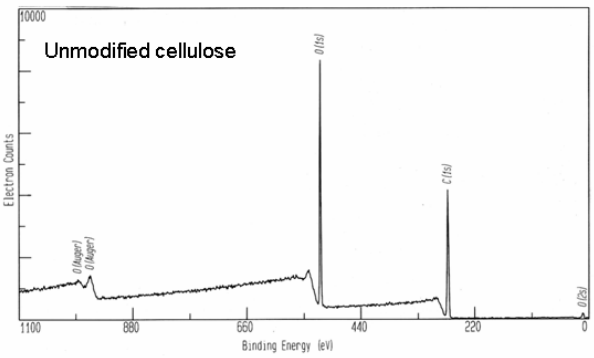
We inked the PDMS stamps with an aqueous solutions of copolymer **2** (10 mg/mL) and let them sit to dry at room temperature. The stamp was, then, pressed against glass slides. After printing copolymer **2**, surfaces were backed at 120°C during 1 hour and finally washed extensively with water.

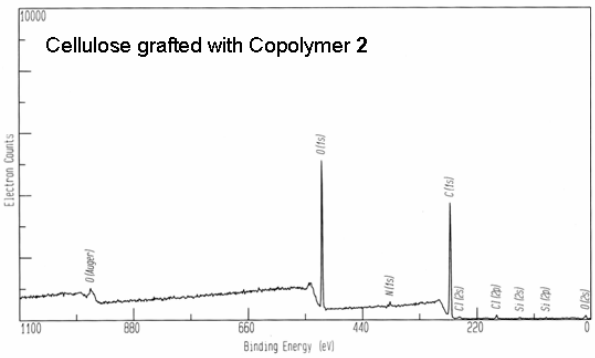
Adhesion of fluorescent nanoparticles to treated surfaces. A 40 µL droplet of 1/10 dilution of fluorescent latex beads of carboxylate modified polystyrene (Sigma, L3530, 0.05 µm, 2.5% solids) was deposed and spread on the studied surfaces. The beads were allowed to sediment on the substrate for 30 minutes. After that, the beads remaining in the solution were washed away by flushing the substrate with distilled water. Then, the surfaces were analysed by the epifluorescence microscope using the 64× water immersion objective. The adsorbed beads appear red.

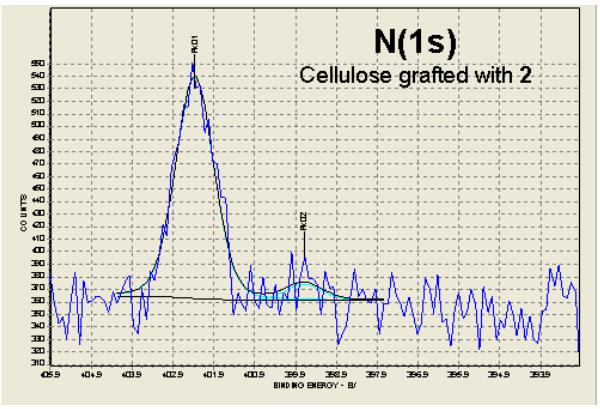
Antimicrobial tests. A 20 µL sessile droplet of *Escherichia coli* (MG1655, 2×10^{8} CFU/mL), *Staphylococcus Epidermidis* (ATCC 12228, 4×10^{8} CFU/mL) or *Streptococcus mutans* (ATCC 25175D, 3×10^{8} CFU/mL) in suspension in distilled water was deposed and spread on the surfaces. Then 1 to 3 µL of a mixture of two fluorescent markers (LIVE/DEAD® *Bac*Ligth™ L7012, stock solution diluted 1000 times in PBS, invitrogen) was added. The treated microscope slides were covered by a thin cover glass whereas cotton and paper samples were squeezed between two untreated glass slides. An epifluorescence microscope (DMR Leica, Germany) equipped with an 64× water immersion or an 100× oil immersion objectives was used for the optical observations, one hour after inoculation. The adsorbed bacteria appear as green dots if still viable and as red/orange dots if their membrane has been damaged following contact with the active substrate. The images were recorded with a color CCD camera (Micropublisher 5.0, QImaging, USA).

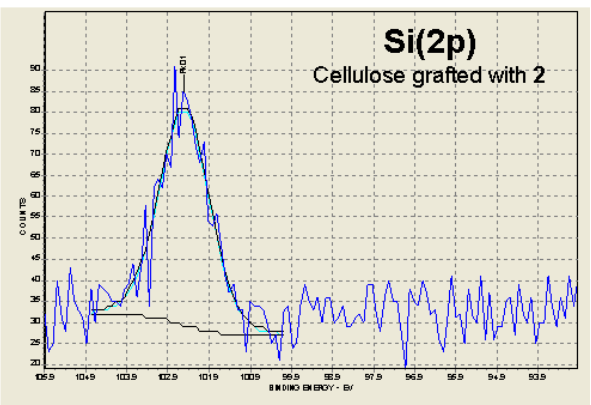
Supplemental Table 1. Elemental Composition of the copolymer **2** on Si/SiO_2 Wafer and Cellulose Measured by XPS.

Substrate	Elemental composition (%)				
	O	N	C	Si	Cl
Copolymer **2**	7	4	83	2	3
Si/SiO_2 grafted with Copolymer **2**	12	74	2	7	5
Unmodified cellulose	41	0	59	0	0
Cellulose grafted with Copolymer **2**	29	1	66	1	2.34





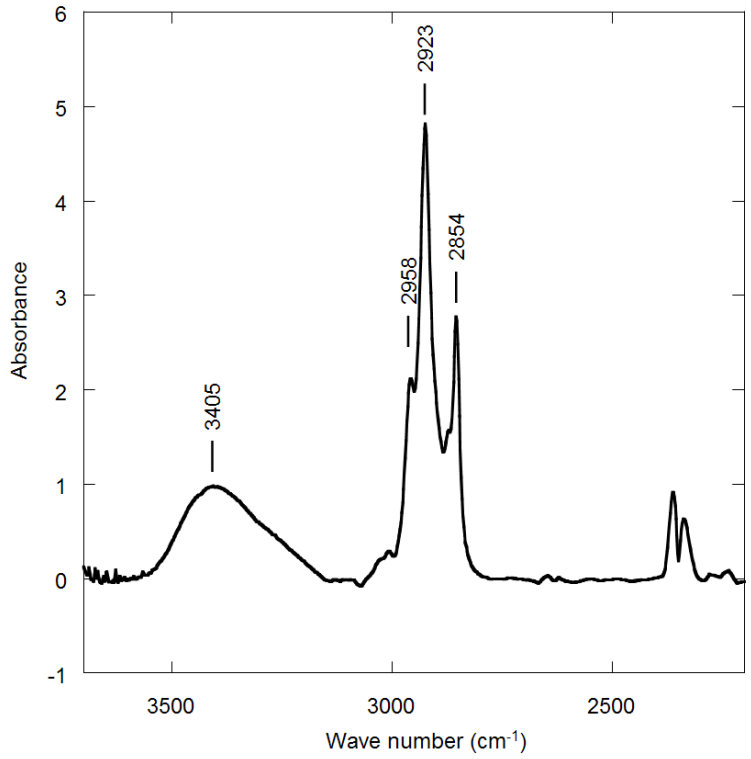




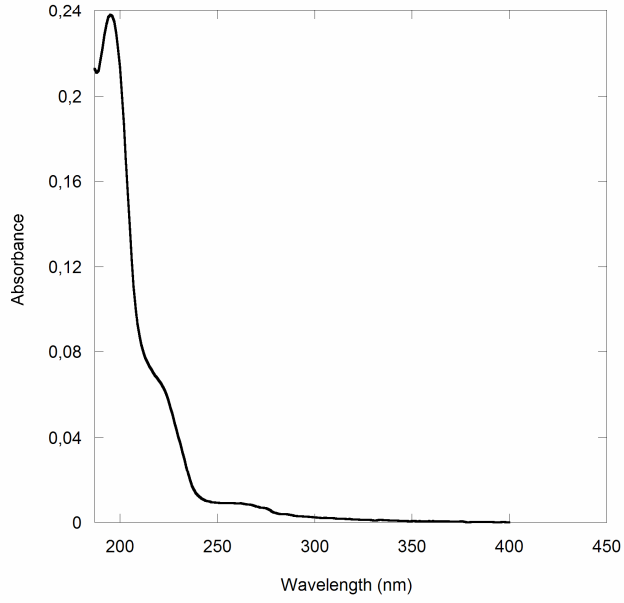
Supplemental Table 2. Thickness and water contact angles of Si wafer and Modified Si wafers.

Substrate	Thickness (nm)	Contact angle (°)	
		advancing	receding
Si/SiO_2	2.16 ± 0.01 (SiO_2 layer)	30±4	14±1
Si/SiO_2 grafted with copolymer **2**	5.95 ± 0.39 (polymer layer)	81±3	44±5

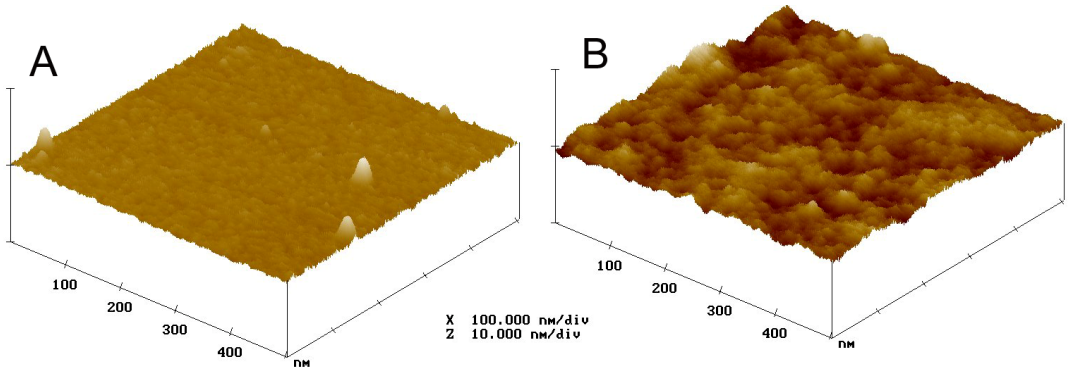
Supplemental Figure 1. IR-ATR spectrum of the APMs grafted on a Si/SiO_2 prism



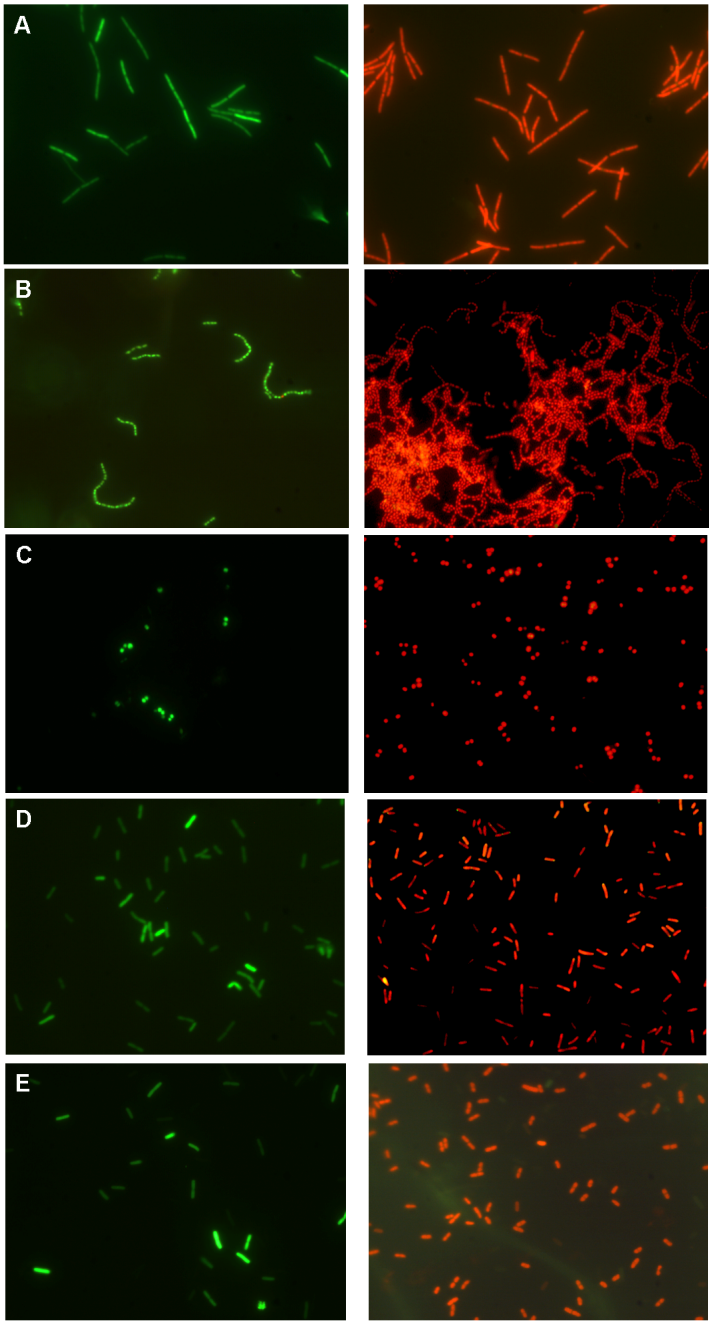
Supplemental Figure 2. UV-vis spectrum of the APMs grafted on a quartz slide.

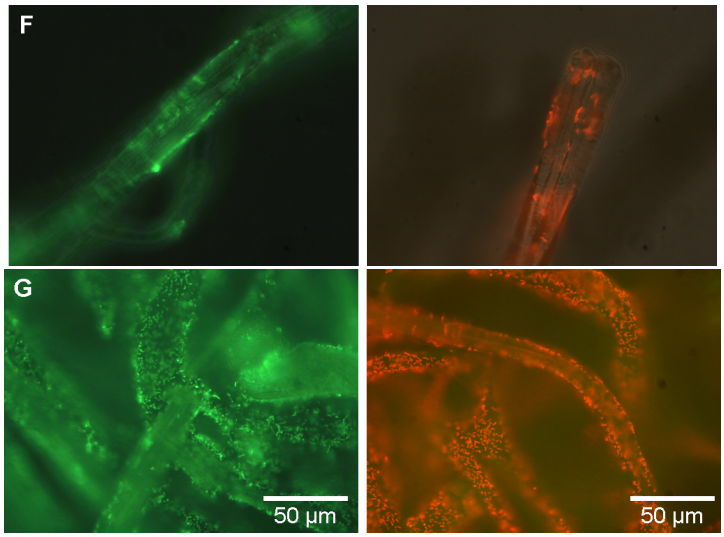


Supplemental Figure 3. Taping mode AFM height images of (A) Si/SiO_2 wafer (B) Si/SiO_2 wafer grafted with copolymer **2**.



Supplemental Figure 4. Fluorescence microscopy of bacteria on untreated (left) and treated (right) surfaces stained with fluorescent cell viability marker (Live and Dead assay, Invitrogen). Viable bacteria appear as green dots and non viable as orange/red dots. (A) *Bacillus subtilis* (ATCC 6633), (B) *Staphylococcus epidermidis* (ATCC 12228), (C) *Streptococcus mutans* (ATCC 25175D), (D) *E. coli* (MG 1655) on treated tile (ceramic), (E) *E. coli* (MG 1655) on treated polydimethylsiloxane elastomer. (F) *E. coli* (MG 1655) on cotton fabrics (cellulose) (G) *E. coli* (MG 1655) on cellulose (paper).





References

1. J. C. Tiller, C.-J. Liao, K. Lewis and A. M. Klibanov, *Proc Natl Acad Sci U S A.*, 2001, **98**, 5981-5985.
2. Y. Xia and G. M. Whitesides, *Angewandte Chem. Int. Ed.*, 1998, **37**, 551-575.