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Antimicrobial and Cell-Compatible Surface-attached Polymer Networks – How the Correlation of Chemical Structure to Physical And Biological Data Leads to a Modified Mechanism of Action.

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Supporting Information

Experimental

General:

All chemicals were obtained as reagent grade from Aldrich, Fluka or Acros and used as received. HPLC grade solvents were purchased dry from Aldrich or Acros and used as received. Gel permeation chromatography (chloroform or THF, calibrated with polystyrene standards) was measured on a PSS SDV or PSS GRAM column (PSS, Mainz, Germany). NMR spectra were recorded on a Bruker 250 MHz spectrometer (Bruker, Madison, WI, USA).

Ellipsometry:

The thickness of the dry polymer layers on silicon wafers was measured with the auto-nulling imaging ellipsometer Nanofilm EP^3 (Nanofilm Technologie GmbH, Göttingen, Germany), which was equipped with a 532 nm solid-state laser. A refractive index of 1.5 was used for all measurements. For each sample, the average value from three different positions was taken.

Attenuated Total Reflection Fourier transform infrared spectroscopy (ATR-FTIR):

Double side polished silicon wafers were used as substrates for the FTIR experiments. The polymer layer was immobilized on one side of a double side polished silicon wafer. The spectra were recorded from 4000 to 400 cm^{-1} with a Bio-Rad Excalibur spectrometer (Bio-Rad, München, Germany), using a spectrum of the blank double side polished silicon wafer as background.

Atomic force microscopy (AFM):

The morphology and roughness of the dry polymer layers on silicon wafers were measured with a Multimode AFM (Nanoscope IIIa, Digital Instruments, Santa Barbara, USA), which was equipped with commercial tips (AppNano). The resonance frequency of the tips was about 180 kHz, and the spring constant 20-95 N/m. All AFM micrographs were recorded in air at ambient conditions. The images were acquired at 512 sample points/line and 512 lines using a scan rate of 0.32 Hz. After capturing the images using the Nanoscope v531r1 software, they were analyzed using Gwyddion 2.26. For each sample, the root mean square (RMS) average roughness from three images of an area of 5x5 μm^2 at different positions was taken.

Contact Angle:

The contact angle system OCA 20 (Dataphysics GmbH, Filderstadt, Germany) was used to measure the static, advancing and receding contact angles of the SMAMP precursors and the activated SMAMP networks. The average value of the contact angle was obtained from four measurements on different positions of one sample. The static contact angles were calculated with the Laplace-Young method, while the advancing and receding contact angles were calculated with elliptical and tangent methods.

Zisman Plot:

To obtain the Zisman plot, the static contact angle of the sample surface was measured using a series of mixtures of water and ethylene glycol in different ratios (Table S1). The cosine of the contact angles was then plotted against the already known surface energy of the water ethylene glycol mixtures and the resulting trend line was extrapolated to the value of cosine equal to one, at which the highest surface tension that would wet the surface entirely can be obtained.

Table S1: Solvent mixtures for the Zisman plot

Ethylene glycol : H_2O / mass ratio	**Surface energy** / J m^{-2}
70:30	53.4
50:50	57.9
30:70	61.2
10:90	69.4
0:100	72.8

Surface Plasmon Resonance Spectroscopy (SPR):

A RT2005 spectrometer (Res-Tec, Framersheim, Germany) was used for SPR measurements. Glass slides (LaSFN9 glass. Hellma) coated with a 1 nm chromium adhesion layer and a 50 nm gold layer were homemade.

Zeta Potential Measurements:

The streaming current measurements for electrokinetic surface characterization were performed with an electrokinetic analyzer with integrated titration unit (SurPASS, Anton Paar GmbH, Austria). The analyzer was equipped with an adjustable gap cell. Ag/AgCl electrodes were used to detect the streaming current. The respective polymers were spin-cast on fused silica substrates (MaTeC, 20x10x1mm lp, Ch.Nr. 13112704) and put into the measuring cell.

Before each measurement the electrolyte hoses were rinsed with ultrapure water until a conductivity of < 0.06 mS/m was reached. The measuring cell was mounted and the electrolyte solution (1mM KCl) was prepared. The pH of the electrolyte solution was adjusted to pH 3.5 with 0.1M HCL prior to filling the electrolyte hoses. The gap height was adjusted to approx. 105 µm while the system was rinsed for 180 sec. at 300 mbar.

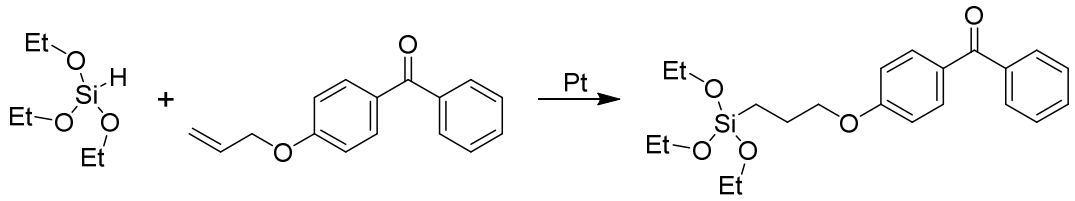
Titration measurement was performed with 0.1M NaOH. The target pressure of the pressure ramp was set to 400 mbar. After titration and before each measurement cycle the system was rinsed for 180 sec. at 300 mbar. The pressure program was: target pressure 400 mbar; max. time 20 s; current measurement; 2 repetitions. The rinse program was: max. pressure 300 mbar; max. time 180s. The parameters for the pH titration were: pH difference = 0.2; volume increment 0.01 mL; pH minimum 2.5; pH maximum 10.5.

Synthesis and Characterization of Surface-Attached Networks of Synthetic Mimics of Antimicrobial Peptides (SMAMPs):

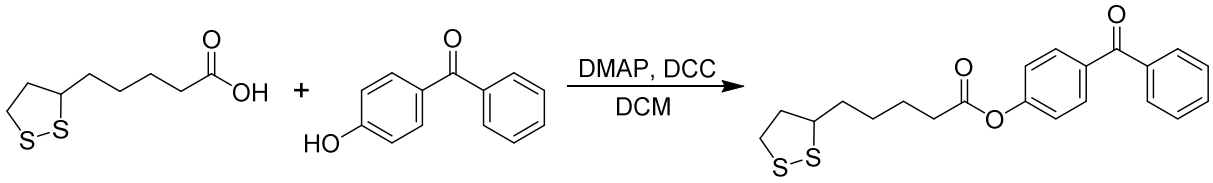
Synthesis of Cross-Linking Agents:

The cross-linking agents triethoxy-benzophenon-silane (3EBP-silane) and alpha-lipoic acid 4-hydroxybenzophenone ester (DS) were synthesized as described in the literature.[1] The general reaction is shown in Scheme S1.

a)



b)

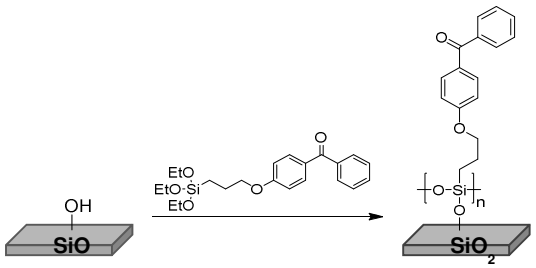


Scheme S1: Synthesis of the cross-linkers: a) triethoxy-benzophenon-silane and b) DS

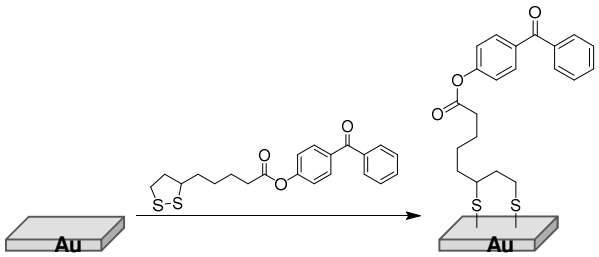
Functionalization of Silicon Wafers and Gold Substrates with Cross-Linking Agents:

Silicon wafer: A solution of 3EBP-silane (20 mg·mL^{-1} in toluene) was spin coated on a (525±25) µm thick one-side-polished 100 mm standard Si (CZ) wafer ([100] orientation, 1000 rpm, 120 s). The wafer was cured for 30 min at 100 °C on a preheated hot plate, washed with toluene and dried under a continuous nitrogen flow. The reaction is shown in Scheme S2.

Gold substrate: For SPR measurements, the LaSFN9 glass slides coated with 1 nm chromium and a 50 nm gold layer were covered with a 5 mM solution of DS in toluene for 24 h. Then the samples were washed with toluene and ethanol, and dried under nitrogen flow. SPR measurements indicated that the thickness of the DS layer was ca. 2 nm. The reaction is shown in Scheme S3.



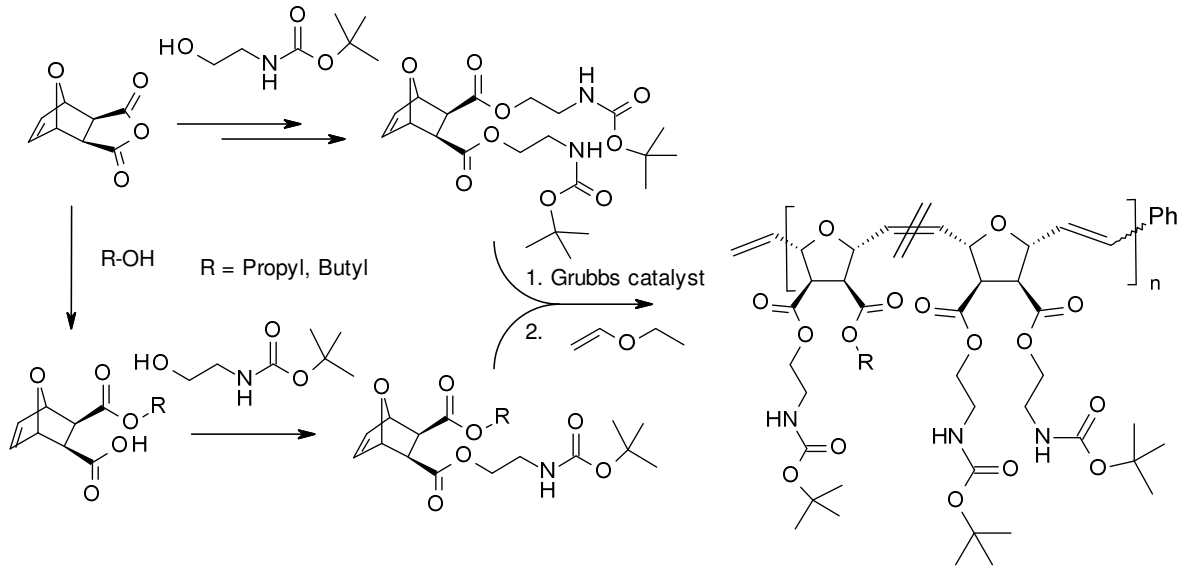
Scheme S2: Functionalization of silicon wafers with 3EBP



Scheme S3: Functionalization gold substrate with DS

Synthesis of SMAMP Precursor Polymers:

The synthesis of SMAMP copolymers is shown in Scheme S4. The synthesis of homopolymers is identical, except that only one monomer kind was used. Monomers, SMAMP precursor homopolymers with R = methyl, ethyl, propyl, butyl and N-*tert*-butyloxycarbonyl-ethanolamine, and SMAMP precursor copolymers were synthesized as described in literature.[2] For the polymerization, about 1 g monomer and Grubbs' 3rd generation catalyst were dissolved in 4 mL dry dichloromehane (DCM) each. In the case of the copolymers, a monomer mixture of the appropriate ratio was dissolved in 4 mL dry DCM. Details are given in Table S2. Both the monomer solution and the catalyst solution were stirred at room temperature for 30 min. The catalyst solution was added to the monomer solution in one shot and the reaction mixture was stirred for another 30 min at room temperature. After adding excess ethylvinyl ether (2 mL) and stirring for 1 h at room temperature, the solution was concentrated by evaporating the solvent under reduced pressure. The SMAMP precursor polymer was then precipitated into n-hexane (1 L) and dried under dynamic high vacuum overnight. The polymer was characterized by ^{1}H-NMR (see below) and gel permeation chromatography (see Table S2). The NMR signals of the polymer matched those in the literature.



Scheme S4: Synthesis of monomers and SMAMP polymer percursors. The di-N-Boc-ethyl-monomers were obtained in a one pot reaction (top left) from oxonorbornene anhydride. The alkyl monomers (R= methyl, ethyl, propyl and butyl) were obtained by first attaching the alkyl group, followed by the N-Boc-ethyl group in the second step. The polymerization with Grubbs catalyst then yielded the desired polymer precursor.

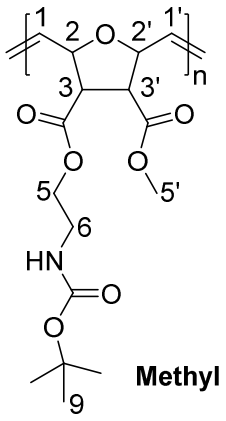
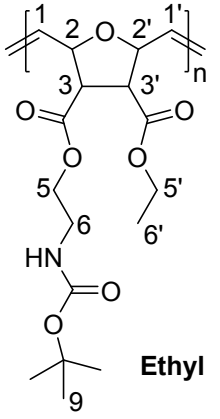
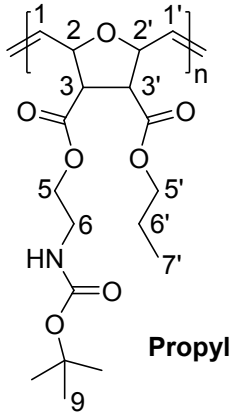
Table S2: Synthesis details for precursor homopolymers and copolymers (suffix –P):

Sample	n_{Alkyl} mmol	m_{Alkyl} / mg	$n_{Diamine}$ / mmol	$m_{Diamine}$ / mg	n_{Cat} mmol	m_{Cat} / mg	$M_{n,\ Target}$ / g·mol^{-1}	GPC M_n, / g·mol^{-1}	M_w/M_n
Diamine-P	-	-	2.55	1200	0.002	1.4	640000	384000	1.3
Methyl-P	2.93	1000	-	-	0.002	1.5	500000	379000	1.1
Ethyl-P	2.82	1000	-	-	0.002	1.5	500000	363000	1.1
Propyl-P	2.71	1000	-	-	0.002	1.5	500000	239000	1.1
Butyl-P	2.61	1000	-	-	0.002	1.5	500000	226000	1.2
P:D = 1:9-P	0.27	100	2.44	1146	0.002	1.5	623000	487000	1.1
P:D = 1:1-P	1.35	500	1.35	637	0.002	1.5	568000	340000	1.2
P:D = 9:1-P	2.44	900	0.27	127	0.002	1.5	514000	332000	1.1
B:D = 1:9-P	0.26	100	2.35	1104	0.002	1.5	602000	300000	1.1
B:D = 1:1-P	1.30	500	1.30	614	0.002	1.5	557000	382000	1.1
B:D = 9:1-P	2.35	900	0.26	123	0.002	1.5	511000	277000	1.2

^{1}H-NMR Data and Spectra for SMAMP Precusor Homopolymers and Copolymers:

The peak positions for each repeat unit are given in Table S3, full spectra are given in Figures S1 to S3.

Table S3: ^{1}N-NMR peak positions for each Precursor Polymer repeat unit.

 **Methyl**	^{1}H-NMR (250 MHz, $CDCl_3$): 1.43 (s, 3 × 9-CH_3), 3.11 (br m, 3-CH & 3'-CH), 3.35 (br m, 6-CH_2), 3.71 (s, 5'-CH_2), 4.15 (s, 5-CH_2), 4.70 (br m, 2-CH & 2'-CH, trans), 5.09 (br m, 2-CH & 2'-CH, cis), 5.27-5.48 (br s, NH), 5.59 (br m, 1-CH & 1'-CH, cis), 5.88 (br m, 1-CH & 1'-CH, trans).
 **Ethyl**	^{1}H-NMR (250 MHz, $CDCl_3$): 0.88 (t, 6'-CH_3), 1.43 (s, 3 × 9-CH_3), 3.10 (br m, 3-CH & 3'-CH), 3.36 (br m, 6-CH_2), 4.06-4.31 (m, 5-CH_2 & 5'-CH_2), 4.70 (br m, 2-CH & 2'-CH, trans), 5.10 (br m, 2-CH & 2'-CH, cis), 5.27-5.48 (br s, NH), 5.59 (br m, 1-CH & 1'-CH, cis), 5.88 (br m, 1-CH & 1'-CH, trans).
 **Propyl**	^{1}H-NMR (250 MHz, $CDCl_3$): 0.91 (t, 7'-CH_3), 1.43 (s, 3 × 9-CH_3), 1.64 (m, 6'-CH_2), 3.11 (br m, 3-CH & 3'-CH), 3.34 (br m, 6-CH_2), 3.94-4.26 (m, 5-CH_2 & 5'-CH_2), 4.69 (br m, 2-CH & 2'-CH, trans), 5.11 (br m, 2-CH & 2'-CH, cis), 5.27-5.48 (br s, NH), 5.58 (br m, 1-CH & 1'-CH, cis), 5.89 (br m, 1-CH & 1'-CH, trans).

	^{1}H-NMR (250 MHz, $CDCl_3$): 0.91 (t, 8'-CH_3), 1.23–1.51 (m, 7'-CH_2 & 3 × 9-CH_3), 1.59 (m, 6'-CH_2), 3.10 (br m, 3-H & 3'-H), 3.35 (br m, 6-CH_2), 4.01-4.28 (m, 5-CH_2 & 5'-CH_2), 4.69 (br m, 2-H & 2'-H, trans), 5.10 (br m, 2-H & 2'-H, cis), 5.30-5.48 (br s, NH), 5.58 (br m, 1-H & 1'-H, cis), 5.88 (br m, 1-H & 1'-H, trans).
Diamine	^{1}H-NMR (250 MHz, $CDCl_3$): 1.43 (s, 6 × 9-CH_3), 3.13 (br m, 2 × 3-H), 3.35 (br m, 2 × 6-CH_2), 4.16 (br m, 2 × 5-CH_2), 4.72 (m, 2-H, trans), 5.10 (br m, 2-H, cis), 5.46 (br m, 2 × NH), 5.59 (br m, 1-H, cis), 5.88 (br m, 1-H, trans).

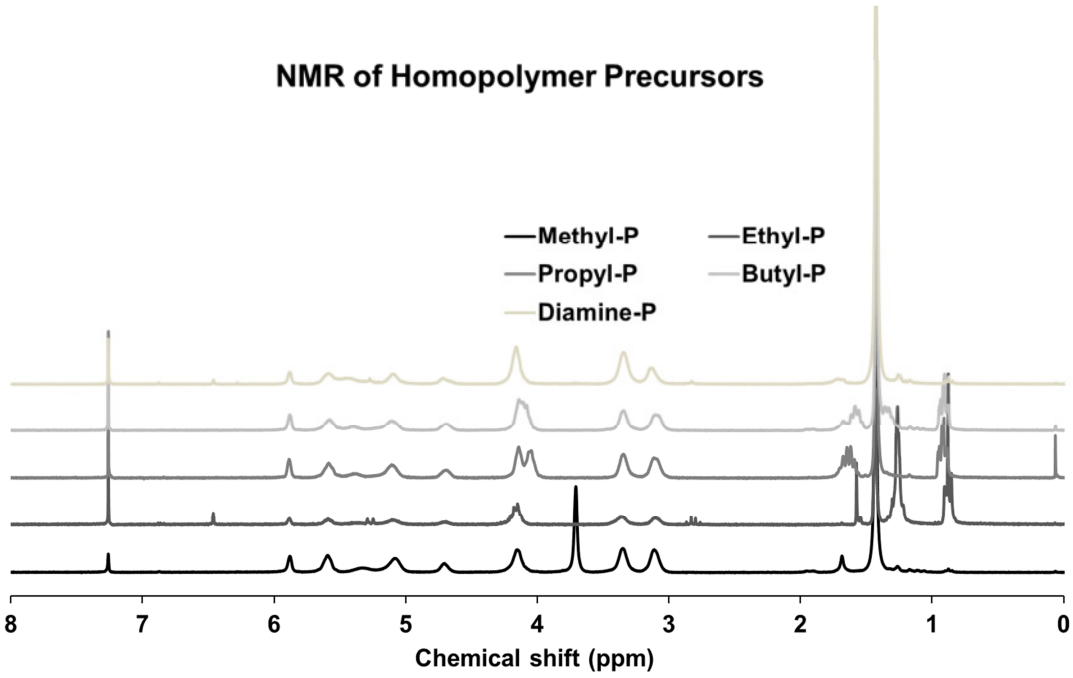


Figure S1: ^{1}H-NMR spectra of the SMAMP homopolymer precursors

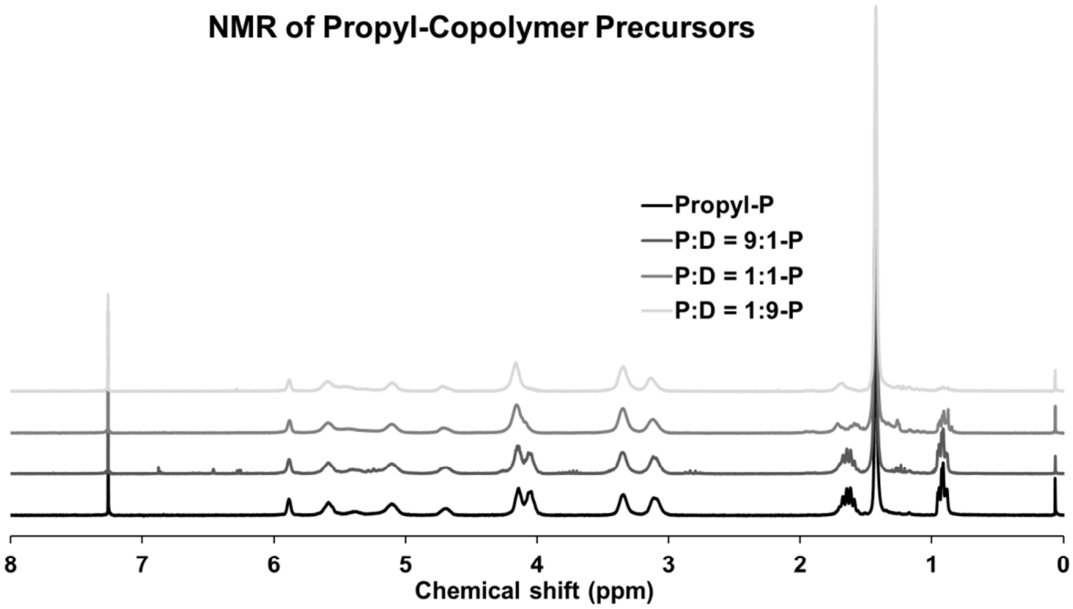


Figure S2: ^{1}H-NMR spectra of the SMAMP propyl copolymer precursors

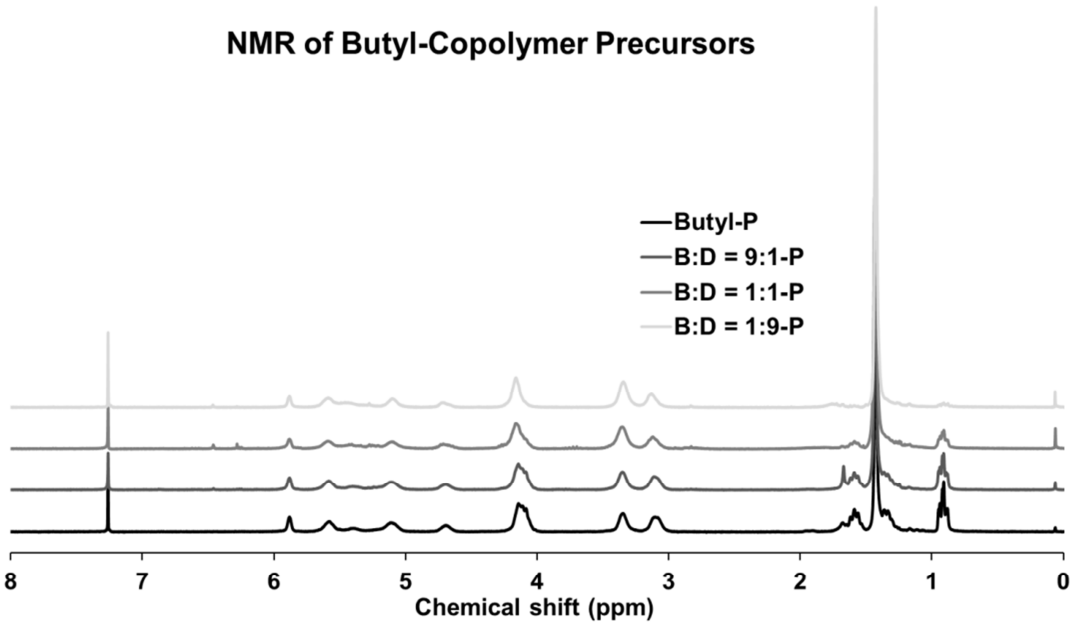


Figure S3: ^{1}H-NMR spectra of the SMAMP butyl copolymer precursors

GPC Data Elugrams:

The GPC data is given in Table S2, the elugrams are given in Figures S4 to S6.

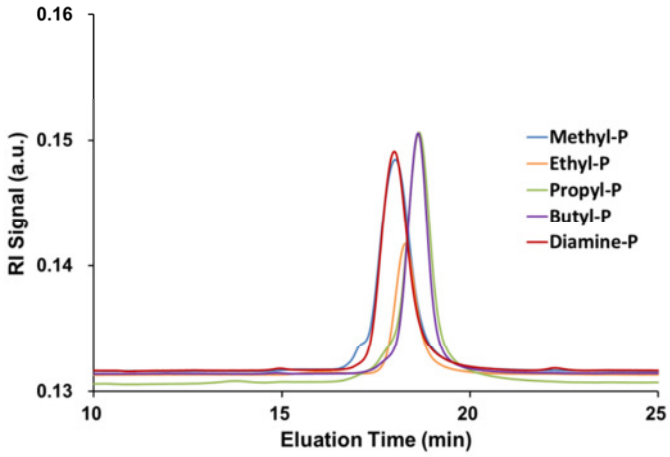


Figure S4: GPC elugrams of the SMAMP homopolymer precursors

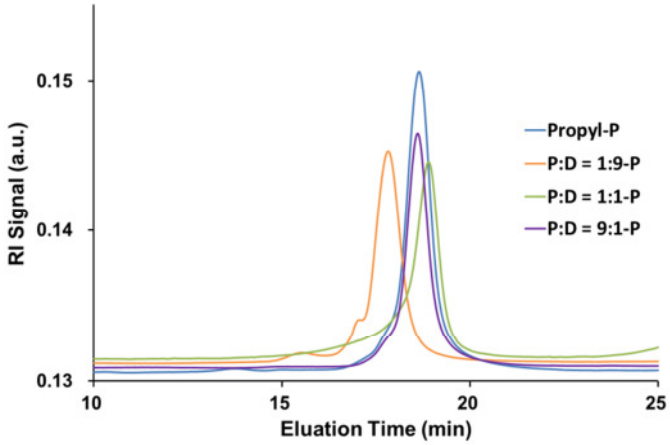


Figure S5: GPC elugrams of the SMAMP propyl copolymer precursors

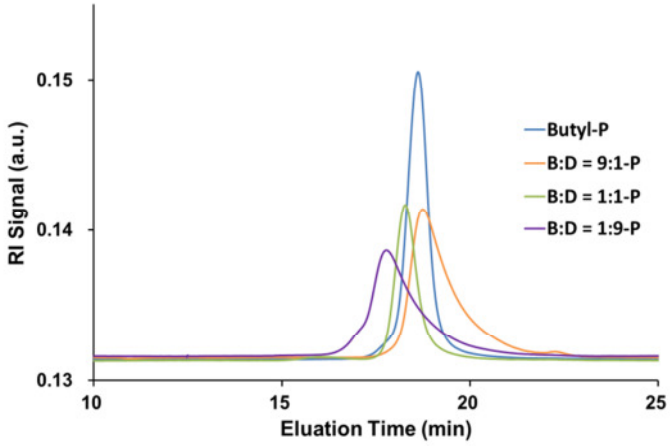


Figure S6: GPC elugrams of the SMAMP butyl copolymer precursors

Immobilization of the SMAMP Precursor Network on the Functionalized Substrates:

The synthesis scheme for the SMAMP network is given in the main text. A stock solution (Solution A) was prepared by dissolving pentaerythritol-tetrakis-(3-mercaptopropionate) (1 mL, 1.3 g, 2.6 mmol) and 2,2-dimethoxy-2-phenylaceto-phenone (4 mg, 0.02 mmol) in DCM (50 mL). After dissolving the SMAMP precursor in Solution A (0.25 mL), a co-solvent was added. The polymer solution was then spin coated (3000 rpm, 30 sec) on the 3-EBP treated silicon wafer pieces or gold substrates (1.5 ×1.5 cm^2). The SMAMP precursor network was formed and immobilized on the wafer surface by cross-linking at 254 nm for 30 min in a BIO-LINK Box (Vilber Lourmat GmbH). The unreacted polymer chains were removed by immersing the network in DCM for 4 h. Details are given in Table S4.

Table S4: Synthesis details for precursor homopolymer and copolymer networks

	m(Polymer) / mg	**V(Solution A) / mL**	**V(Co-Solvent) / mL**
Diamine-P	12.7	0.25	Chloroform: 0.6
Methyl-P	9.3	0.25	Chloroform: 0.6
Ethyl-P	9.6	0.25	Chloroform: 0.6
Propyl-P	10.0	0.25	Toluene: 0.4
Butyl-P	10.4	0.25	Toluene: 0.4
P:D = 1:9-P	12.5	0.25	Chloroform: 0.6
P:D = 1:1-P	11.4	0.25	Toluene: 0.4
P:D = 9:1-P	10.3	0.25	Toluene: 0.4
B:D = 1:9-P	12.5	0.25	Chloroform: 0.6
B:D = 1:1-P	11.6	0.25	Toluene: 0.4
B:D = 9:1-P	10.6	0.25	Toluene: 0.4

Activation of SMAMP Networks by Removal of the Boc Protective Group:

The wafer covered with SMAMP precursor monolayer or network was immersed in HCl (4 M in dioxane) for 12 hours to obtain the complete deprotection of the Boc-protected amine groups. Before antimicrobial test, the sample was washed for three times with ethanol and dried under a flow of N_2.

Deterimination of Layer Thickness by Ellipsometry:

The layer thickness results obtained from ellipsometry are given in Table S5.

Table S5: Layer thickness from ellipsometry.

Layer Thickness from ellipsometry / nm		
	SMAMP precursor	SMAMP
Diamine	197 ± 2	157 ± 4
Methyl	173 ± 3	147 ± 3
Ethyl	163 ± 2	143 ± 3
Propyl	171 ± 2	149 ± 3
Butyl	178 ± 3	153 ± 4
P:D = 1:9	179 ± 3	142 ± 4
P:D = 1:1	183 ± 3	155 ± 3
P:D = 9:1	169 ± 2	147 ± 3
B:D = 1:9	184 ± 3	148 ± 3
B:D = 1:1	185 ± 2	158 ± 3
B:D = 9:1	177 ± 3	152 ± 4

Attenuated Total Reflection Fourier Transform Infrared Spectroscopy (ATR-FTIR) of SMAMP Precursor Networks and SMAMP Networks:

The ATR-FTIR spectra for the SMAMP Precursors networks (Fig. S7 to S9) and SMAMP networks (Fig. S10-S12) are given below.

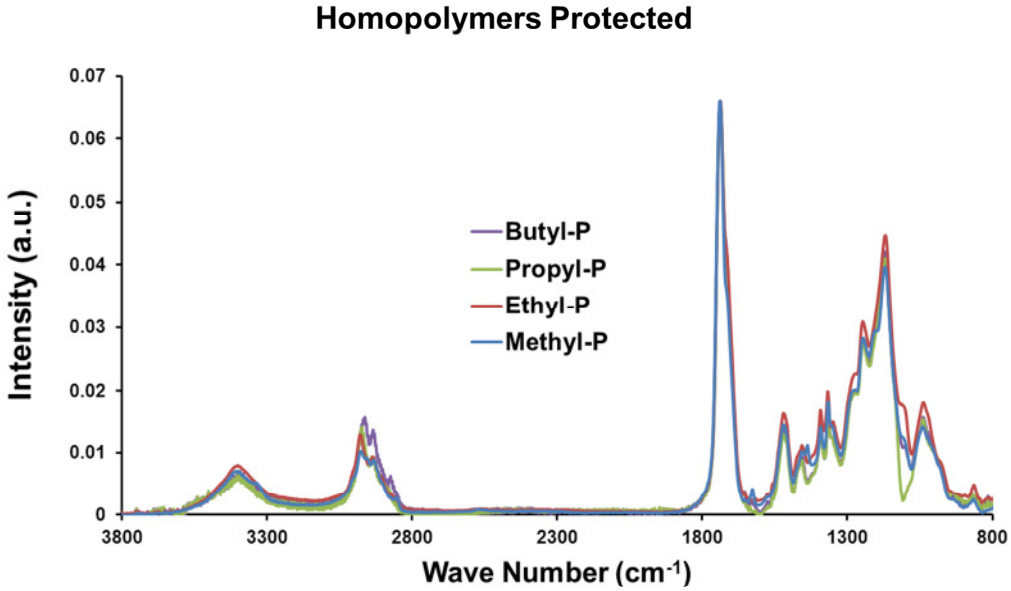


Figure S6: ATR-FTIR spectrum of the SMAMP homopolymer precursor networks

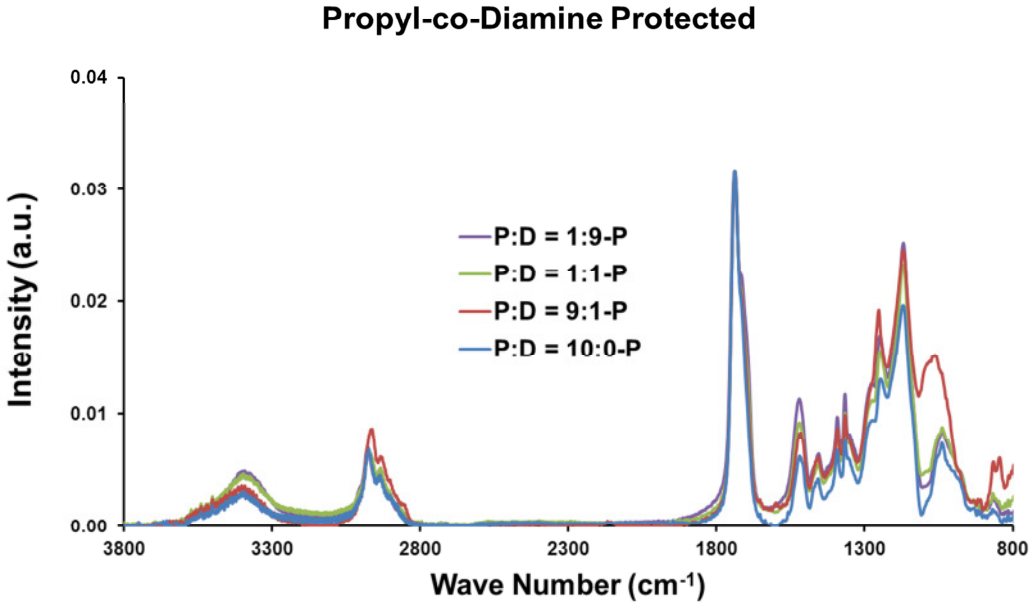


Figure S7: ATR-FTIR spectrum of the SMAMP propyl copolymer precursor networks

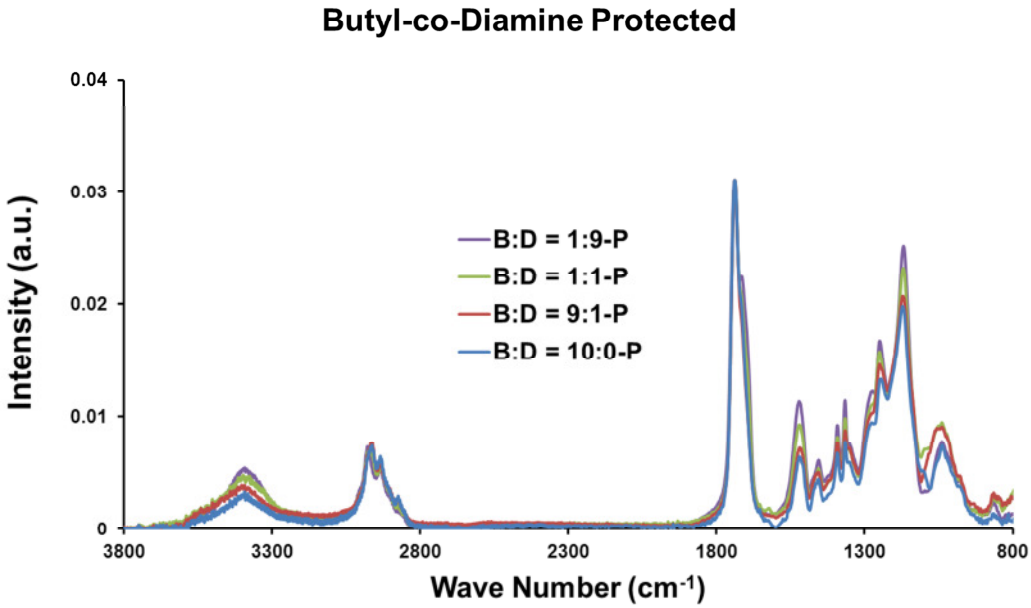


Figure S9: ATR-FTIR spectrum of the SMAMP butyl copolymer precursor networks

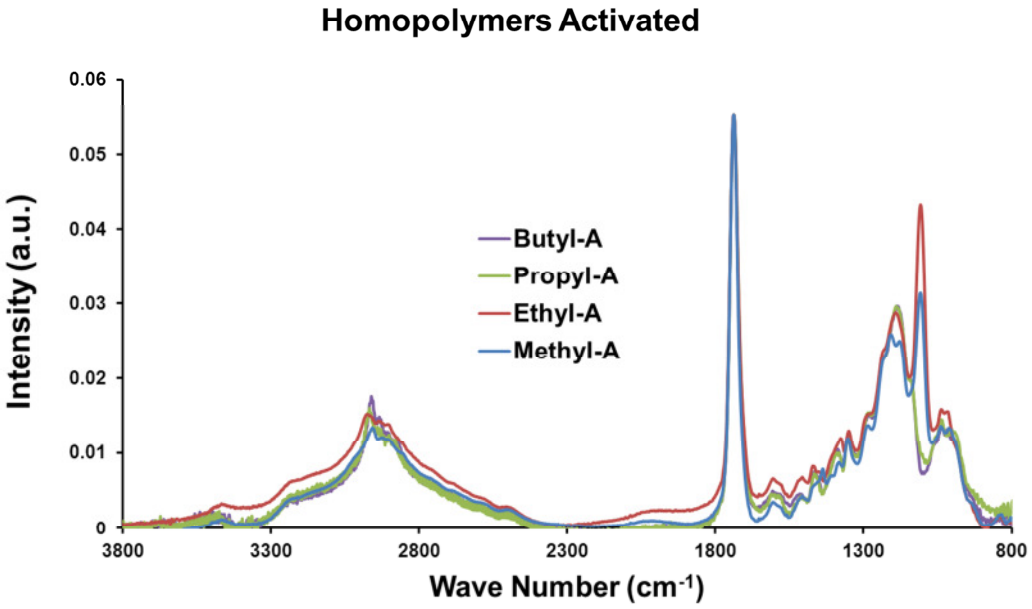


Figure S10: ATR-FTIR spectrum of the SMAMP homopolymer networks

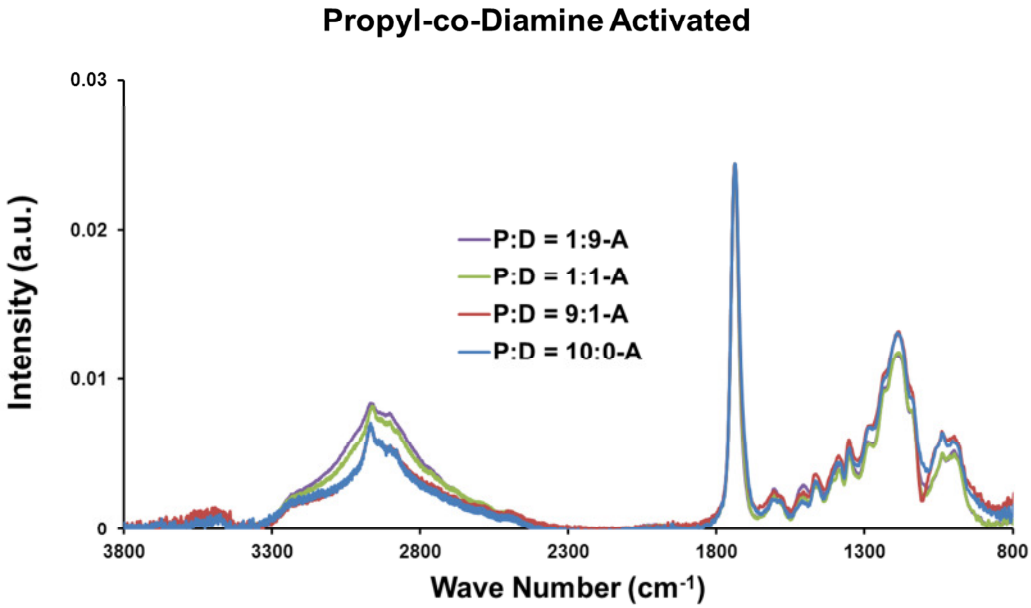


Figure S11: ATR-FTIR spectrum of the SMAMP propyl copolymer networks

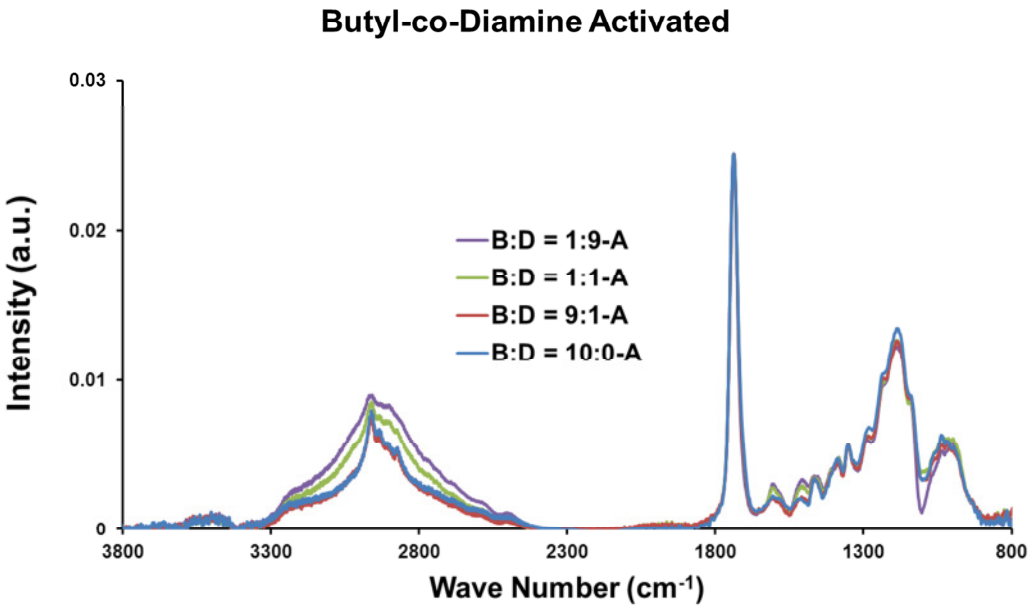


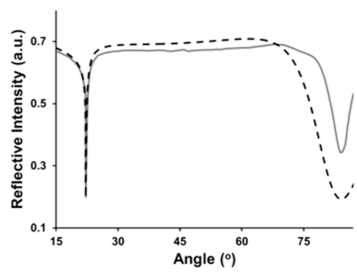
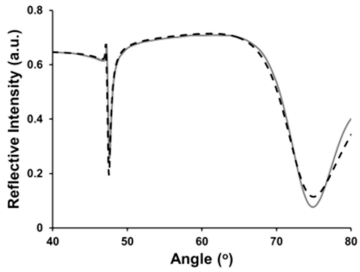
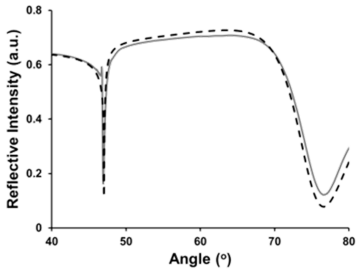
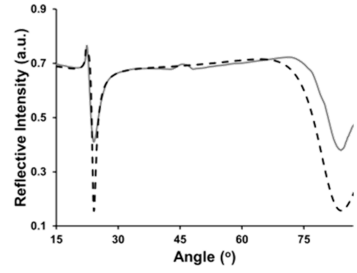
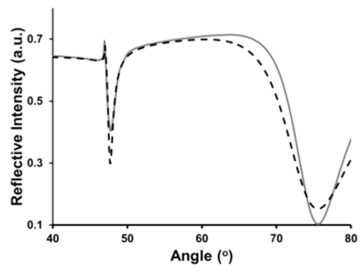
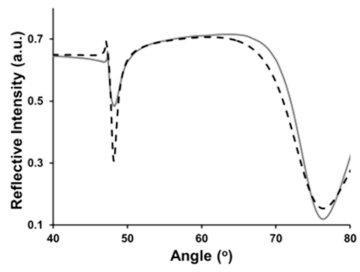
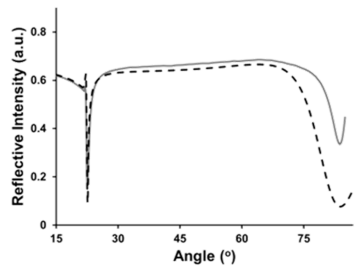
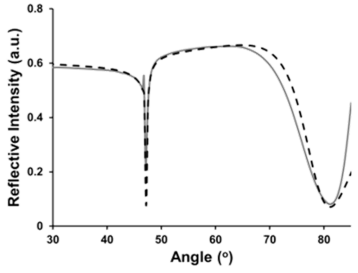
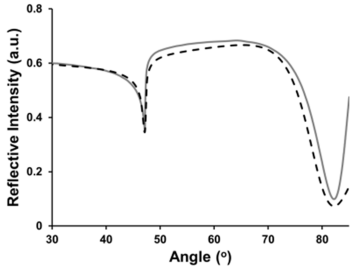
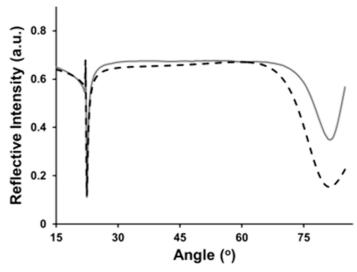
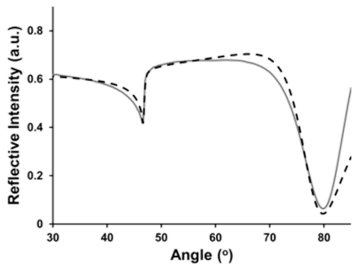
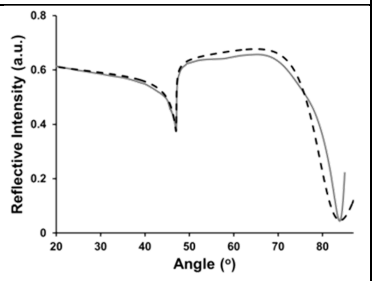
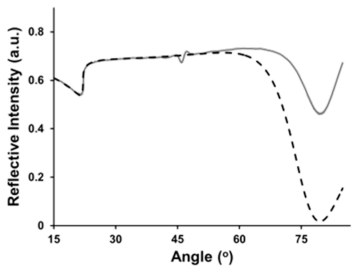
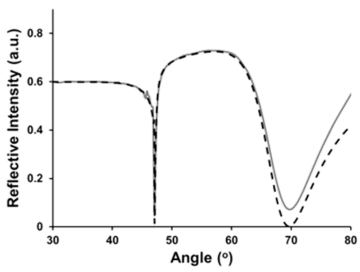
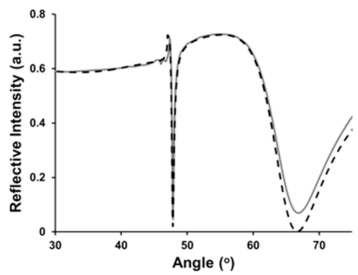
Figure S12: ATR-FTIR spectrum of the SMAMP butyl copolymer networks

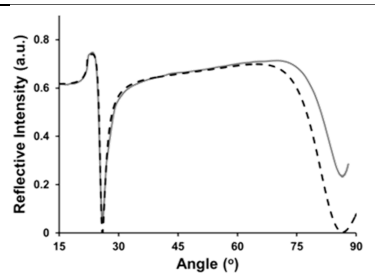
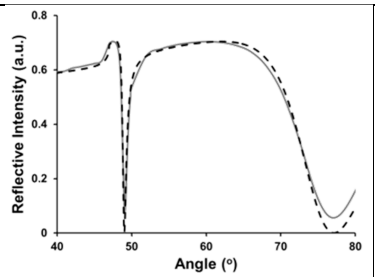
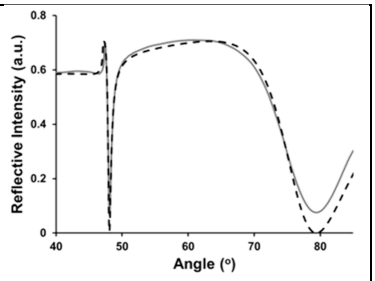
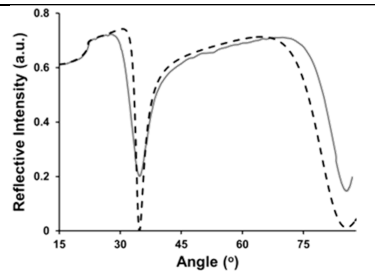
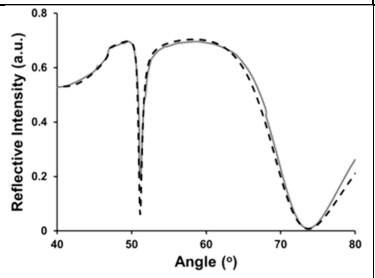
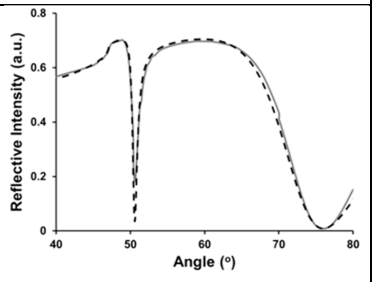
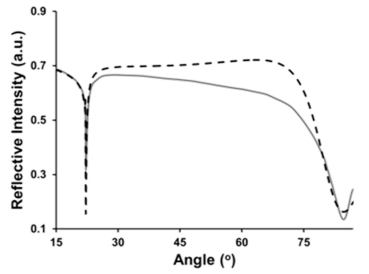
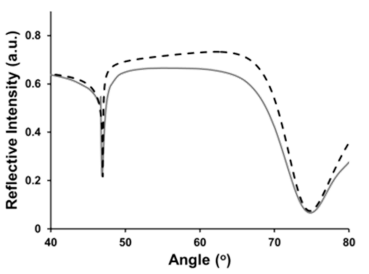
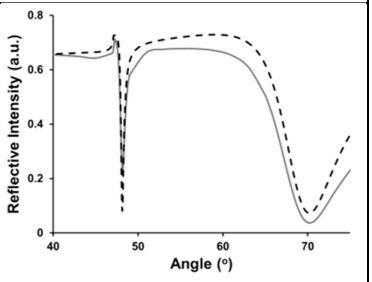
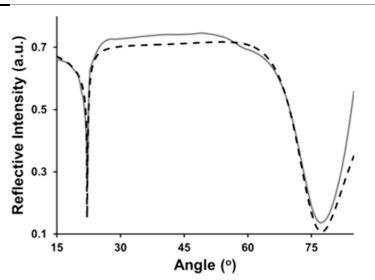
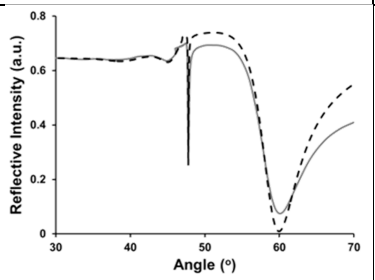
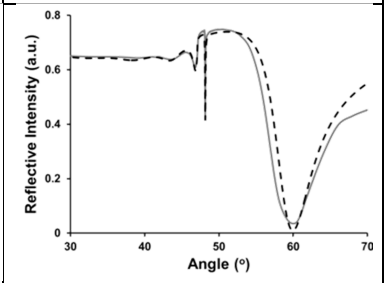
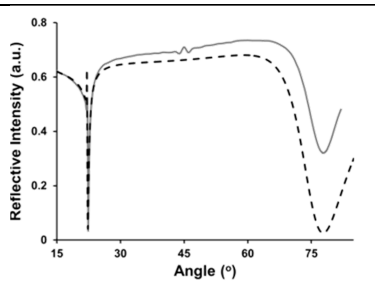
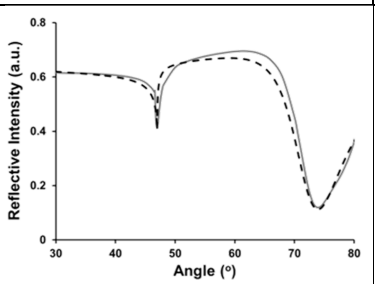
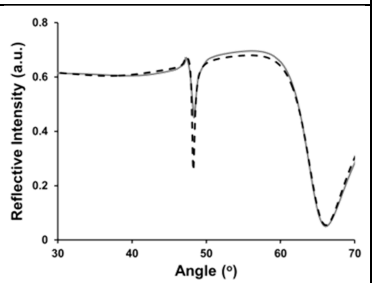
Determination of the Swelling Ratio of the SMAMP Precursor and SMAMP Networks:

The swelling behavior of the polymer networks was measured with surface plasmon resonance (SPR) spectroscopy. In a swelling experiment, the polymer network was first covered with solvent for 5 min and then dried under constant nitrogen flow. The SPR reflectivity curve was recorded until the polymer network was completely dried, which was evident by a constant plasmon and waveguide mode position. Then, the flow cell was again filled with solvent, and another SPR reflectivity curve was recorded after complete swelling. Each scanning curve obtained was simulated with Fresnel calculations and the two unknown parameters, the thickness *d* and the corresponding real permittivity *ε'* of the polymer network, were obtained by fitting the calculated curve to match the SPR minimum as well as the minimum of the wave guide mode. The swelling ratio of the polymer network was calculated by: $Q = \frac{d_{solvent}}{d_{dry}}$

The SPR reflectivity curves (grey) are shown together with the simulation curves (black dashed) for each polymer layer in Table S6. The respective layer thickness and real permittivity are listed below the curves.

Table S6: SPR results for SMAMP Networks:

	Dry layer	In MeOH	In H_2O
Methyl			
	d = 235 nm, ε' = 2.303	d = 381 nm, ε' = 2.157	d = 433 nm, ε' = 2.127
Ethyl			
	d = 273 nm, ε' = 2.288	d = 437 nm, ε' = 2.156	d = 452 nm, ε' = 2.150
Propyl			
	d = 244 nm, ε' = 2.287	d = 366 nm, ε' = 2.218	d = 343 nm, ε' = 2.236
Butyl			
	d = 245 nm, ε' = 2.227	d = 338 nm, ε' = 2.182	d = 302 nm, ε' = 2.278
Diamine			
	d = 193 nm, ε' = 2.262	d = 471 nm, ε' = 2.018	d = 630 nm, ε' = 1.958

B9D1			
	d = 291 nm, ε' = 2.258	d = 519 nm, ε' = 2.107	d = 442 nm, ε' = 2.146
B1D1			
	d = 369 nm, ε' = 2.235	d = 761 nm, ε' = 2.045	d = 634 nm, ε' = 2.084
B1D9			
	d = 235 nm, ε' = 2.312	d = 471 nm, ε' = 2.125	d = 543 nm, ε' = 2.040
Acrylate			
	d = 242 nm, ε' = 2.190	d = 1493 nm, ε' = 1.822	d = 2140 nm, ε' = 1.815
Vinylpyrindie			
	d = 250 nm, ε' = 2.189	d = 379 nm, ε' = 2.094	d = 700 nm, ε' = 1.944

Contact Angle Data of SMAMP Precursor Networks and SMAMP Networks:

The static, advancing and receding contact angle data for the SMAMP precursor networks (Table S7 to S10) and SMAMP networks are given below (Table S11 to S13).

Table S7: Contact angles of SMAMP homopolymer precursor networks

Contact Angle (°)	Static	Advancing	Receding
Methyl-P	79 ± 3	76 ± 3	20 ± 2
Ethyl-P	82 ± 3	86 ± 2	22 ± 2
Propyl-P	88 ± 3	90 ± 2	38 ± 2
Butyl-P	87 ± 2	83 ± 3	34 ± 2
Diamine-P	86 ± 3	81 ± 3	22 ± 3

Table S8: Contact angles of SMAMP propyl copolymer precursor networks

Contact Angle (°)	Static	Advancing	Receding
P:D = 9:1-P	82 ± 2	79 ± 2	25 ± 2
P:D = 1:1-P	80 ± 3	81 ± 2	24 ± 3
P:D = 1:9-P	85 ± 2	82 ± 2	24 ± 2

Table S9: Contact angles of SMAMP butyl copolymer precursor networks

Contact Angle (°)	Static	Advancing	Receding
B:D = 9:1-P	85 ± 2	82 ± 2	23 ± 2
B:D = 1:1-P	86 ± 2	80 ± 3	20 ± 2
B:D = 1:9-P	85 ± 2	81 ± 2	20 ± 3

Table S10: Contact angles of SMAMP homopolymer networks

Contact Angle (°)	Static	Advancing	Receding
Methyl-A	47 ± 3	56 ± 3	10 ± 2
Ethyl-A	52 ± 2	58 ± 2	14 ± 2
Propyl-A	56 ± 2	59 ± 2	14 ± 2
Butyl-A	70 ± 3	68 ± 2	17 ± 2

Table S11: Contact angles of SMAMP propyl copolymer networks

Contact Angle (°)	Static	Advancing	Receding
P:D = 9:1-A	50 ± 2	53 ± 3	12 ± 2
P:D = 1:1-A	46 ± 2	45 ± 2	8 ± 3
P:D = 1:9-A	37 ± 2	28 ± 2	4 ± 2
Diamine-A	32 ± 2	28 ± 2	4 ± 2

Table S12: Contact angles of SMAMP butyl copolymer networks

Contact Angle (°)	Static	Advancing	Receding
B:D = 9:1-A	61 ± 2	57 ± 2	14 ± 2
B:D = 1:1-A	55 ± 2	52 ± 3	11 ± 2
B:D = 1:9-A	43 ± 2	44 ± 2	6 ± 2
Diamine-A	32 ± 2	28 ± 2	4 ± 2

Zisman Plots for SMAMP Homopolymer and Copolymer Networks

The Zisman Plots for the SMAMP homopolymer networks (Figure S13) and copolymer networks (Figure S14 and S15) are given below.

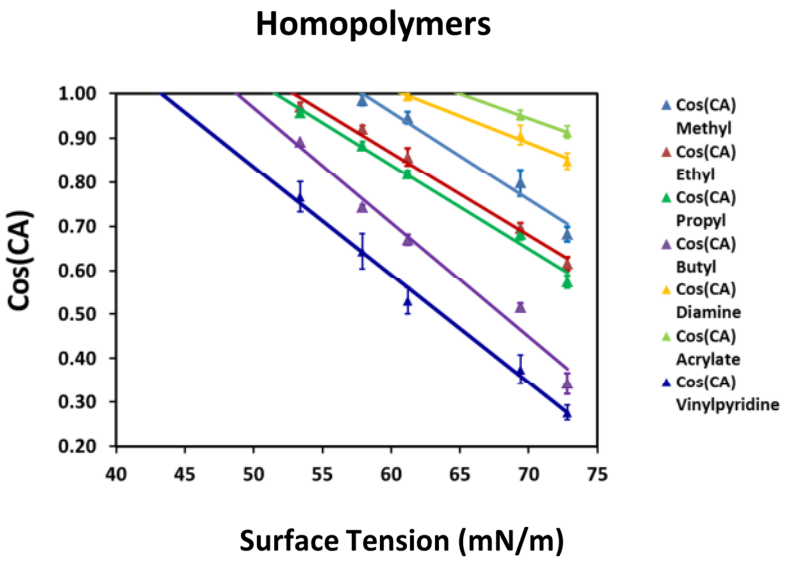


Figure S13: Zisman plot for the SMAMP homopolymer networks and reference polymers

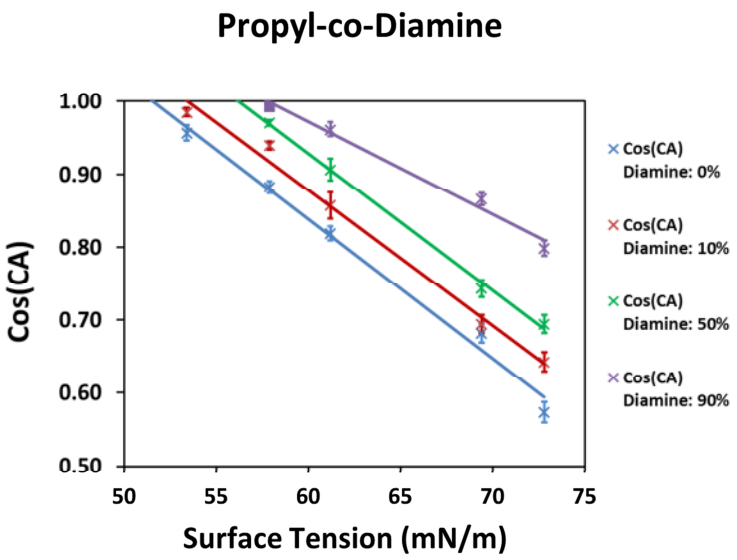


Figure S14: Zisman plot for the SMAMP propyl copolymer networks

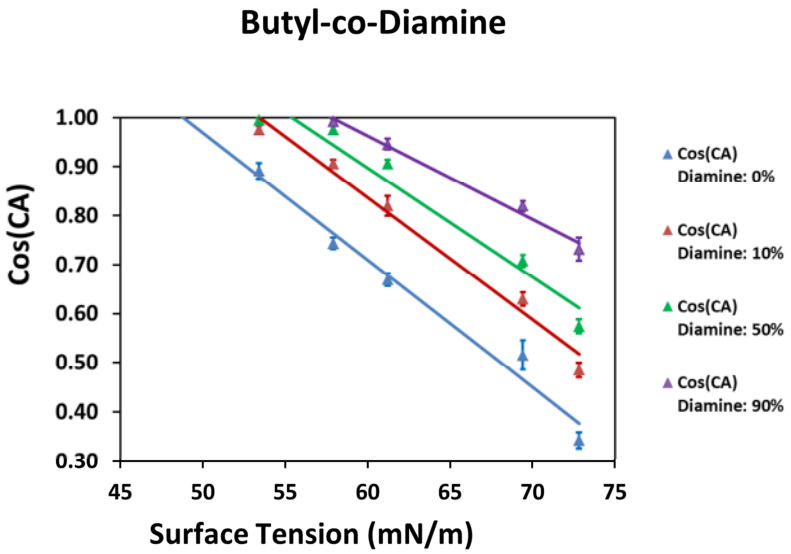
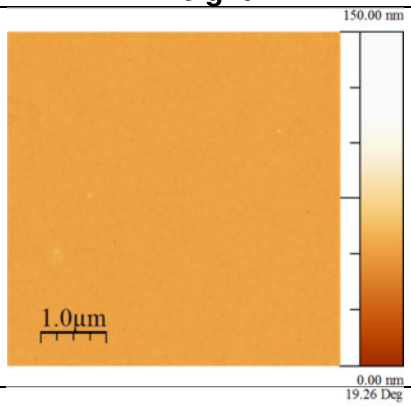
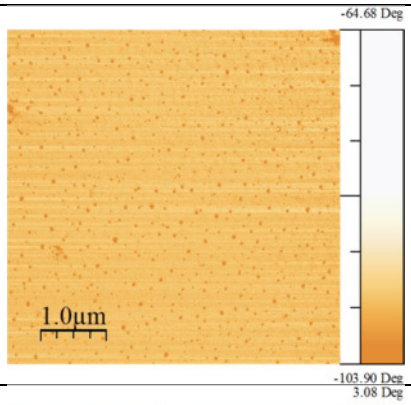
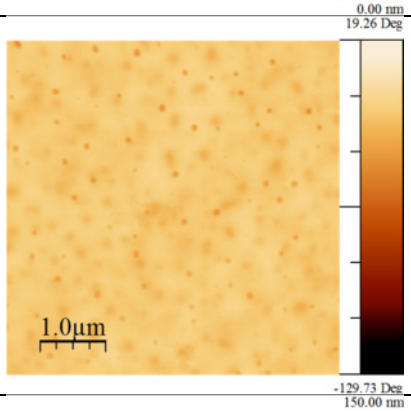
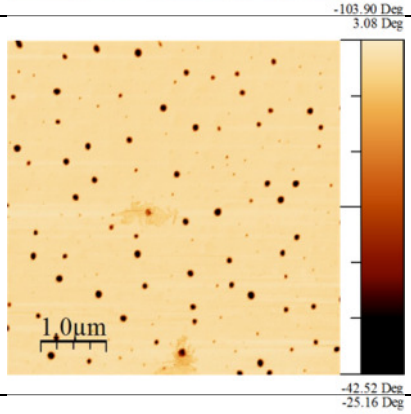
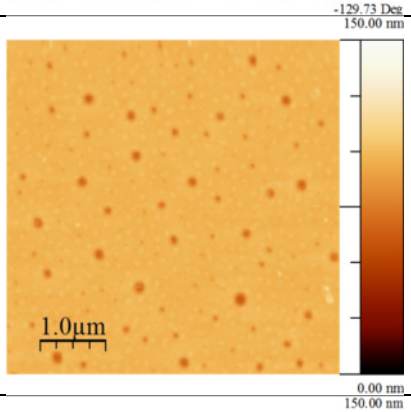
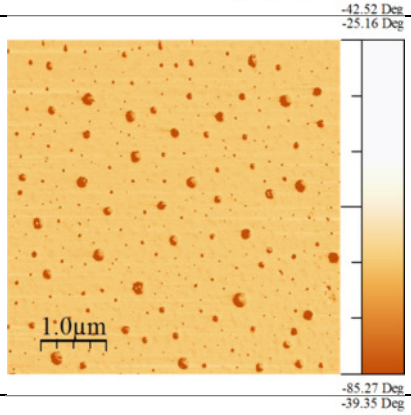
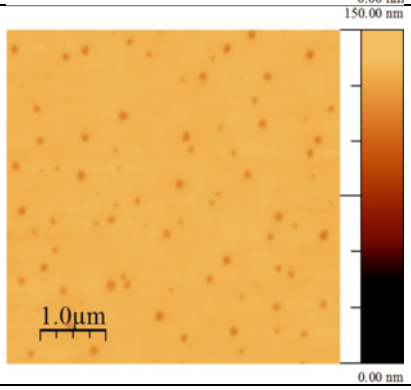
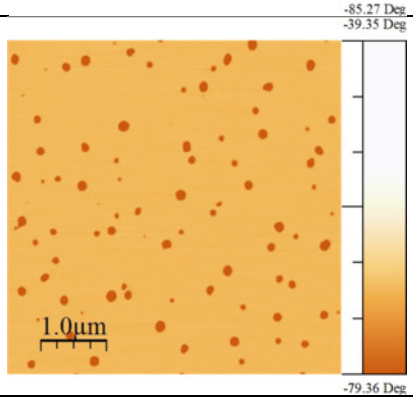


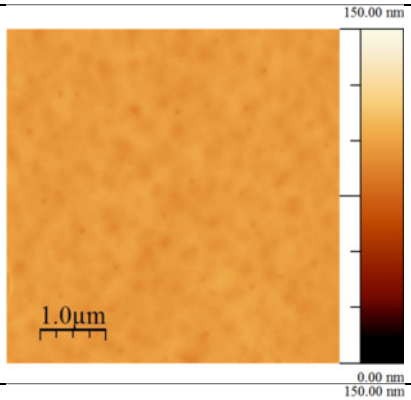
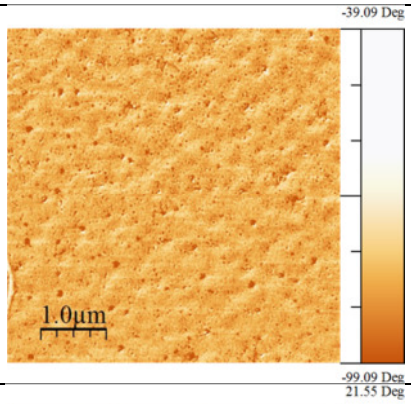
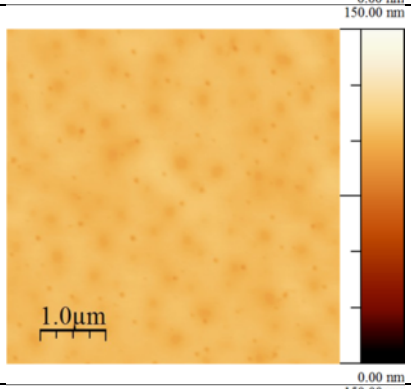
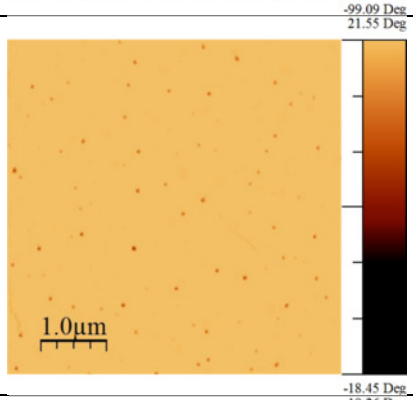
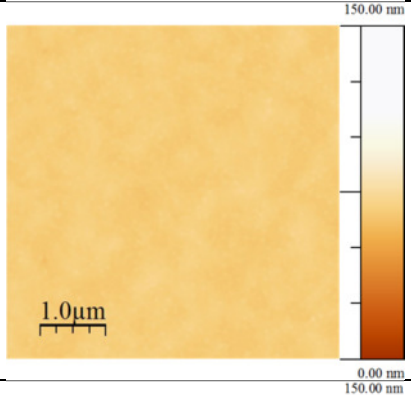
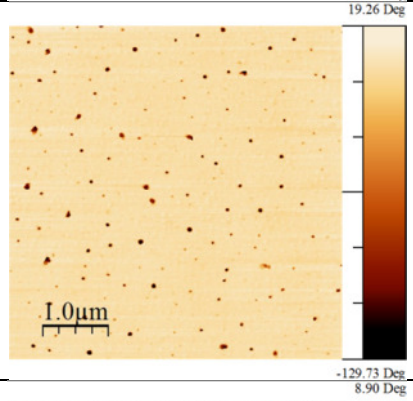
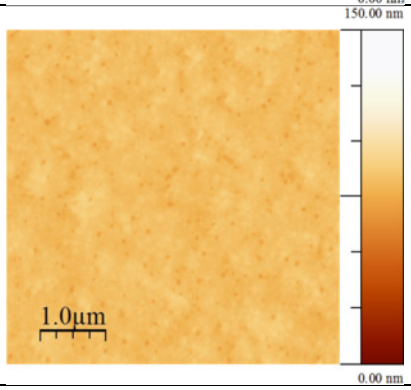
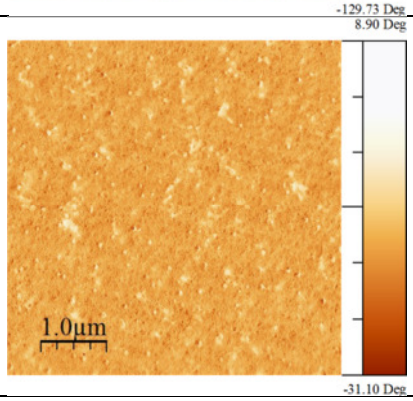
Figure S15: Zisman plot for the SMAMP butyl copolymer networks

Atomic Force Microscopy Results for the SMAMP Precursor Networks and SMAMP Networks

The results of the AFM investigations of the SMAMP precursor networks (Table S13) and SMAMP networks (Table S14) are given below. Each image represents a 5 µm x 5 µm scan. The RMS roughness was calculated from the images using the Gwyddion software package and is included in the first column of the respective Table.

Table S13: Atomic force microscopy results of the SMAMP precursor polymer networks

	Height	Phase
Methyl-P R = 1 nm		
Ethyl-P R = 1 nm		
Propyl-P R = 2 nm		
Butyl-P R = 2 nm		

Diamine-P R = 2 nm		
P:D=9:1-P R = 2 nm		
P:D=1:1-P R = 1 nm		
P:D=1:9-P R = 2 nm		

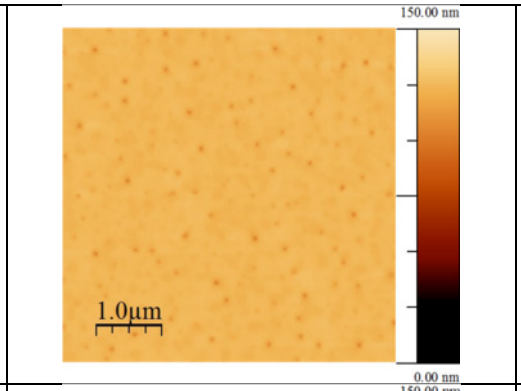
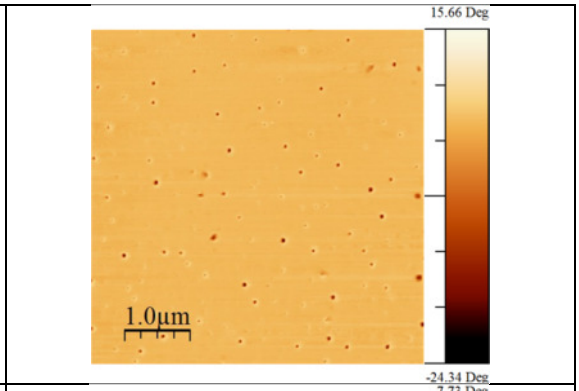
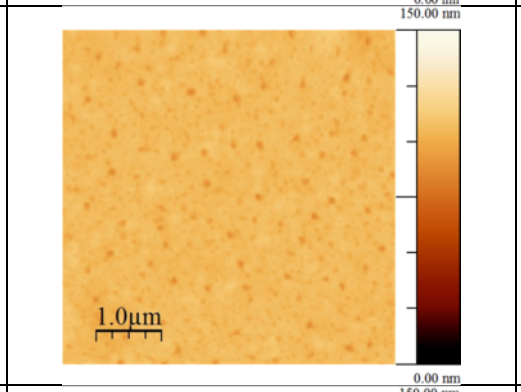
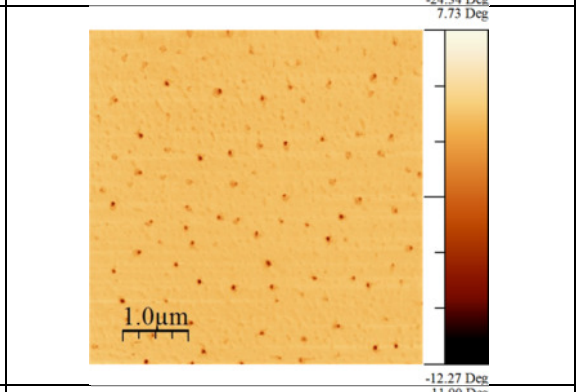
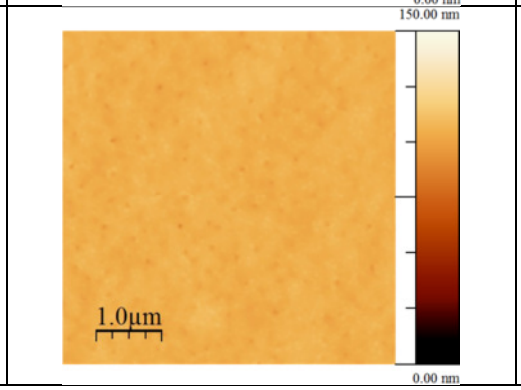
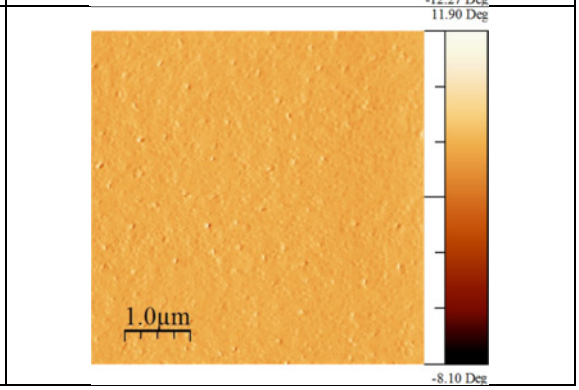
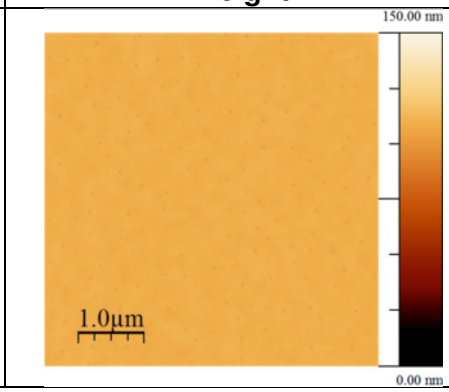
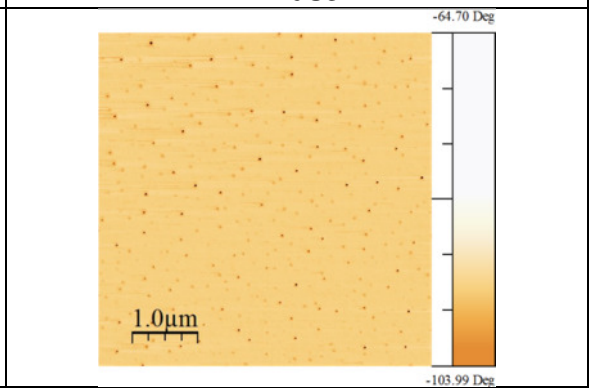
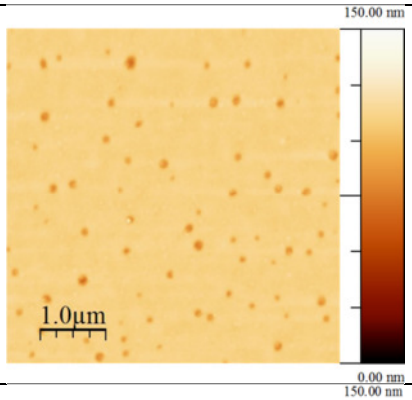
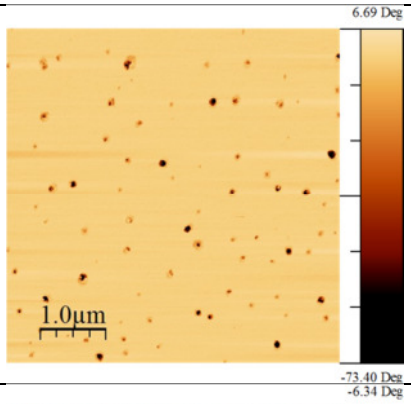
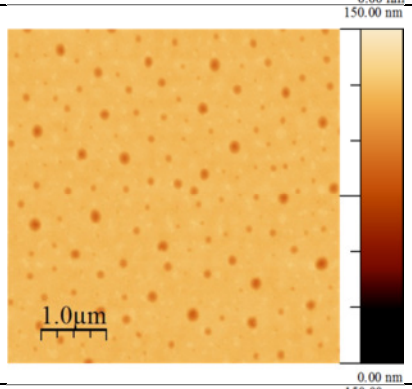
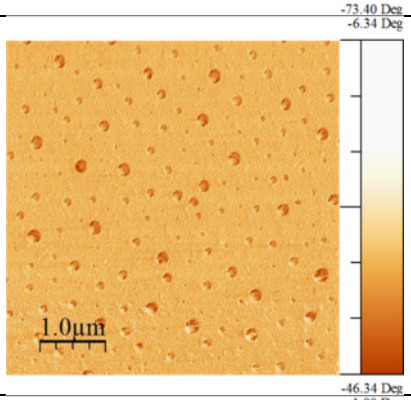
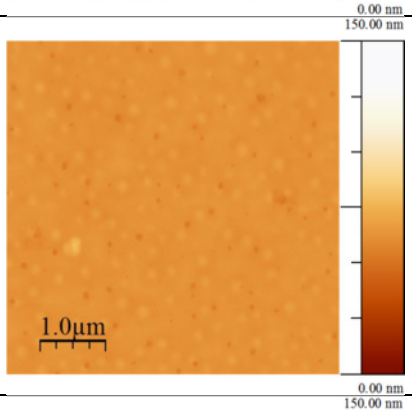
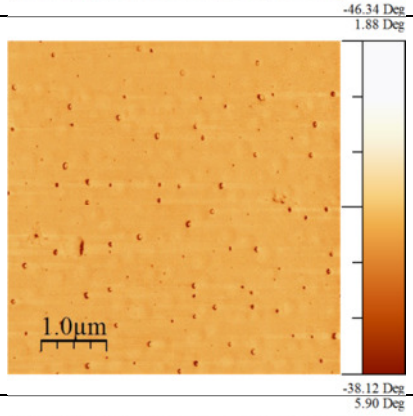
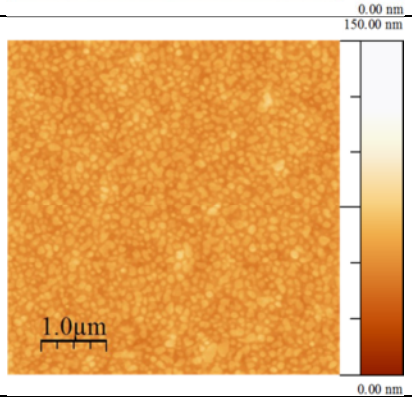
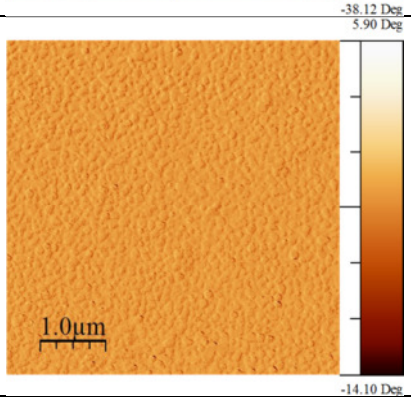
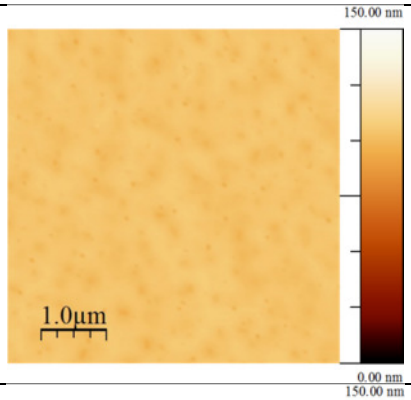
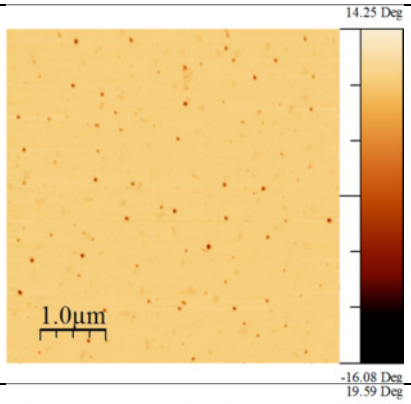
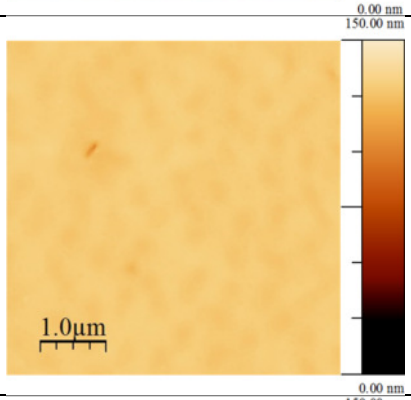
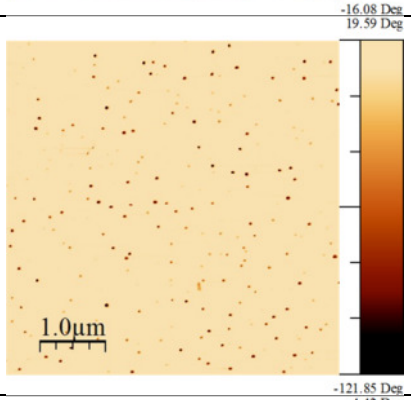
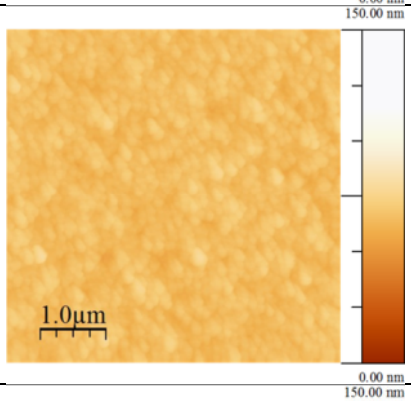
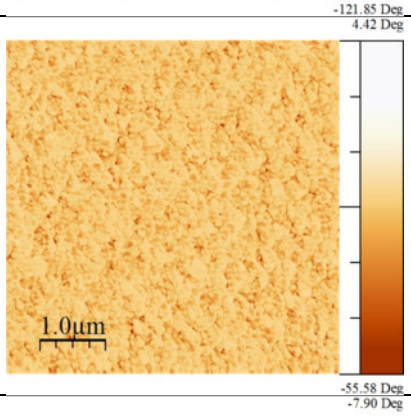
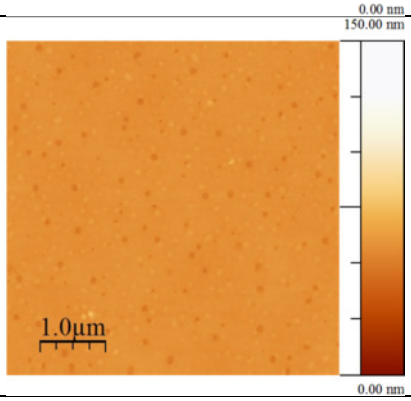
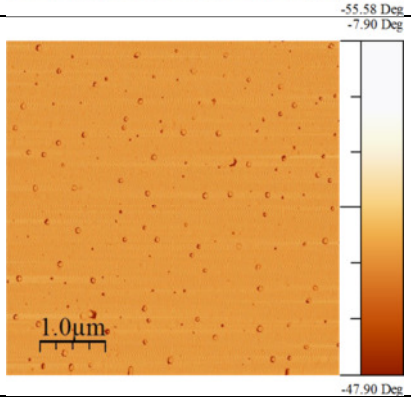
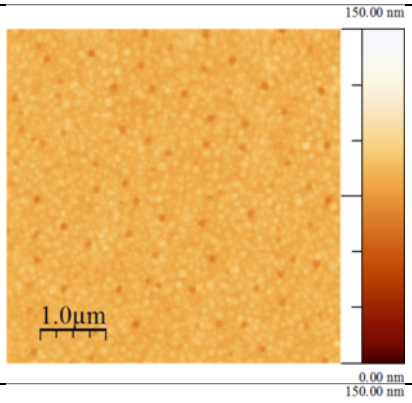
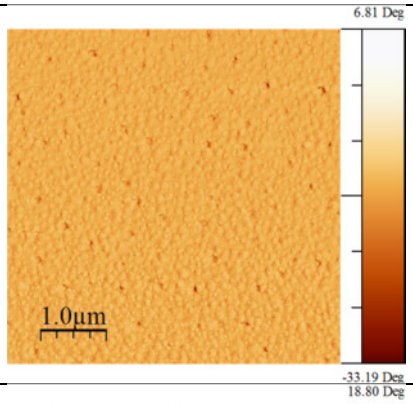
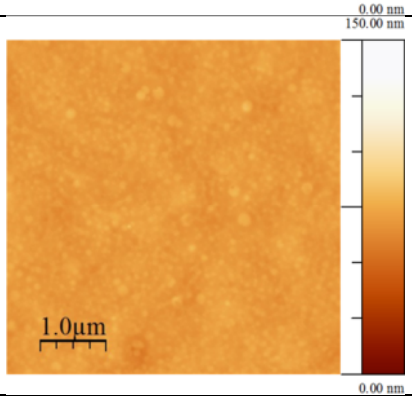
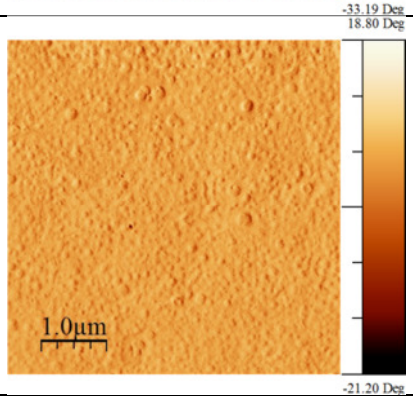
B:D=9:1-P R = 1 nm		
B:D=1:1-P R = 2 nm		
B:D=1:9-P R = 1 nm		

Table S14: Atomic force microscopy results of the SMAMP polymer networks

	Height	**Phase**
Methyl-A R = 1 nm		

Ethyl-A R = 2 nm		
Propyl-A R = 2 nm		
Butyl-A R = 2 nm		
Diamine-A R = 4 nm		

P:D=9:1-A R = 3 nm		
P:D=1:1-A R = 1 nm		
P:D=1:9-A R = 3 nm		
B:D=9:1-A R = 1 nm		

B:D=1:1-A R = 3 nm		
B:D=1:9-A R = 3 nm		

Catheter Coating Process

As proof-of-concept for a real-life substrate, medical grade silicone rubber tubing (=PDMS tubing, 2.0 mm inner diameter, 0.3 mm wall thickness, 2.6 mm outer diameter) from Eur. Pharm. (FDA §177.2600, cytotoxicity ISO-10993-1) was used. For testing, the tubing was cut into 4-6 cm long pieces and the ends were sealed with a silicone humidity curing or air drying adhesive (RS 692-542, RS Components). The pieces were then rinsed several times with distilled water, ultrasonicated for 5 min (Ultrasonic bath 5510, Branson) and dried (first in air and, then under nitrogen).

The PDMS tubing was then activated by plasma oxidation (6 s, Diener electronics). Immediately after plasma oxidation, the surface was dip-coated with 3EBP silane solution and then heated for 1 h at 120 °C in an oven. Excess 3EBP silane was removed by rinsing with toluene. The substrate was allowed to dry in air at room temperature, then dip-coated into activated SMAMP polymer and cross-linker solution (containing 60 mg/mL of polymer and 16 mg/mL of cross-linker in methanol) at 500 mm/second (maximum speed). Then, the substrate was allowed to dry in air again and the SMAMPs coating was cross-linked by UV light irradiation (254 nm, 3 J). Typically, the tubing was exposed to a dose of 1.5 J of

radiation, then turned around and irradiated by another dose of 1.5 J to make sure all sides were equally well covered with covalently attached coating. After cross-linking, the surface was immersed in ethanol for 2 h, and then rinsed thoroughly with ethanol. The sealed ends of the substrate were discarded. For the antimicrobial testing, the PDMS tubing was then sliced by its length in half and cut into 1.5 cm long pieces. The halves were placed in an Eppendorf tube and sterilized. For maximum activity, two layers of SMAMP coating were applied.

The antimicrobial activity of the tubing was tested with the spraying assay described below. Catheter pieces with 100 nm or 200 nm thick SMAMP coatings were cut in half. They were then either immersed into ethanol or autoclaved. The samples were sprayed with *E. coli* bacteria and incubated for 2 h (see Experimental). The results are shown in Fig. S16. In appearance, there is no marked change between the 100 nm or 200 nm thick SMAMP coating. However, the antimicrobial data reveals that the 200 nm thick coatings were fully bactericidal, while the 100 nm thick coatings had only 97-98% activity. The two sterilization processes (ethanol and autoclaving) did not compromise the performance of the coating, as both 200 nm thick coatings were bactericidal (activity > 99.9%). The physical characterization of the 3D tubing was difficult. However, flat model surfaces that underwent the modified SMAMP coating process were studied, and had a static water contact angle of 64°, and a thickness of 100 nm (measured by ellipsometry). This contact angle is very close to the contact angle of the SMAMP after surface-activation in the previous process, indicating that the coating composition is very similar.

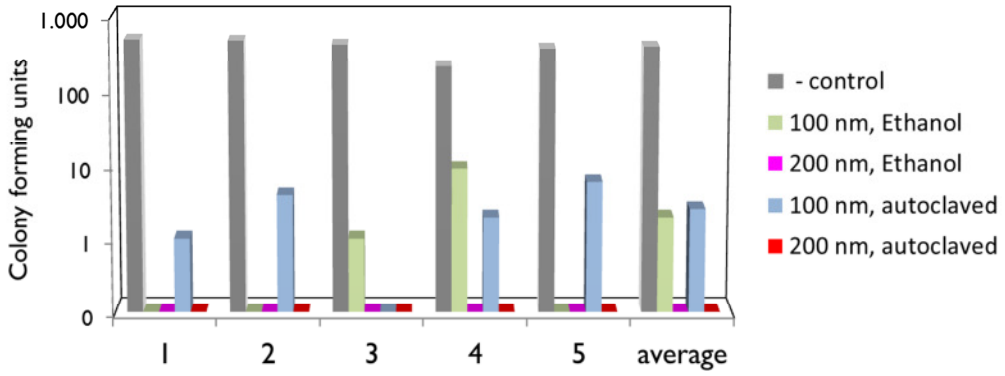


Fig. S16: Results of the antimicrobial spraying assay of PDMS tubing. Four different kinds of SMAMP coatings were tested. Each experiment was conducted with 5 replicas. On average, there were 392 ± 110 colony forming units (CFUs) on the negative control (uncoated wafer). For the 100 nm thick coatings, an average of 2.0-2.6 ± 3 CFUs was obtained, for the 200 nm coatings, 0 ± 0 CFUs were recorded, indicating bacterial killing > 99.9%. In other words, the PDMS tubings were bactericidal.

Synthesis and Characterization of Reference Polymer Networks:

Three reference polymer networks were synthesized and tested for their antimicrobial activity and cell compatibility: an antibiofouling poly(dimethyl acrylamide) based network (=PDMAA), the two antimicrobial networks based on poly(butyl acrylate-co-ethylamminium acrylate) and poly(vinyl buylpyridinium), respectively. The structures of the polymers are shown in Fig. S17.

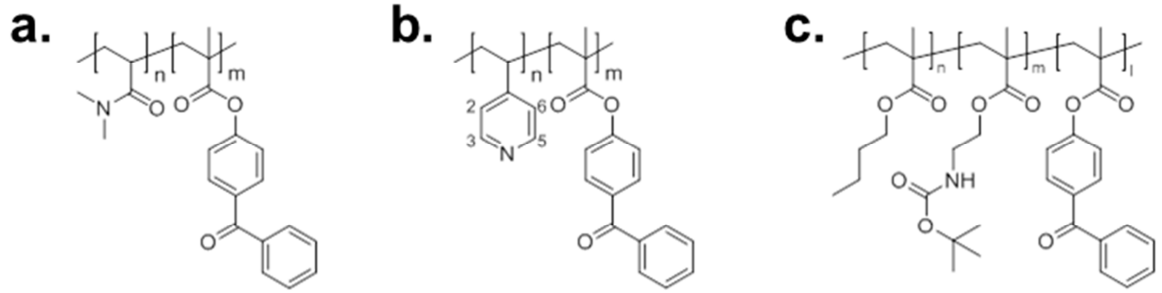


Fig. S17: Chemical structure of the reference copolymers: a) poly(DMAA-1%MABP), b) poly(vinylpyridine-co-1%MABP), c) poly(butyl methacrylate-co-Boc-ethylamine methacrylate-co-MABP).

Synthesis and Characterization of Poly(DMAA-1%MABP) (=PDMAA)

The synthesis of 4-methacryloyl benzophenone (MABP) was reported elsewhere.[3] MABP and dimethylacrylamide (DMAA) were distilled before use. For polymerization, MABP (107 mg, 0.4 mmol) and DMAA (39.6 mmol) were dissolved in DMF (39 mL). A solution of azo-iso-butyronitrile (AIBN) in dimethylformamide (0.04 mmol/mL, 1 mL) was added and the reaction mixture was degassed by three freeze-pump-thaw cycles. After the mixture was stirred at 60 °C for 16 h, the polymer was precipitated by dropping the reaction mixture into diethyl ether (500 mL). For purification, the product was dissolved in chloroform and precipitated again in diethyl ether. The polymer poly(DMAA-1%MABP) (2.0 g, 50%) was obtained as colorless product after drying under high vacuum.

^{1}H-NMR (250 MHz, $CDCl_3$): 1.20-1.80 (m, -CH_2), 2.80-3.05 (m, 2 × CH_3), 3.10-3.25 (br s, CH)

GPC (PSS GRAM column, DMF, 35 °C, 1 mL/min): M_n = 36,000 g mol^{-1}, M_w = 82,000 g mol^{-1}, PDI = 2.3.

Synthesis and Characterization of PDMAA networks

A solution of PDMAA-*co*-1%MABP in ethanol (15 mg/mL) was spin-coated (3000 rpm, 60 sec) onto a silicon wafer functionalized with 3EBP. The resulting film was cross-linked at 254 nm for 10 min in the BIO-LINK Box. The unattached polymer chains were removed by immersing the sample in ethanol for 4 h.

The AFM images of the PDMAA network are shown in Fig. S18.

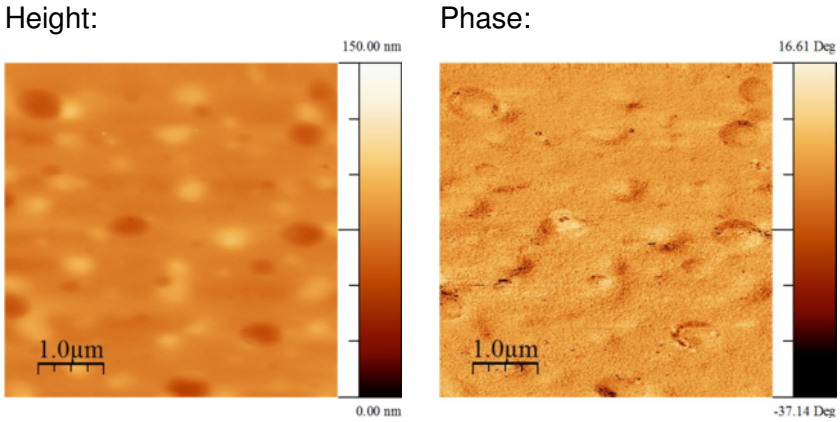


Fig. S18: Atomic force microscopy results of the PDMAA networks, demonstrating uniform coatings with a RMS roughness of 4 nm.

The ATR-FTIR results for the PDMAA network are shown in Fig. S19.

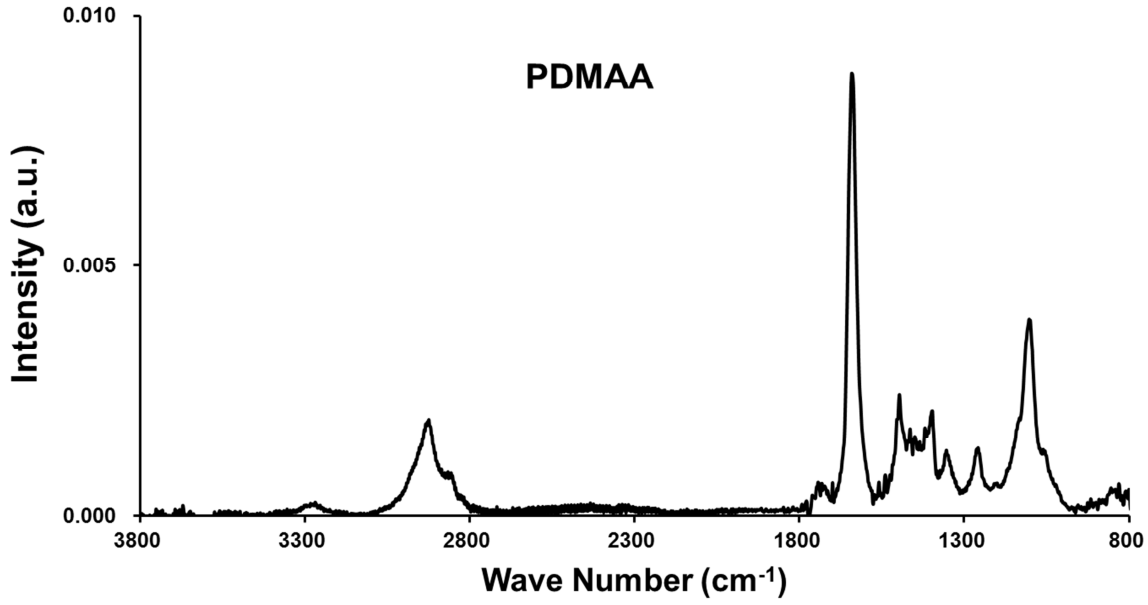


Fig. S19: ATR-FTIR spectrum of the PDMAA networks.

Synthesis and Characterization of Poly(vinylpyridine-1%MABP)

Fresh distilled 4-vinylpyridin (2.2 g, 19.8 mmol) and MABP (53 mg, 0.2 mmol) were dissolved in DMF (9 mL). A solution of AIBN in DMF (0.02 mmol/mL, 1 mL) was added and the reaction mixture was degassed by three freeze-pump-thaw cycles. The reaction mixture was then stirred at 60°C for 20 hours and the polymer was precipitated into diethyl ether. For purification, the product was dissolved in methanol and precipitated again in diethyl ether. In the end, the polymer poly(vinylpyridine-1%MABP) (0.6 g, 27%) was obtained as white product after drying under high vacuum.

^{1}H-NMR (250 MHz, methanol-D4): 1.42-1.89 (m, -CH & -CH_2), 6.53-6.81 (m, 2-CH & 6-CH), 8.04-8.35 (m, 3-CH & 5-CH).
GPC (PSS GRAM column, DMF, 35 °C, 1 mL/min): M_n = 125,000 g/mol, M_w = 200,000 g/mol, PDI = 1.6.

Synthesis and Characterization of Poly(vinylpyridine-1%MABP) Networks (=Vinyl pryridine), followed by Quarternization

PVP-*co*-1%MABP (20 mg) was dissolved in a mixture of ethanol (0.6 mL) and acetone (0.9 mL). The solution was filtered and spin-coated a silicon wafer functionalized with 3EBP. The film was then cross-linked in BIO-LINK Box at 254 nm for 10 min and the unattached polymer chains were removed by washing with ethanol. For quaternization, the sample was immersed in a solution of butyl bromide in nitromethane (Vol.: 20%) at 75 °C for 72 h. After the reaction, the sample was washed with nitromethane, methanol and dried under nitrogen flow.

The AFM results of the vinyl pyridine network after quarternization are shown in Fig. S20.

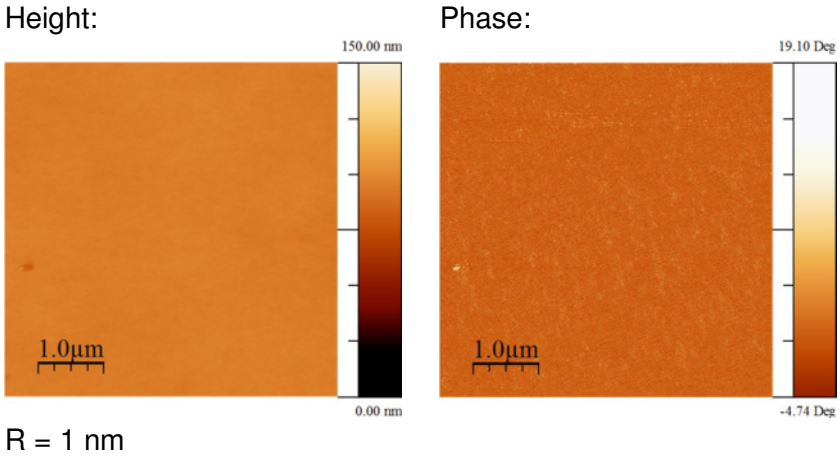


Fig. S20: Atomic force microscopy results of the vinyl pyridine networks, demonstrating very smooth, uniform coatings with a RMS roughness of 1 nm.

The ATR-FTIR spectra of the vinyl pyridine networks after quarternization are shown in Fig. S21.

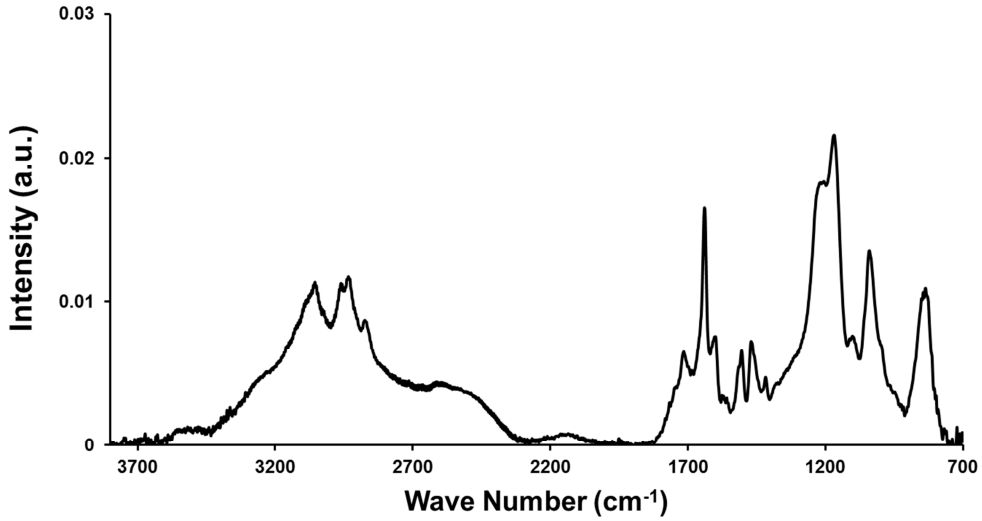


Fig. S21: ATR-FTIR spectrum of the vinyl pyridine networks.

Synthesis and Characterization of Poly(butyl methacrylate-co-N-Boc-ethylamine methacrylate-co-MABP) Polymer (=Acrylate).

N-Boc ethylamine methacrylate (0.92 g, 4.0 mmol) and 0.16 mL (1.0 mmol) butyl methacrylate (0.16 mL, 1.0 mmol) were dissolved in acetonitrile (anhydrous, 1.5 mL) containing AIBN (4 mg, 0.025 mmol, 0.5%). The flask was closed and the reaction mixture was degassed by three freeze-pump-thaw cycles. Afterwards the mixture was stirred for 24 h at 60 °C. The oily residue was diluted with 20 mL DCM and dropped into cold hexane. The precipitate was filtered off, dissolved again and once more precipitated into hexane. The polymer was dried in high vacuum and obtained as colorless solid (0.91 g, 86%).

^{1}H NMR (250MHz, $CDCl_3$): 0.75-1.15 (m, $(CH_2)_2CH_3$), 1.48 (s, *tert*-butyl), 1.61 (s, C-CH_3), 1.70-2.10 (br m, C-CH_2), 3.4 (br s, NH- CH_2), 4.04 (bs, O-CH_2), 5.48 (bs, NH).
GPC (PSS SDV column, chloroform, r.t., 1 mL/min): M_n = 27,000 g/mol, M_w = 116,000 g/mol, PDI = 4.3.

Synthesis and Characterization of the Acrylate Network:

The acrylate polymer was dissolved in $CHCl_3$ (15 mg mL^{-1}). The solution was filtered, spin-coated (3000 rpm, 30 sec) onto a silicon wafer functionalized with 3EBP and cross-linked at 254 nm for 10 min in the BIO-LINK Box. The unattached polymer chains were removed by immersing the sample in $CHCl_3$ for 4 h. The surface was immersed into 4 M HCl in dioxane to remove the Boc protective group.

The AFM results of the acrylate network after deprotection is shown in Fig. S22

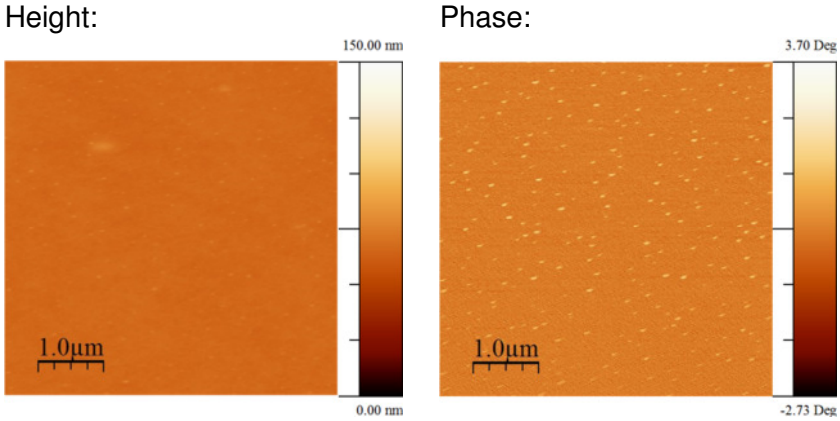


Fig. S22: Atomic force microscopy results of the acrylate networks, demonstrating very smooth, uniform coatings with a RMS roughness of 1 nm.

The ATR-FTIR spectrum of the Acrylate Network after deprotection is shown in Fig. S23.

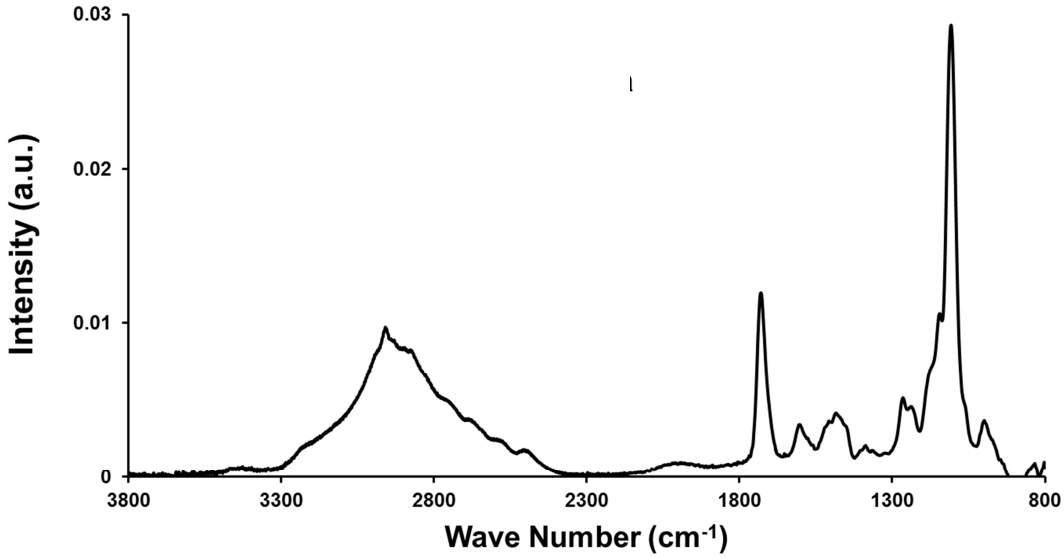


Fig. S23: ATR-FTIR spectrum of the acrylate networks.

Antimicrobial Activity Testing:

All bacterial strains used in this study were maintained routinely with weekly sub-culturing on Columbia blood agar (CBA, Oxoid, Wesel, Germany). Long-term storage of these bacteria was at - 80°C in basic growth medium containing 15% (v/v) glycerol according to Jones et al. [4] and as described earlier [5].

The basis of the antimicrobial activity assay was the Japanese Industrial Standard JIS Z 2801:2000, a water-borne test for antibacterial activity and efficacy, as described by Madkour et al. [6]. Importantly, the bacterial inoculation through pipetting a bacterial suspension onto the test material in the JIS assay was replaced by spraying the bacterial suspension on the material in the airborne antimicrobial assay. All other steps were similar. The assay was conducted as described previously[2d]: *Staphylococcus aureus* ATCC29523, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Enteriococcus faecalis* T9 were tested. Overnight cultures were prepared in tryptic soy broth (TSB, Merck, Darmstadt, Germany). A log-phase culture was prepared from an overnight-culture by transferring a specific volume into fresh TSB culture medium and incubating for 3-4 h. The optical density of this culture was measured using a Smart-Spec plus spectrophotometer (Bio-Rad, Life Science Group, Hercules, USA) at 595 nm. A bacterial solution with a concentration of ca. 10^6 colony forming units (CFU) per ml was prepared for each bacterial strain by dilution in 0.9 % saline solution. The bacterial solution was transferred into the sterilized sprayer containing a stirring bar. The sprayer was then positioned in the spraying set-up described previously[2d] at a distance of 15 cm from the sample. The device was placed on a magnetic stirrer, and the bacterial suspension was sprayed using compressed air from a 50 ml syringe.

The test samples were then transferred into a sterile petri dish and incubated for 30 min and 2 h in a humid chamber at 37°C under aerobic conditions and 5% CO_2 (capnophilic conditions). For each incubation period, five test samples, five positive controls coated with the antiseptic chlorhexidine, and five negative controls (uncoated samples), were tested. After incubation, 50 µl of sterile 0.9% saline solution was added with a pipette onto the sprayed area of the tested surface, and left for 2 minutes. To ensure removal of the bacteria from the surface, the solution was pumped back and re-pipetted twice, then pumped back a third time and spread on a Columbia blood agar plate (CBA). The agar plates were incubated for two days at 37°C with 5% CO_2. The colony forming units (CFUs) were counted using the Gel Doc EQ Universal Hood (Bio-Rad Life Science Group, Hercules, USA). The killing effects of the coated surface were measured by comparing the CFU number with the results from the uncoated wafers (negative control) and with the wafers which were previously immersed

in 0.2 % chlorhexidine digluconate (positive control). The percent killing was calculated by the following method:

$$\%\, Survival = \frac{Test\ sample\ CFU - pos.control\ CFU}{neg.control\ CFU - pos.control\ CFU} \times 100.$$

The results of the antimicrobial activity tests are shown in Fig. 6 of the main text.

For the PDMS tubing, the test was slightly modified: 150 µL of the *E. coli* bacterial culture was mixed in a chromatography sprayer bottle with 100 mL of sterile NaCl solution (0.9 %) and continuously stirred. One half of a 1.5 cm long PDMS tubing was glued to the center of a Petri dish, immediately covered and transferred to a sterile laminar flow hood (Astec Microflow). The Petri dish was placed 15 cm from the sprayer. Then the bacterial suspension was sprayed on the Petri dish with the sample by quickly pressing an air volume of 60 cm^3 through a plastic syringe. Immediately after spraying, the Petri dishes were covered and then incubated for 2 h at 37 ºC. After this, the catheter was taken out from the Petri dish and immersed in 500 µL of sterile NaCl solution (0.9%) in an Eppendorf tube and vortexed for 10 s. The sample was then ultrasonicated for 2 minutes (Grant ultrasonic bath). 250 µL of solution was taken from the Eppendorf tube and plated out over Columbia agar dishes. These were incubated overnight at 37 ℃ without agitation. Viable bacterial colonies were counted with the computer program Quantity One. A summary of the typical results obtained are shown in Tables S15 and Fig. S16.

Table S15: Antimicrobial activity test for PDMS tubing.

Negative control	SMAMPs coating, 100 nm, ethanol	SMAMPs coating, 200 nm, ethanol	SMAMPs coating, 100 nm, autoclave	SMAMPs coating, 200 nm, autoclave
488	0	0	1	0
473	0	0	4	0
417	1	0	0	0
214	9	0	2	0
369	0	0	6	0

Cell compatibility testing:

Two kinds of toxicity tests were carried out with immortalized human keratinocytes and primary human fibroblast cells: the Alamar Blue cell viability assay, and a PCR based viability assay.

Ethics Statement:

Human gingiva mucosal keratinocytes and gingival fibroblasts were obtained using the explant technique.[7] Patients whose tissue was harvested had given their informed consent in accordance with the Helsinki declaration.

PCR Assay:

Sample Preparation. Silicon wafers (1.5 cm x 1.5 cm) coated with test samples and non-coated wafers (negative control) were washed in 70 % ethanol for 10 minutes in order to sterilise. Thereafter, wafers were allowed to dry under sterile conditions, and then washed in sterile PBS 3 times for 10 minutes to remove all traces of ethanol. Four silicon wafers per coating treatment were placed in a cell cultivation petri dish (diameter ~ 54 mm) (Greiner, onto which cells were seeded out.

Cell Treatment:

Immortalised gingival mucosal keratinocytes (HPV-16 E6/E7 [8], passages between 80 - 110) in keratinocyte growth medium (KGM) (Cat. No. C-20011, Promocell, Heidelberg, Germany) and human primary gingival fibroblasts (passages 6 – 14) in DMEM (PAA, Pasching, Austria) were cultivated in a Hera Cell 150 cell incubation chamber (Thermo Electron Corporation, Langenselbold, Germany) at 37 °C and 5% CO_2 until required. Cells were then trypsinized and resuspended in the appropriate medium at to give 7.3 x 10^4 cells / mL. 5 mL of the cell suspension were pipetted onto the antimicrobial polymers surfaces to give a cell density of ~ 3200 cells / cm^2. Control samples were generated by plating cells onto cultivation dishes, and cells were cultivated further until RNA was extracted after 6 hours.

RNA Extraction and Quantification:

RNA extraction was undertaken, by removing silicon wafers from the cell cultivation dishes and immediately treating with 600 µl RTL lysis buffer (Qiagen, Hilden, Germany) combined with 12 µl of 2M dithiothreitol (DTT). Lysis buffer was pipetted gradually onto the silicon wafers with the overflow being collected in a fresh cultivation dish underneath the sample and at an incline to allow the lysis buffer to flow across the silcon wafer.This procedure was repeated 3 times with a recycling of the lysis buffer. Finally, RNA was isolated in 30 µl RNase free water by running the 600 µl extracted RNA through a Qiagen QIAcube robot (Qiagen, Hilden, Germany) using the RNAeasy extraction kit (Qiagen, Hilden, Germany).

RNA quantification and quality assessment was made by Bio-Rad Experion automated electrophoresis system (Experion BioRad, München, Germany). cDNA was synthesized using 500 ng total RNA aliquots written to DNA with the Fermentas RevertAid First Strand cDNA Synthesis Kit (ThermoScientific, MA, USA). cDNA concentration was measured by Quant-iT™ PicoGreen ® dsDNA Kit (Life TEchznologies GmbH, Darmstadt, Germany), according to the manufacturer's instructions. Thereafter, each cDNA sample was normalized with nuclease free water in order to obtain samples of the same concentration (generally 5 – 10 ng / µl cDNA for each PCR reaction).

qPCR analysis was performed on normalized cDNA with the CFX96 real-time PCR detection system (BioRad, München, Germany). Quantitative amplification detection was accomplished using SABiosciences qPCR SYBR Green Master Mix (SABiosciences, Qiagen, Frederick, USA). Relevant biomarkers were examined with the aid of commercially available primers of Caspase 3 (CASP3), Annexin A5 (ANXA5), Interleukin 6 (IL6), Protein Ki67 (MKi67) and the housekeeping genes Ubiquitin C (UBC) and Ribosomal protein L13A (RPL13A) from (SABiosciences, Qiagen, Frederick, USA). The temperature profile protocol recommended by SABiosciences for primers was applied involving an initial denaturation step for 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 60s.

The relative gene expression levels for each biomarker was calculated using the $\Delta\Delta C_T$ equation and normalized against the C_T index of the UBC and RPL13A non-modulated housekeeping genes, while being referenced against the expression of control cells grown on standard plastic cell culture surfaces. The presented data represent the mean (n=2, ±SD) for three independent experiments.

Results for Fibroblasts:

Relative concentrations of RNA extracted from the fibroblasts are shown below in Fig. S24. Because initial cell density was the same for each sample, RNA quantity could be used as indicative of cell numbers on the samples. This suggests that samples B:D=5:5 and P:D=5:5 were the most conducive to cell proliferation. It is important to note that the RNA concentration (each in 30 µl) for the control sample is the result of extracting RNA from all cells on a cultivation dish. Normalisation to give a comparable area to the silicon wafer samples, suggests a concentration in the region of 47 ng / ml (for an area of 6 cm^2 = 4 x 1.5 cm^2 silicon wafer samples). This in turn could indicate that the antimicrobial polymer surfaces are in fact inducing an increased (abnormal) proliferation; alternatively this phenomenon may

be the result of superior adhesion properties of the antimicrobial polymers allowing cells to adhere more rapidly and to begin to proliferate sooner.

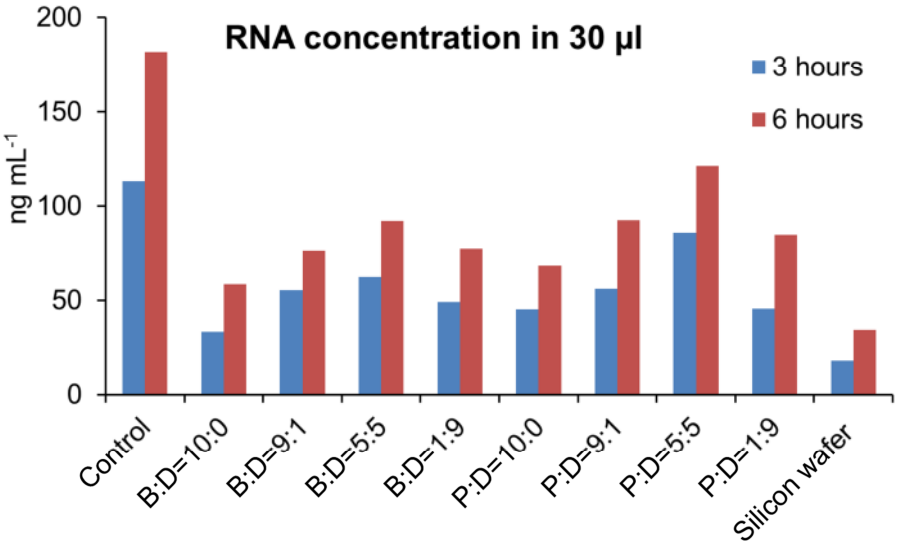


Fig. S24: RNA concentration (in total of 30 µl) in samples extracted from cells growing on 4 x 1.5 cm^2 silicon wafers.

PCR results for the fibroblasts presented in Fig. S25 reveal that the proliferation marker, MKi67 was consistent across all samples compared to the control (except for the negative control, the untreated silicon wafer), confirming a normal proliferation for each sample. In fact, all markers for apoptosis (ANXA5, CASP3), inflammation (IL6) and proliferation (MKi67) showed little if any deviation from the control sample indicating that cells on the antimicrobial polymers behaved similarly to control samples.

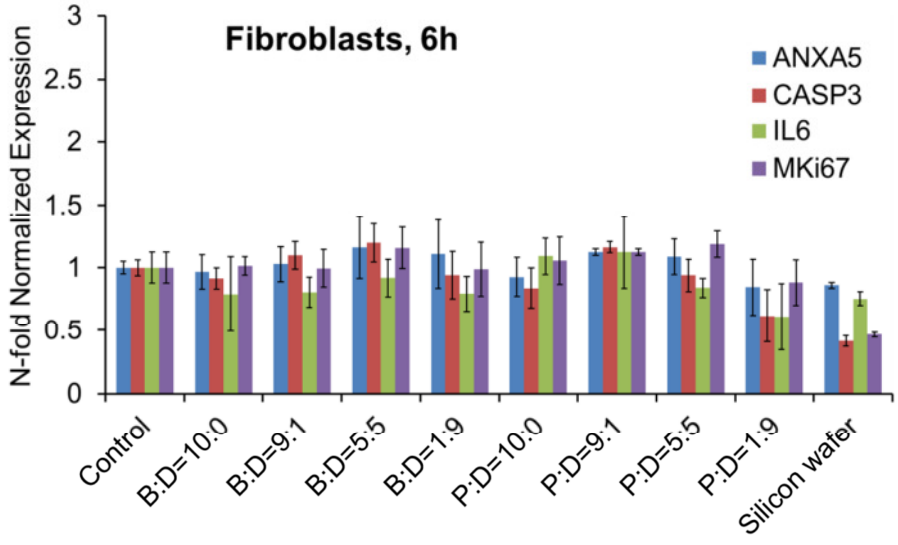


Fig. S25: PCR results for fibroblasts after 6 hours: ANXA5 = apoptosis marker, CASP3 = apoptosis marker, IL6 = inflammation marker, MKi67 = proliferation marker.

Results for Keratinocytes:

As displayed in Fig. S26, interleukin 6 (IL6) gene expression (marker for inflammation [9]) in keratinocytes cultivated on the antimicrobial polymer surfaces was significantly up-regulated with respect to the control cells growing on the standard plastic cultivation plate surface for all but P:D = 1:9 and B:D =1:9. In the more hydrophobic samples, upregulation of IL6 was seen to approach 20x that of the control sample. This suggests that those surfaces with a higher ratio of hydrophobic constituents are more conducive to eliciting an inflammatory response in the keratinocyte cells. Apparent also from the graph is that lower overall quantities of the RNA were extracted from cells on antimicrobial surfaces, where IL6 expression was highest. This result is sensible if one postulates that the cells are in a state where they are unable to initiate normal adhesion and proliferation mechanisms, whereby they would normally spread and expand. Results suggest that surface B:D = 1:9, and more particularly, P:D = 1:9 are the surfaces least likely to cause an inflammatory response in keratinocytes. The other RNA markers are normally regulated, indicating that proliferation and apoptosis are not increased. In summary, the data presented here indicates that polymer surfaces B:D = 1:9 and P:D = 1:9 causes the least deviation from normal keratinocyte gene expression *in vitro*.

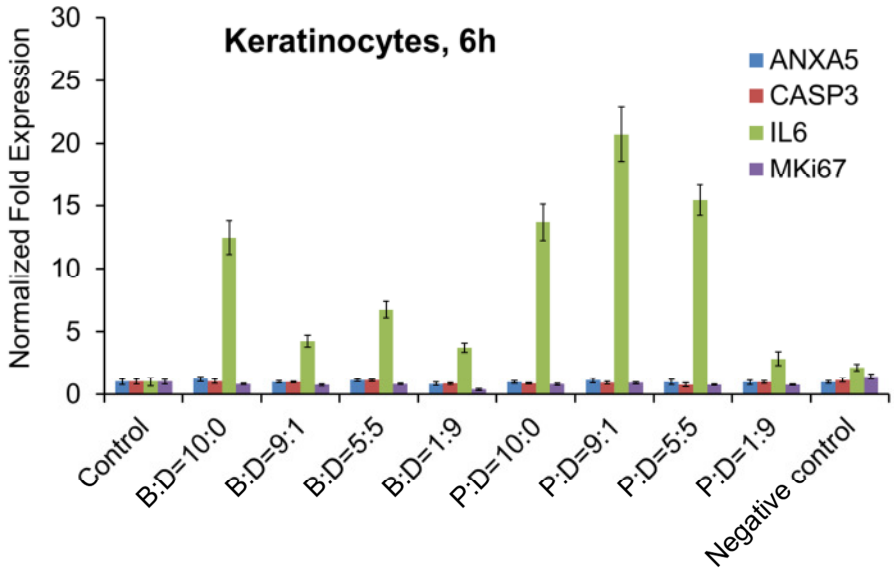


Fig. S26: PCR results for kerationcytes after 6 hours: ANXA5 = apoptosis marker, CASP3 = apoptosis marker, IL6 = inflammation marker, MKi67 = proliferation marker.

Alamar Blue Assay:

Sample preparation:

Alamar Blue experiments were performed on round glass microscope coverslips (22 mm diameter, thickness 0.5 mm; Langenbrick, Emmendingen, Germany), which had been coated with the test networks. Coverslips without coating were used as controls. Before commencing the experiment, coverslips to be used as controls were washed 30 minutes in 100% isopropyl alcohol, then 30 minutes in 100% acetone to emulate the process used for the spin-coated samples. Thereafter, both control and sample coverslips were sterilized for 15 minutes in 70% ethanol. All coverslips (test samples and controls) were subsequently washed 3 times with PBS in order to remove residual ethanol. Coverslips (samples and controls) were tested in triplicate and placed in 12-well plates (Cat. No. 655-180, bio-one Cellstar, Greiner, Frickenhausen, Germany), which themselves possess a 22 mm diameter, creating a near perfect fit between coverslip and well.

Cell treatment:

Immortalised HPV-16 gingival mucosal keratinocyte (GM-K) cells [8] were cultivated in Keratinocyte Growth Medium (KGM) (Promocell, Heidelberg, Germany) with accompanying supplements prepared at concentrations supplied by the manufacturer: bovine pituitary extract – 0.004 ml / ml; epidermal growth factor (EGF) – 0.125 ng / ml; insulin – 5 µg / ml; hydrocortison – 0.33 µg / ml; epinephrine – 0.39 µg / ml; transferin - 10 µg / ml; $CaCl_2$ – 0.06 mM; in addtion to the antibiotic kanamycin at 100 µg / ml. Cells were trypsinised at between 70 – 90 confluency and resuspended in supplement / antibiotic free KGM. Cells were seeded out onto test and control surfaces in 1 ml medium at 5.0 x 10^4 cells / ml (132 cells / mm^2) in supplement / antibiotic free medium. Thereafter, the 12 well plates containing the cells were incubated at 37°, 5% CO_2 for 6 hours allowing cells to settle and begin adhesion. At this time 500 µl of medium above the cells was carefully aspirated and replaced by 500 µl medium containing double normal supplement concentration yielding a normal supplement concentration medium as described above. This procedure was undertaken to ensure an initial direct contact between cell and surface, which otherwise could have been hindered by the adsorption of medium supplements onto the surface at the initial seeding out phase. Cells on test surfaces and controls were cultivated for a further 18 hours (total 24 hours), at which time positive control samples were generated by aspirating 500 µl medium from 3 wells and adding 500 µl 60 % iso-propyl alcohol to give a 30 % iso-

propyl alcohol solution. A negative control was generated by aspirating 500 µl medium and returning the medium to the well. All samples and controls were cultivated for a further 30 minutes, after which 110 µl pre-warmed (37°C)

Alamar Blue (AbD Serotec, Oxford, UK) was slowly pipetted into each well (samples and controls) with gentle agitation to ensure homogeneous dispersion giving a 10 % solution. Cells were returned to the incubation chamber for 2 hours, after which time all medium containing Alamar Blue was aspirated and collected into 2 ml Eppendorf tubes. Tubes were centrifuged at 1,000 g for 5 minutes to exclude cells, then the fluorescence intensity of the supernatant was measured (excitation at 540 nm and measurement at 590 nm) on a Tecan, Infinte 200 plate reader and data analysed according to the Alamar Blue manufacturer's instructions. The experimental procedure was repeated at 48 hours and 72 hours to give time dependent data. The results of the Alamar Blue assay are shown in Fig. 6 of the main text.

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