Electronic Supplementary Information

Bacteria Repelling Poly(methylmethacrylate-*co*-dimethylacrylamide) Coatings for Biomedical Devices

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1. Screening for bacteria repellent substrates on polymer microarrays

Polymer microarrays were fabricated by contact printing of preformed polymers as reported previously.[1,2,3]

1.1. Bacteria culture for microarray screening

Clostridium strains were cultured in an anaerobic environment (80% N_2,10% CO_2, 10% H_2) at 37 °C. All other strains were cultured in a microaerobic environment (85% N_2, 10% CO_2, 5% O_2) at 37 °C.

Clostridium perfringens and *Clostridium difficile*

C perfringens (NCTC 8257) and *C difficile* (NCTC 630) were grown in brain-heart infusion (BHI) supplemented with L-cysteine (0.5 mg/mL), sodium bicarbonate (1 mg/mL), anhemin (5 mg/L) and menadione (0.5 mg/L).

Campylobacter jejuni

C jejuni strains CH4 and NCTC 11168 were cultured in Brucella broth, supplemented with *Campylobacter* growth supplement (FBP) consisting of iron (II) sulphate (0.15 mg/mL), sodium pyruvate (0.15 mg/mL), sodium metabisulphite (0.15 mg/mL), vancomycin (2500 U/L) and trimethoprim (5 mg/L).

Streptococcus mutans

S mutans (NCTC 10923) was grown in Luria–Bertani (LB) broth.

Bacteria mixtures. The strains used in BacMix-1 and BacMix-2 were isolated from endotracheal tubes from ICU patients and genotyped by PCR analysis. BacMix-1 consisted of *Klebsiella pneumoniae* (*K pneumoniae*), *Staphylococcus saprophyticus* (*S saprophyticus*), and *Staphylococcus aureus* (*S aureus*), which were all grown in LB broth. BacMix-2 consisted of *Streptococcus mutans (S mutans)*, *S aureus*, *K pneumoniae* and *Enterococcus faecalis* (*E faecalis*), which were grown in brain-heart infusion (BHI).

1.2. Bacterial adhesion on the microarrays

For the microarray experiments, each bacterial strain was grown in 5 mL of the appropriate medium (see above, section 1.1) and incubated at 37 °C overnight with shaking to a constant density. For experiments with a single bacteria strain, 2 mL of each culture was collected by centrifugation (6000 rpm, 3 min), washed, resuspended in 20 mL of fresh medium. The microarrays (n = 2) were placed in a rectangular 4-well plate. 6 mL of the diluted bacteria culture (~2×10^8 CFU/mL) was added to the polymer microarrays (the arrays were submerged in medium), and the microarrays were incubated overnight. For the anaerobic and microaerophilic strains, the 4-well plate was placed in a gas jar. Overnight cultures of bacteria in BacMix-1 were combined in equal amounts and then diluted fourfold with fresh media prior to incubation on the polymer microarrays. Bacteria in BacMix-2 were prepared similarly but were incubated on microarrays at 37 °C over 5 days, under microaerophilic conditions with agitation (30 rpm).

After incubation, polymer microarrays were washed gently with 6 mL of PBS, and bacteria stained with DAPI (1 μg/mL in PBS, 6mL/well) for 20 min. Subsequently, the polymer microarrays were washed with PBS (3 × 6 mL) and deionised water (1 × 6 mL), and then dried with a stream of air. A GeneFrame and coverslip (1.9 × 6.0 cm, AB-0630) were applied to each slide and sprayed with 1% Virkon.

1.3. Analysis of bacterial attachment

The polymer microarrays were analysed using a LaVision Bioanalyser 4F/4S scanner with a DAPI filter using an exposure time of 30 ms and an automated fluorescence microscope (with an X-Y-Z stage running Pathfinder™; IMSTAR), which allowed capture of single images for each polymer spot. Brightfield and fluorescence channel (DAPI) were used for imaging under a ×20 objective.

Bacterial adhesion was evaluated *via* calculating the average fluorescence intensity of the quadruplicate polymer features after background correction. The population standard deviation of the eight features was also measured (four features for each polymer on the microarray, two microarrays used for each strain). Reproducibility between two identical microarrays was evaluated by a student t-test. Polymers with p-values < 0.001 and 6 degrees of freedom were considered statistically significant. Figures S1–S8 show bacteria repellence of the 'hit' polymer features on the polymer microarrays when compared to features with strong bacterial adhesion. Table S1 shows the comparison of fluorescence intensity from bacterial adhesion on 'hit' polymers and Table S2 the chemical composition of the 'hit' polymers.

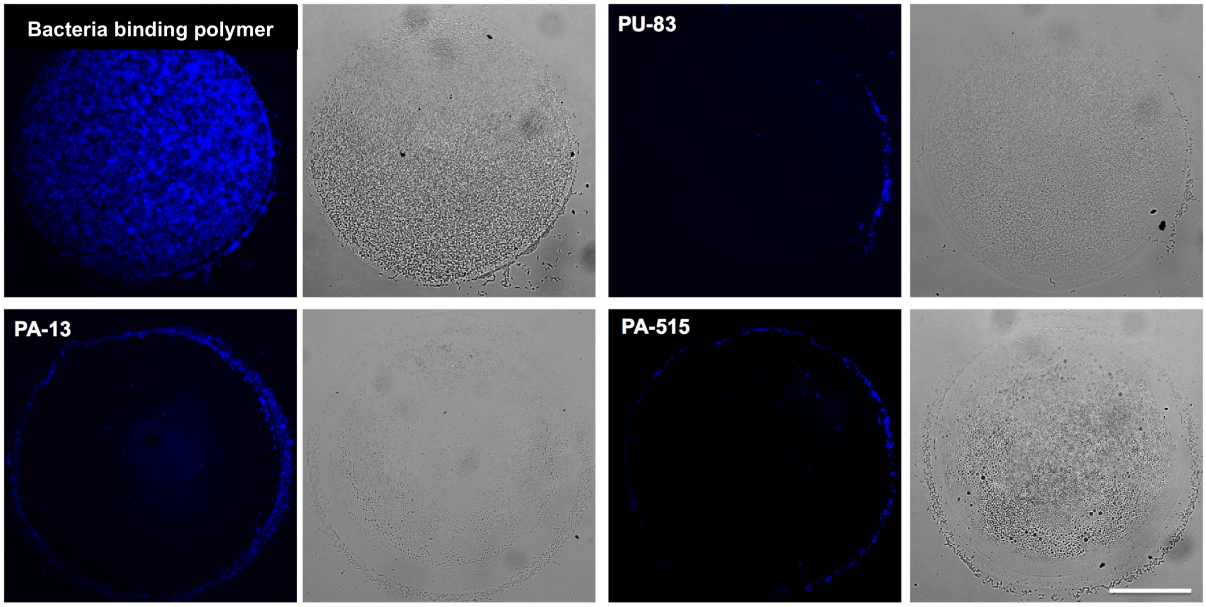

Fig. S1 Fluorescence (DAPI channel) and brightfield microscopy images of *C jejuni* 11168 attachment on a representative polymer feature exhibiting strong bacteria binding and on the *C jejuni* 11168 non-binding polymers. Scale bar = 100 µm.

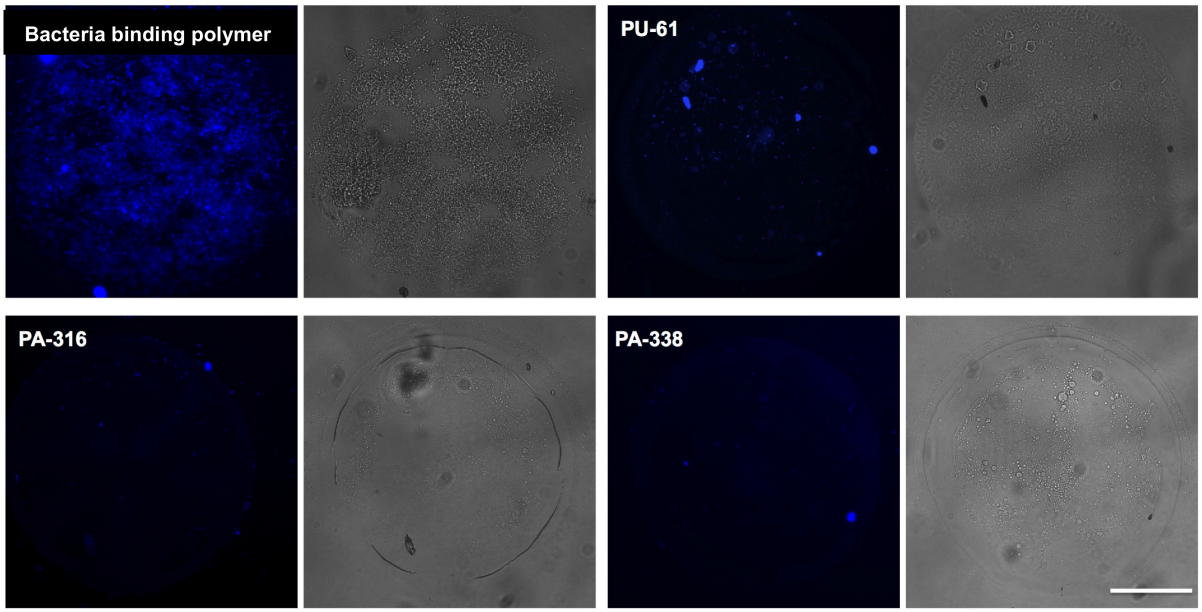

Fig. S2 Fluorescence (DAPI) and brightfield microscopy images of *C jejuni* CH4 attachment on a representative polymer feature exhibiting strong bacteria binding and on *C jejuni* CH4 non-binding polymers. Scale bar = 100 µm.

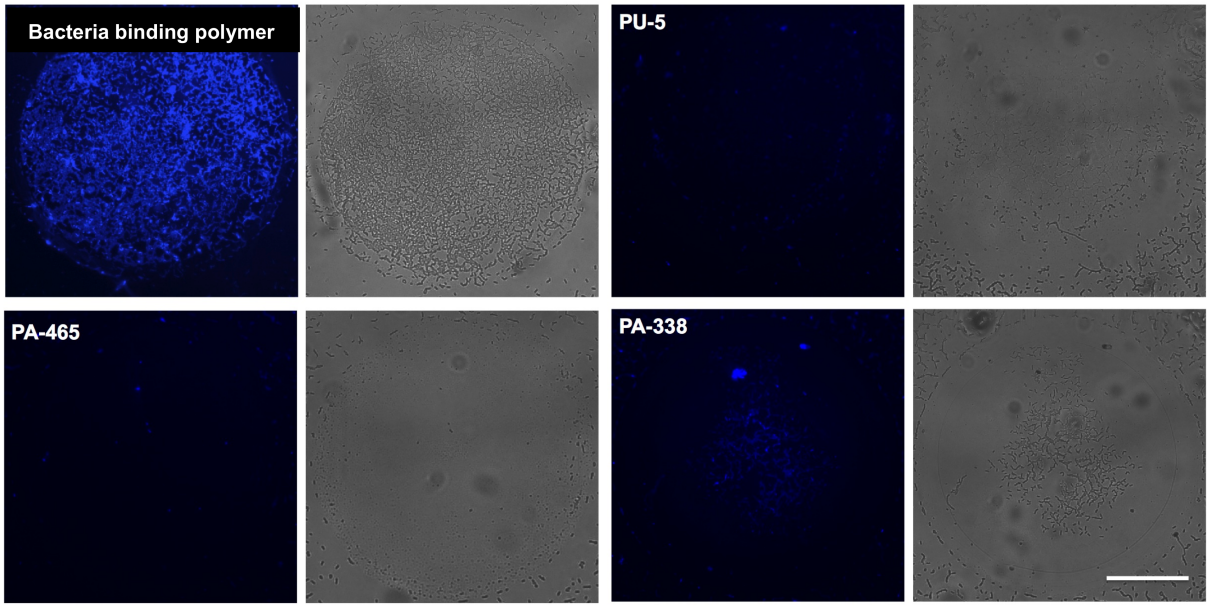

Fig. S3 Fluorescence (DAPI) and brightfield microscopy images of *C perfringens* on a representative polymer feature exhibiting strong bacteria binding and on the *C perfringens* non-binding polymers. Scale bar = 100 µm.

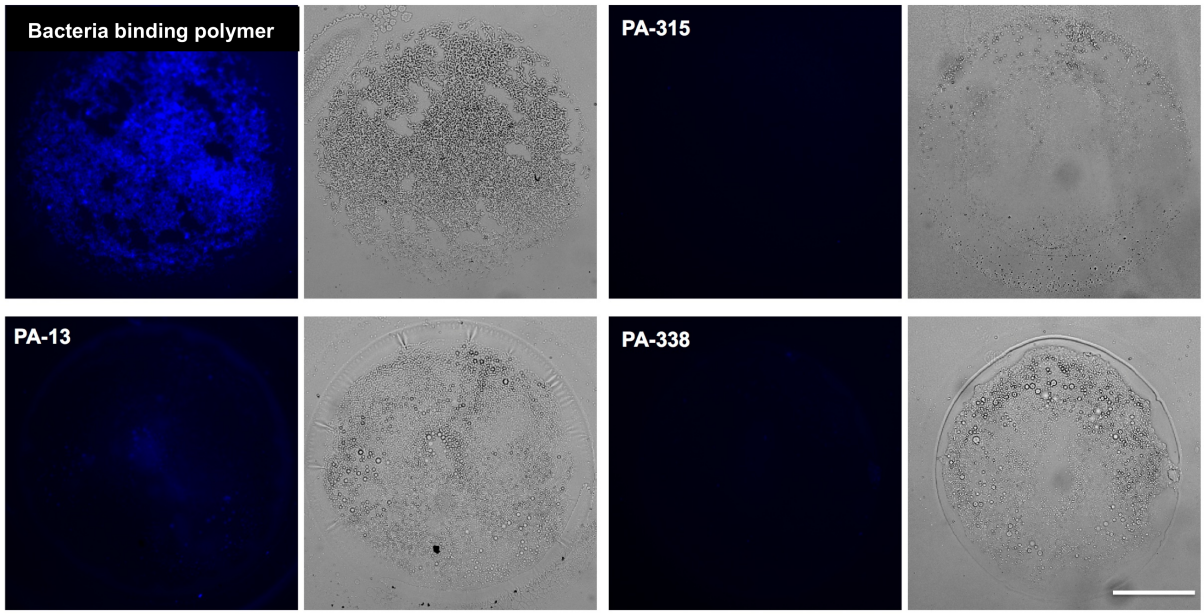

Fig. S4 Fluorescence (DAPI) and brightfield microscopy images of *C difficile* attachment on a representative polymer feature exhibiting strong bacteria binding and on *C difficile* non-binding polymers. Scale bar = 100 µm.

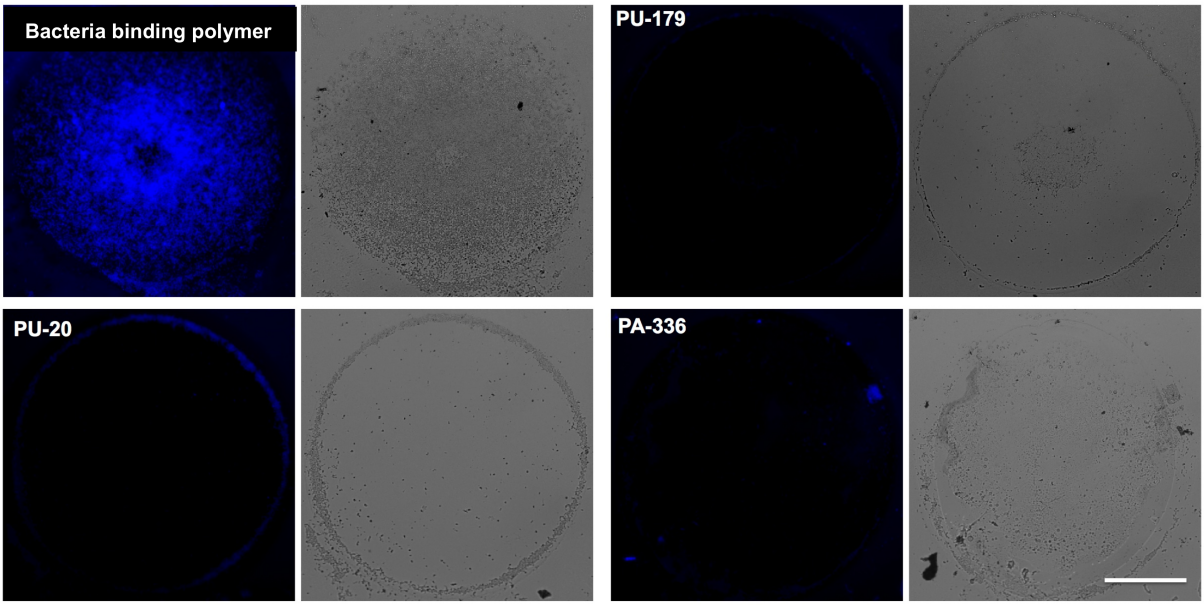

Fig. S5 Fluorescence (DAPI) and brightfield microscopy images of *S mutans* attachment on a representative polymer feature exhibiting strong bacteria binding and on *S mutans* non-binding polymers. Scale bar = 100 µm.

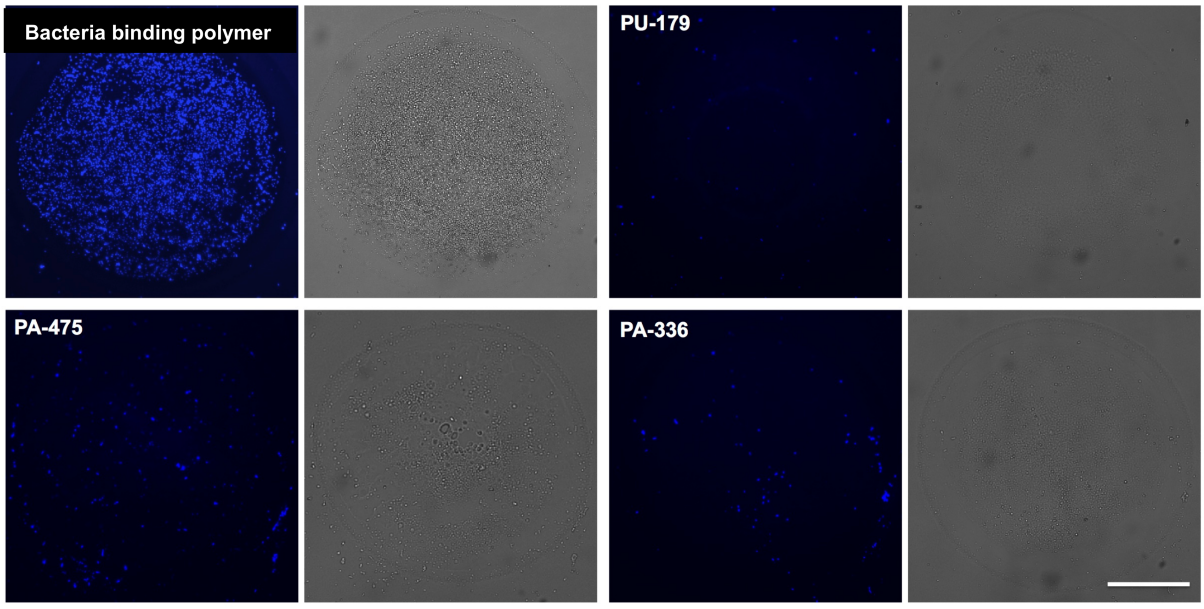

Fig. S6 Fluorescence (DAPI) and brightfield microscopy images of BacMix-1 attachment on a representative polymer feature exhibiting strong bacteria binding and BacMix-1 non-binding polymers. Scale bar = 100 µm.

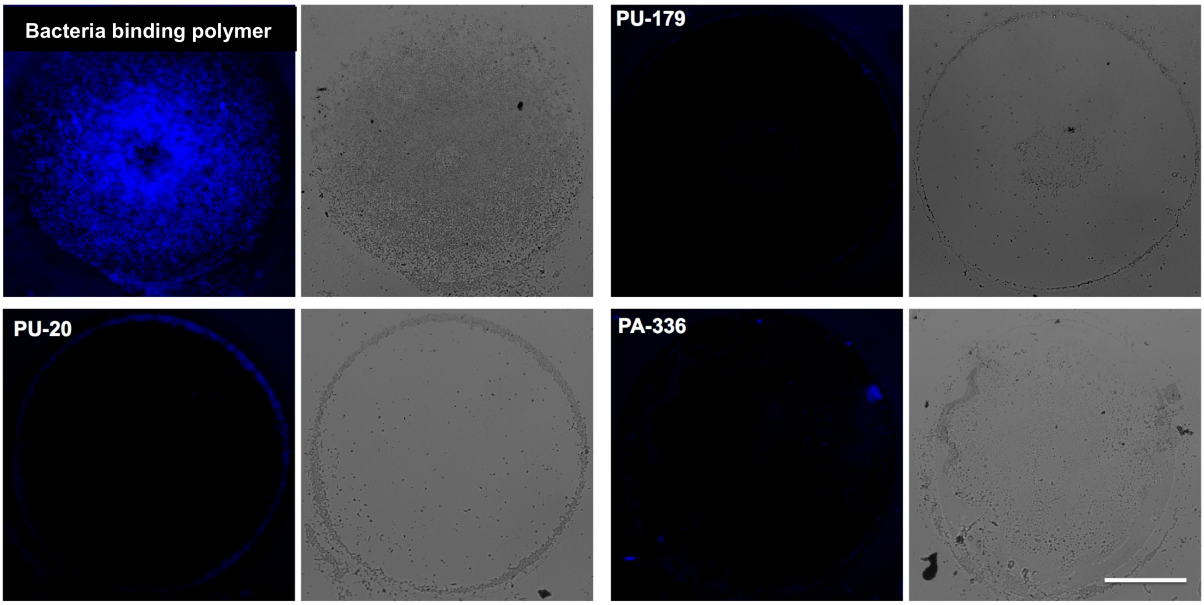

Fig. S7 Fluorescence (DAPI) and brightfield microscopy images of BacMix-2 attachment on a representative polymer feature exhibiting strong bacteria binding and the BacMix-2 non-binding polymers. Scale bar = 100 μm.

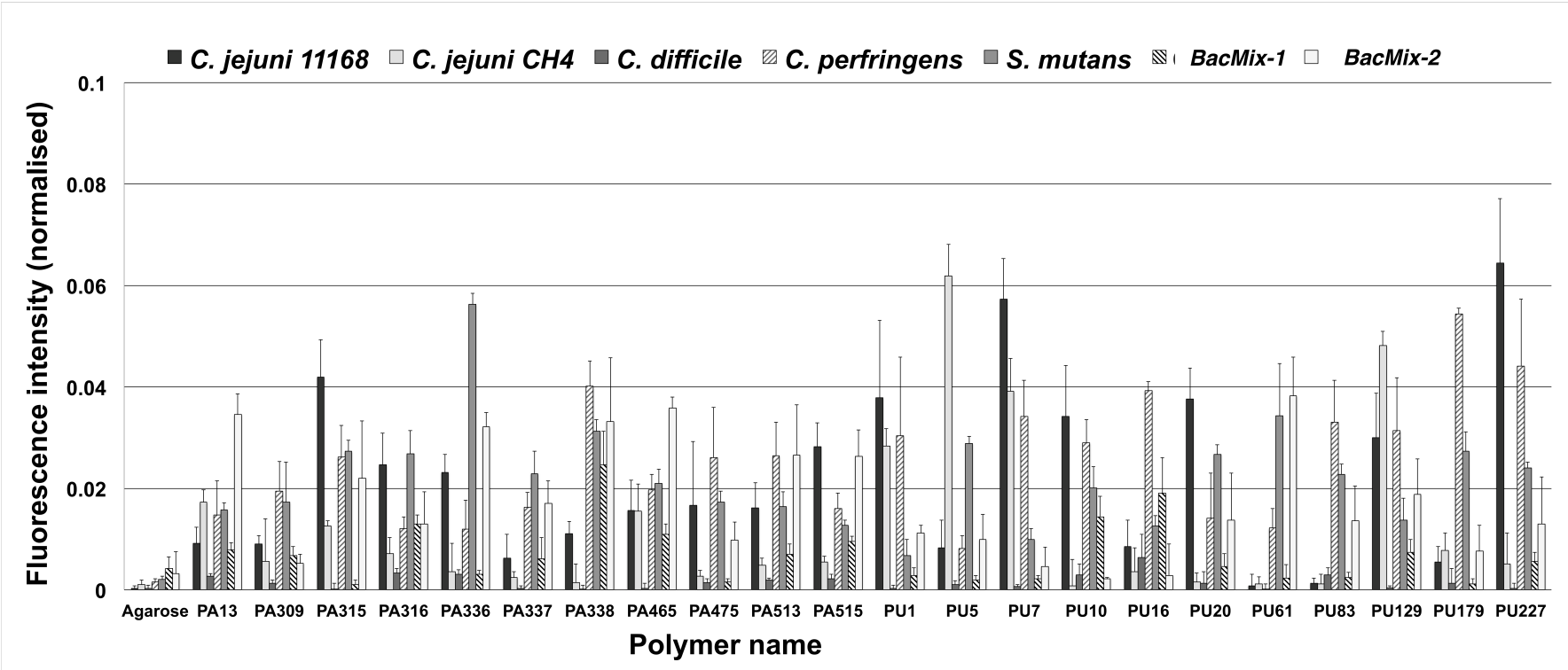

Figure S8. Polyacrylates/acrylamides (PA) and polyurethanes (PU) showing low binding of bacteria (*C jejuni, C difficile, C perfringens, S mutans,* and BacMix-1 and BacMix-2) expressed as background corrected mean DAPI fluorescence intensity (unit = arbitrary units).

Table S1. Analysis of bacterial adhesion on 'hit' polymers. Fluorescence intensity of DAPI stained bacteria on each polymer across bacteria strains and bacteria mixtures. Unit = arbitrary units (au) $\times 10^4$.

Polymer	*C jejuni* 11168	*C jejuni* CH4	*C perfringens*	*C difficile*	*S mutans*	BacMix-1	BacMix-2
Agarose	2	10	15	3	20	42	31
PA13	90	170	14	26	150	79	350
PA309	89	56	190	12	170	67	50
PA315	410	120	260	2	270	10	210
PA316	240	71	120	32	260	120	130
PA336	230	35	110	30	560	30	320
PA337	61	24	160	3	220	0	170
PA338	110	13	400	1	310	240	330
PA465	150	150	190	3	200	100	350
PA475	160	27	260	13	170	14	100
PA513	160	48	260	19	160	70	260
PA515	280	54	150	21	120	95	260
PU1	440	440	300	3	67	27	110
PU5	83	800	81	10	120	18	98
PU7	520	160	340	6	99	21	45
PU10	340	8	140	28	200	140	20
PU16	84	35	130	63	120	190	27
PU20	370	15	140	13	260	45	130
PU61	7	11	120	0	340	22	380
PU83	12	11	330	29	220	23	130
PU129	290	270	310	4	130	73	180
PU179	55	77	110	12	270	12	76
PU227	710	510	440	2	240	55	120

Table S2. Monomers and monomer ratios used in the synthesis of the bacteria non-binding 'hit' polymers.

Polymer	Monomer 1	Monomer 2	Monomer 3	Ratio of Monomers		
PA465	MEMA	DEAEMA	HEA	8.0	1.0	1.0
PA475	MEMA	DEAEA	HEMA	6.0	1.0	3.0
PA513	MEMA	DEAEMA	MMA	8.0	1.0	1.0
PA515	MEMA	DEAEA	MMA	6.0	1.0	3.0
PA13	MMA	DMAA	-	9.0	1.0	-
PU1	PEG2000	HDI	-	4.9	5.2	-
PU16	PEG2000	MDI	-	4.9	5.2	-
PU161	PEG2000	MDI	BD	2.5	5.2	2.3
PU7	PEG900	BICH	-	4.9	5.2	-
PU83	PEG900	HMDI	BD	2.5	5.2	2.3
PU227	PPG-PEG-1900	HDI	-	4.9	5.2	-
PU129	PPG425	BICH	DMAPD	2.5	5.2	2.3
PU10	PTMG2000	BICH	-	4.9	5.2	-
PU179	PTMG2000	HDI	NMAPD	2.5	5.2	2.3
PU20	PTMG2000	MDI	-	4.9	5.2	-
PU5	PTMG2000	HDI	-	4.9	5.2	-

Abbreviations

MEMA	2-methoxyethylmethacrylate
HEMA	2-hydroxyethylmethacrylate
HEA	2-hydroxyethylacrylate
MMA	methyl methacrylate
DMAA	dimethylacrylamide
DEAEA	2-(diethylamino)ethyl acrylate
DEAEMA	2-(diethylamino)ethyl methacrylate
PEG	poly(ethyleneglycol)
PPG	poly(propyleneglycol)
PTMG	poly(butyleneglycol)
HDI	1,6-diisocyanatohexane
MDI	4,4'-methylenebis(phenylisocyanate)
BICH	1,3-bis(isocynanatomethyl)cyclohexane
HMDI	4,4'-Methylenebis(cyclohexyl isocyanate)
BD	1,4-butanediol
EG	ethylene glycol
DMAPD	3-dimethylamino-1,2-propanediol
NMPD	2-nitro-2-methyl-1,3-propanediol

Table S3. Polyacrylates/acrylamides that were functionalised post-polymeriation via a reaction between an epoxy group in GMA and various amines (GMA = glycidyl methacrylate).

Polymer	MMA/GMA ratio	Fuctionalisation amine
PA309	9:1	Di-*n*-hexylamine
PA315	9:1	Dibenzylamine
PA316	7:3	Dibenzylamine
PA336	9:1	*N*-methylaniline
PA337	7:3	*N*-methylaniline
PA338	1:1	*N*-methylaniline

2. Scale-up and validation of 'hit' polymers

Polymers were spin-coated onto circular glass coverslips (∅ 13 mm), placed on 24-well plates, and incubated with BacMix-1 and BacMix-2 as described earlier (section 1.2), and imaged by fluorescence microscopy and scanning electron microscopy (SEM). Uncoated coverslips and agarose coated coverslips were used as controls.

Scanning electron microscopy

After incubation with bacteria, uncoated and coated coverslips were washed twice with 0.1 M cacodylate buffer (pH 7.4) and then fixed with 2.5% (w/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h. Samples were post-fixed with 1% (w/v) osmium tetroxide for 1 hour at room temperature, dehydrated stepwise with ethanol (50, 70, 90 and 100% (v/v)), critical point dried in CO_2, and gold coated by sputtering. The samples were examined with a Philips XL30CP Scanning Electron Microscope. Figure S9 and S10 show the SEM images of bacteria binding / non-binding on the surfaces.

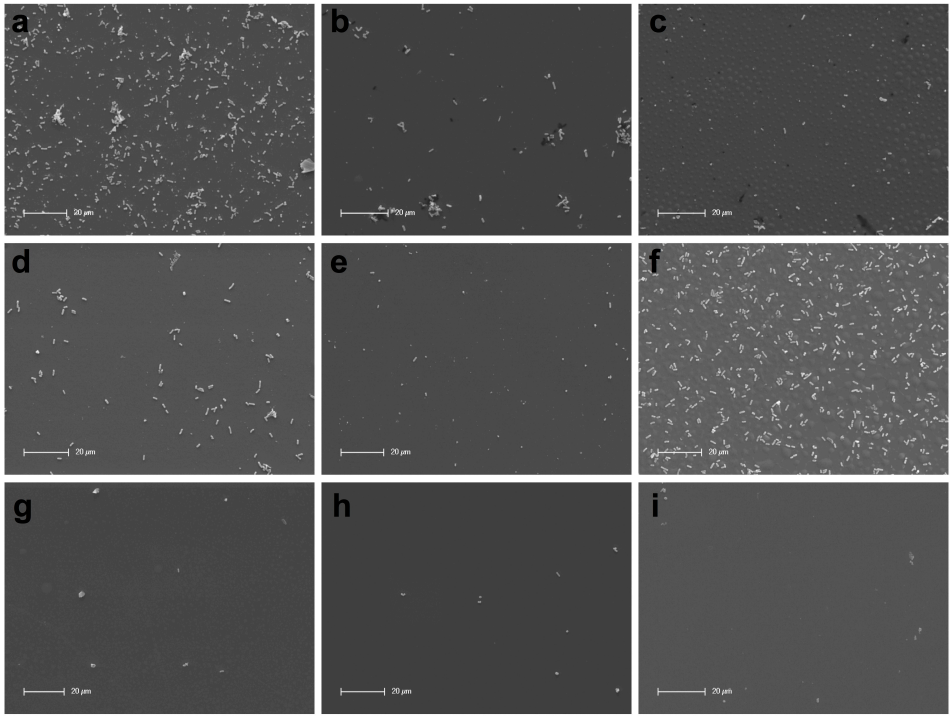

Fig. S9 SEM images of the coated/uncoated coverslips after incubation with BacMix-1. (a) uncoated (glass); (b) **PA338**; (c) **PA515**; (d) agarose; (e); **PU5** (f) **PU20**; (g) **PA13**; (h) **PU83**; (i) **PU179**. Scale bar = 20 μm.

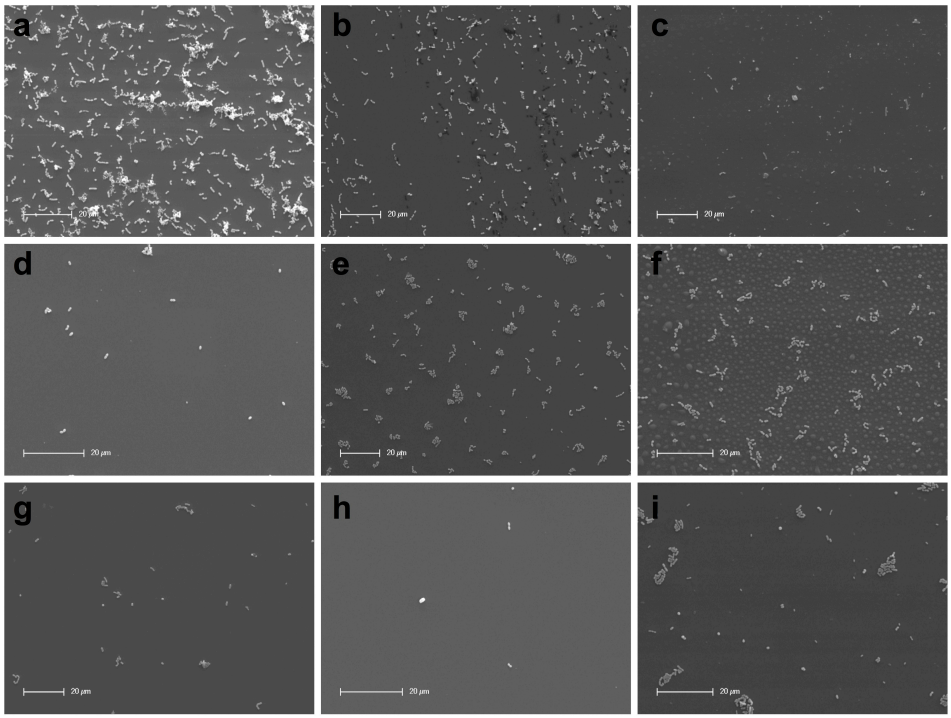

Fig. S10 SEM images of the coated/uncoated coverslips after incubation with BacMix-2. (a) uncoated (glass); (b) **PA338**; (c) **PA515**; (d) agarose; (e); **PU5** (f) **PU20**; (g) **PA13**; (h) **PU83**; (i) **PU179**. Scale bar = 20 μm.

3. Coating of catheters

3.1 Polymer synthesis and characterisation

For coating of the catheters, **PA13, PA515** and **PA155** were resynthesised by free-radical polymerisation, using 2,2'-azobis(2-methylpropionitrile) (AIBN) as an initiator. Reaction conditions for each polymer are summarised in Table S4. The monomers, AIBN and the solvent were added to a glass vessel, and polymerisation carried out for 48 h under N_2 atm. The polymers were precipitated by dropwise addition into hexane (**PA13** and **PA515**) or 50% Et_2O–hexane (**PA155**), collected by filtration, washed with the solvent, and dried overnight *in vacuo* at 40 °C. The polymers were characterised by GPC (Table S5) and IR (Fig. S11).

Table S4. Synthesis of **PA155**, **PA515** and **PA13**.

Polymer	**Monomers**			**AIBN** (mmol)	**Solvent**	**T** (ºC)	**Yield** %
PA13	**MMA** 36 mmol	**DMAA** 4 mmol	–	0.100	Toluene (4 mL)	60	88
PA515	**MEMA** 18 mmol	**DEAEA** 3 mmol	**MMA** 9 mmol	0.075	Toluene (6 mL)	60	84
PA155	**HEMA** 4 mmol	**DMAEMA** 3.5 mmol	–	0.008	DMF (19 mL)	80	54

Table S5. Molecular weight (Mw and Mn) and polydispersity index (PDI) of the polyacrylates/acrylamides **PA155**, **PA515** and **PA13**.

Polymer	**M_w**	**M_n**	**PDI**
PA13	411000	121000	3.4
PA515	90000	33500	2.4
PA155	9450	7910	1.2

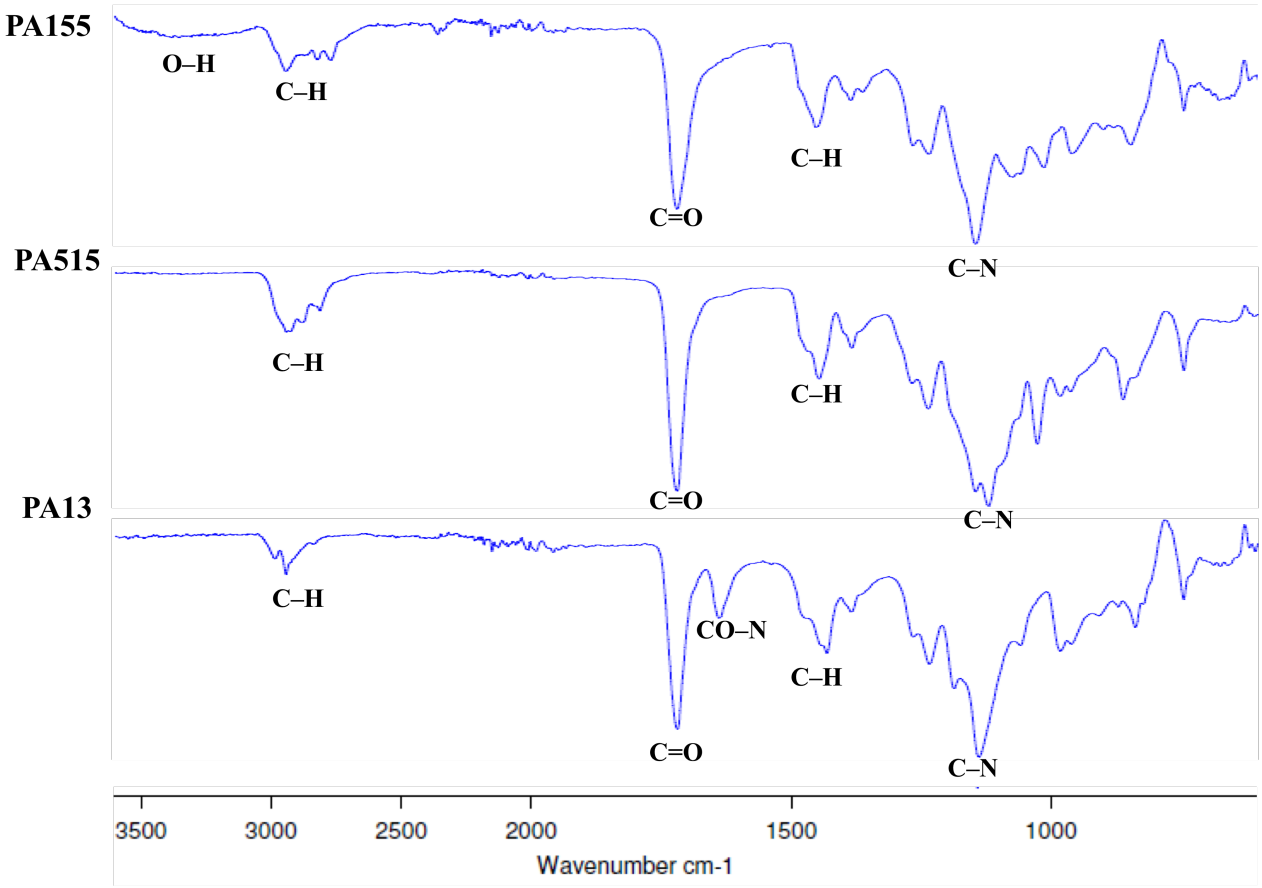

Fig. S11 FT-IR spectra of **PA155**, **PA515** and **PA13**.

3.2 Stability and solubility of the catheters in organic solvents

Indwelling parts of **Cath-1** (Arrow International CS12123E) and **Cath-2** (Baxter Healthcare Corporation ECS1320) were cut into small cylindrical pieces along their length with a razor blade, and measured with a digital Vernier. Average length of the catheter pieces was found to be 5.10 ± 0.42 mm for **Cath-1** (n = 36) and 5.05 ± 0.45 mm for **Cath-2** (n = 71).

Solvents evaluated included acetic acid, ammonia, acetone, acetonitrile, diethyl ether, tetrahydrofuran (THF), dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), ethanol, methanol, ethylene glycol, toluene and xylene. Catheter pieces were immersed in the solvents, left for 12 hours, and evaluated visually (Fig. S12). Acetic acid, DMF, THF and NMP caused **Cath-1** to swell or disintegrate or resulted in cloudy solvent. **Cath-2** exhibited good resistance to the solvents tested. Acetone was found to be benign for both catheters.

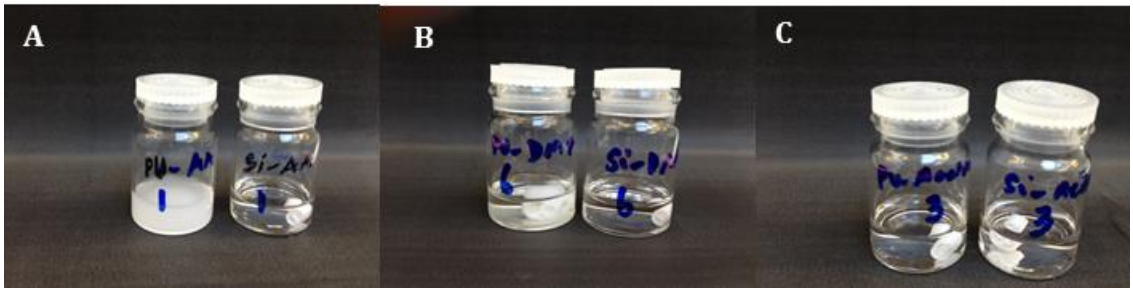

Fig. S12 Stability test of catheters in various solvents (A) **Cath-1** (left) and **Cath-2** (right) in acetic acid. (B) **Cath-1** (left) and **Cath-2** (right) in DMF. (C) **Cath-1** (left) and **Cath-2** (right) in acetone.

IR Analysis. Pieces of **Cath-1** and **Cath-2** were placed in acetone for 12 h. For **Cath-1** there was no evidence for the elution of urethane containing components (~1700 cm^{-1}). For **Cath-**2, the IR spectra showed no bands in the mid-infrared spectrum range where silicones exhibit strong absorption bands.

3.3 Thickness of the coating

The catheters **Cath-1** and **Cath-2** were coated with 10% solution of **PA13** in acetone, cut, and the thickness of the polymer coating measured by SEM (FEI Quanta 3D FEG) (Fig. S13).

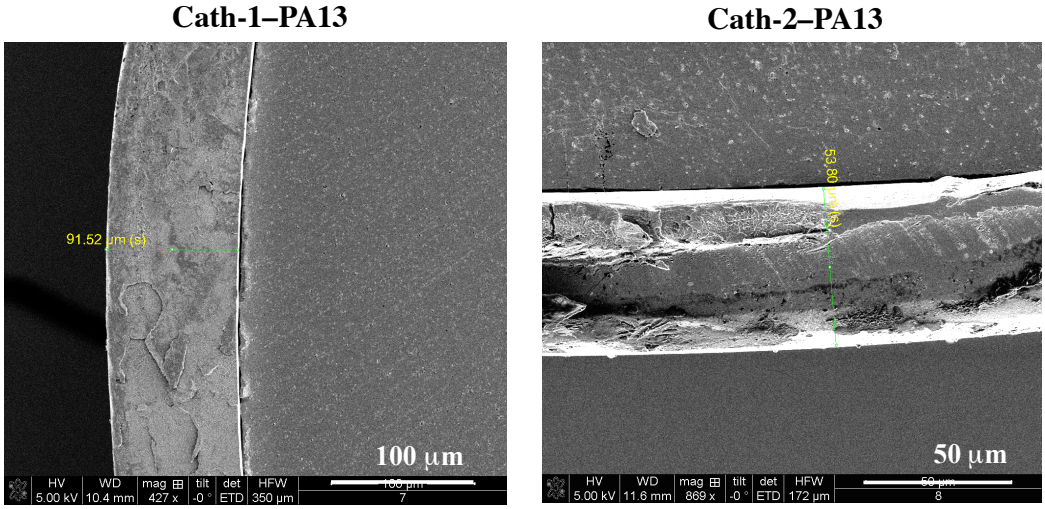

Fig. S13. SEM (FEI Quanta 3D FEG) images showing the polymer coating on the catheter surfaces.

4. Bacteria binding studies on coated catheters

4.1 Confocal microscopy and image analysis

Confocal imaging was performed on a Leica SP5 confocal microscope (405nm blue diode laser set at 10%. Emitted fluorescence was detected using a hybrid detector, with filter a range of 414 to 502 nm, Pin hole – Airy1, Image Size – 1024 × 1024 pixels, Voxel width – 105.2nm. Z stack were produced using 0.5µm spacing. Magnification – 40 × 1.25 Oil UV). Confocal imaging was completed through z-stacking 50 images across 100 µm length of the catheter. Confocal images were analysed using Image-pro plus 7.0. The z-stacked images were flattened in the z plane using the function 'extended depth of field', to create a single image of a catheter piece. This image was then analysed to obtain the area of the catheter covered by bacteria. The total area of the image was approximately 100 × 100 µm. Images were background corrected using the 'flatten background' function within Image-pro.

4.2 SEM images of Cath-1–PA13 with BacMix-1

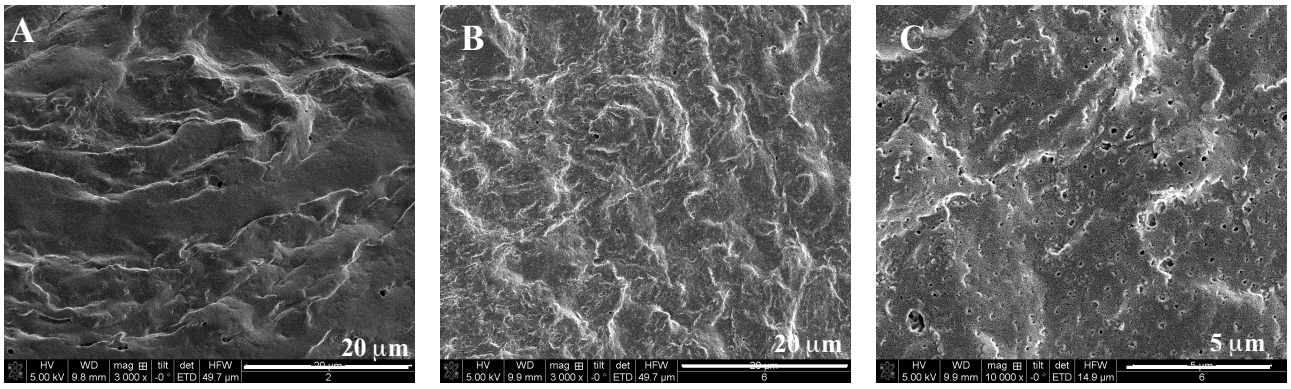

Fig. S14. SEM images (FEI Quanta 3D FEG) of **Cath-1–PA13**. (A) No bacteria, scale bar 20 µm; (B) Incubated with BacMix-1, scale bar 20 µm; (C) Incubated with BacMix-1, scale bar 5 µm.

5. References

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