Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Cytocompatible, Soft and Ultrathin Brush Modified Scaffolds with Prolonged Antibacterial Effect to Mitigate Wound Infections

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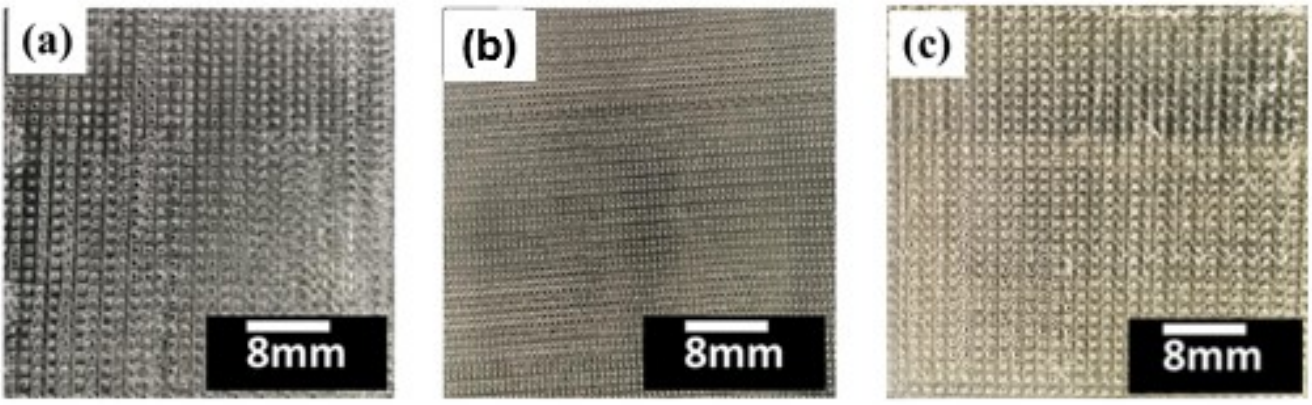


Figure S1. Pictures of Scaffold fabricated by 3D printer (a) PLA (b) Scaffold P2 (P1/PLA=5/95) Pore size: 0.5 mm(c) Scaffold P2-g-polyDMAPS-*co*-polyPEGMA (after 48 h of brush growth) Mn of PEGMA = 950, at 50/50 copolymer ratio; Pore size: 0.456 mm (porosity ~85%)* (3 samples)

*Porosity of Scaffold P2 (before and after brush modifications) was found to be ~85% and calculated using following reference.

X. Li, Y. Wang, M. Guo, Z. Wang, N. Shao, P. Zhang, X. Chen, Y. Huang, Degradable three dimensional-printed polylactic acid scaffold with long-term antibacterial activity, ACS Sustainable Chemistry & Engineering 6(2) (2018) 2047-2054.

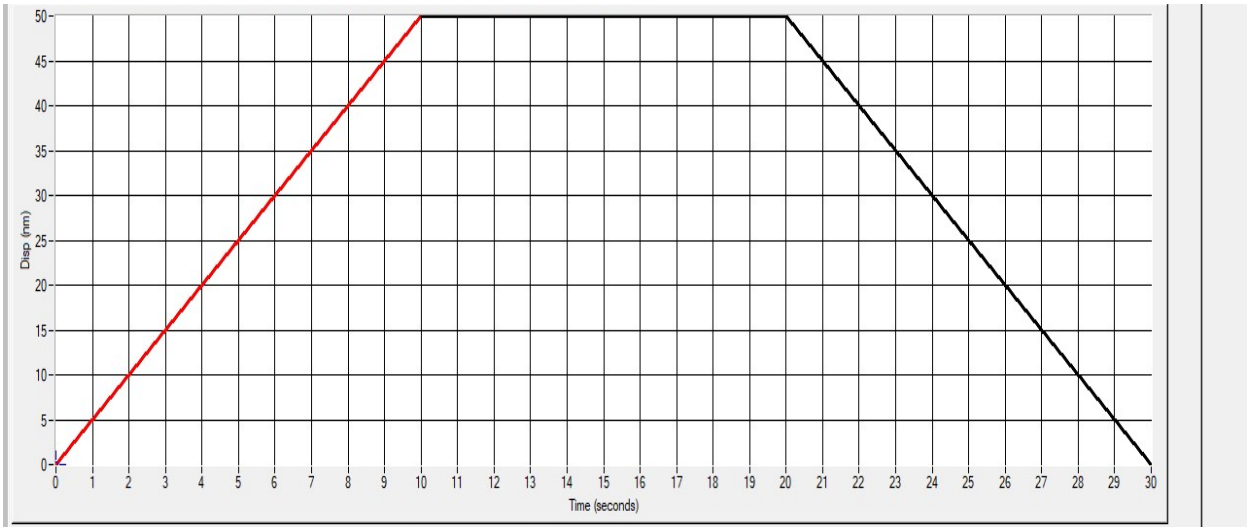


Figure S2. Displacement versus time-controlled loop to penetrate into the first 50 nm of the polymer brush modified surface (P2-g-polyDMAPS and P2-g-polyPDMAPS-*co*-polyPEGMA) representing the loading-unloading cycle curve

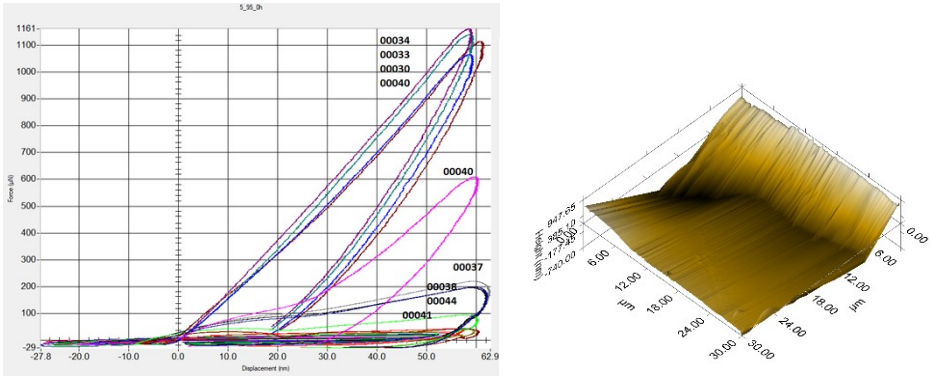
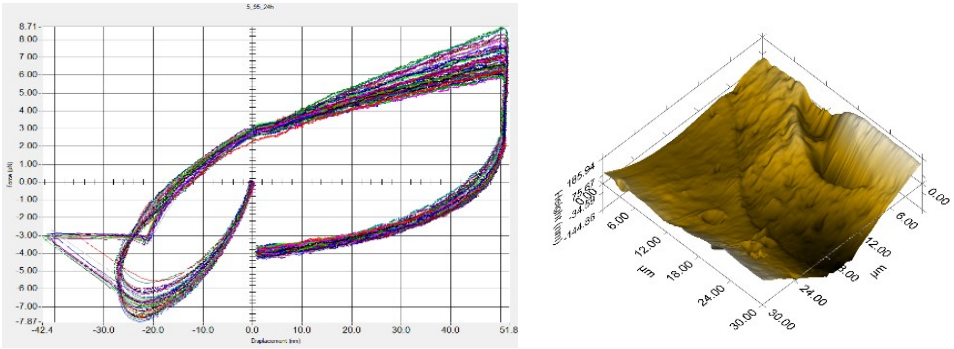
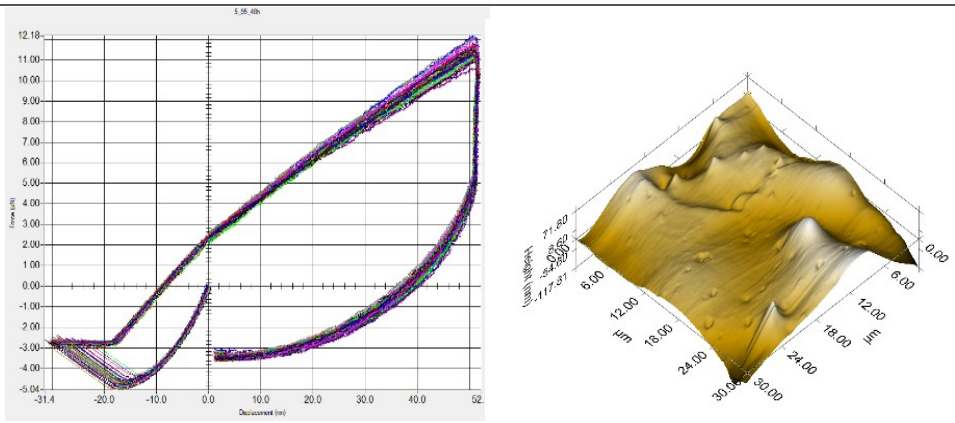
Reaction time	Load displacement curves for P2-g-polyDMAPS
0 hour	
24 hours	
48 hours	

Figure S3. Representative load-displacement curves (45 each) and Post-Test SPM images for 1st area (00000-00008) on P2 (5:95 blend) modified with polyDMAPS brush polymerized for 24 and 48 h.

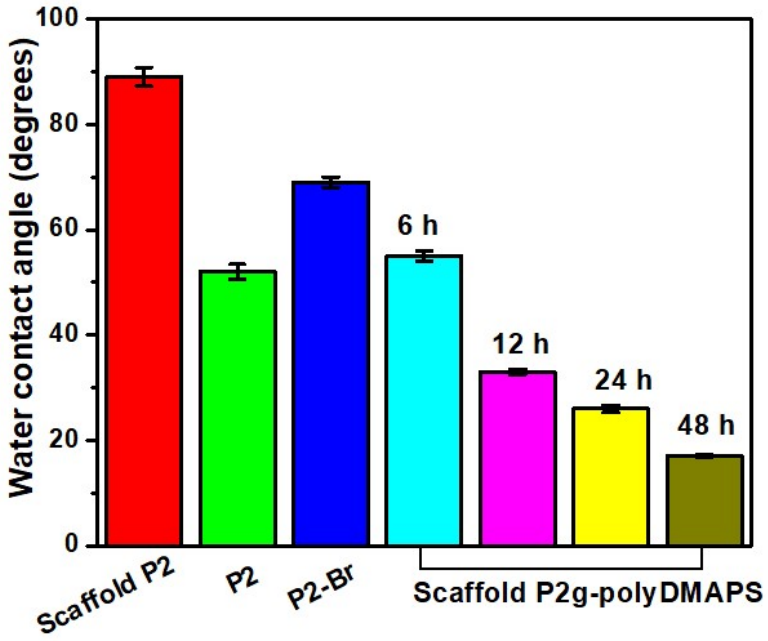


Figure S4. Variation of water contact angle after each step of surface modifications of Scaffold P2 (P1/PLA=5/95) substrate. 'P2' denotes the same Scaffold surface with unmasked hydroxyl functionality. The number placed on top of the column represents polymerization time to graft polyDMAPS onto Scaffold P2 surface. All data are shown as average + standard deviation (error bar) (7 samples). Note: Measurement was carried out on surfaces immobilized with thickest brush (i.e., polymerized for maximum time period).

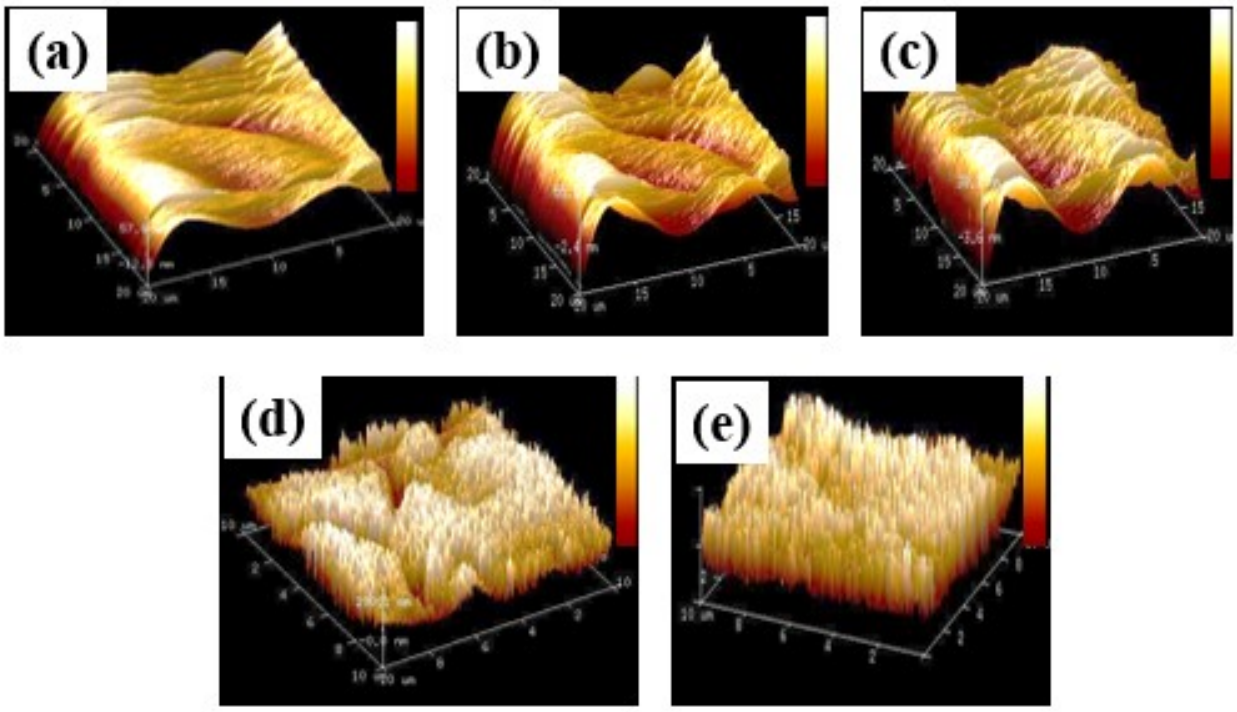


Figure S5. AFM topography images obtained for (a) polymer P Ra=10nm, (b) P1 (c) P1-Br (Scale bar = 20 micron) Ra=20nm (d) P1-g-polyDMAPS (12h) Ra= 98nm and (e) P1-g-polyDMAPS (24h) Ra=156nm. (Scale bar = 10 micron) Information in bracket for (d) and (e) represent polymerization time.

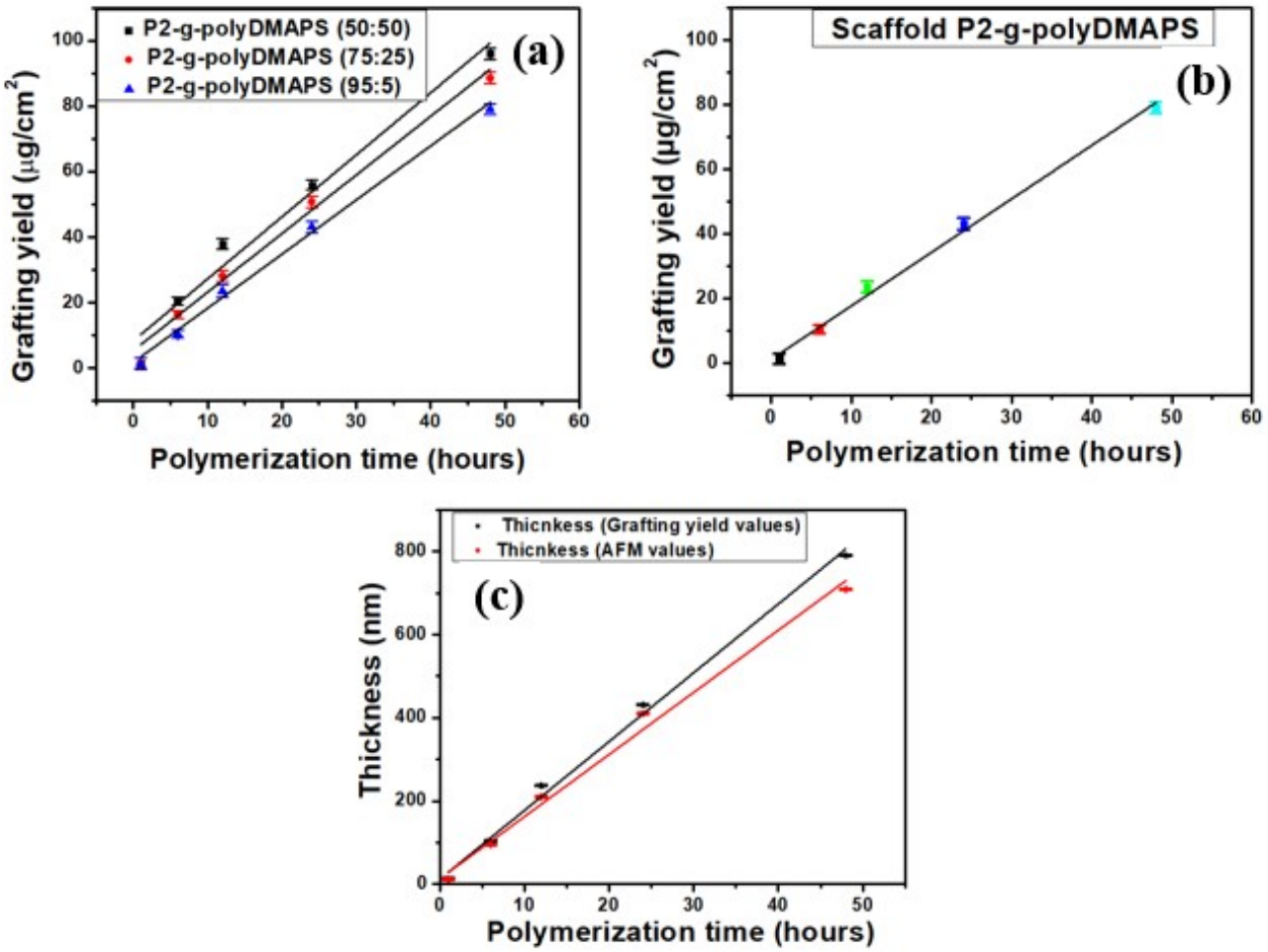


Figure S6. Surface initiated polymerization of polyDMAPS on P2-Br surface represented by measuring 'Grafting yield' with respect to polymerization time for various blend compositions (PLA:P1) coated on glass surface: (a) 50:50;(5 samples) , 75:25;(5 samples) and 95:5;(5 samples). All data points are shown as average + standard deviation (error bar). (b) Surface initiated polymerization of polyDMAPS on Scaffold P2-Br surface (P1/PLA=5/95) represented by measuring 'Grafting yield' with respect to polymerization time. All data points are shown as average + standard deviation (error bar).(5 samples) (c)Evolution of thickness of polyDMAPS brush on the Scaffold **P2** (P1/PLA=5/95) surface for different polymerization times. Thickness is calculated using Grafting yield values as shown below and from AFM images as well. All data points are shown as average + standard deviation (error bar).

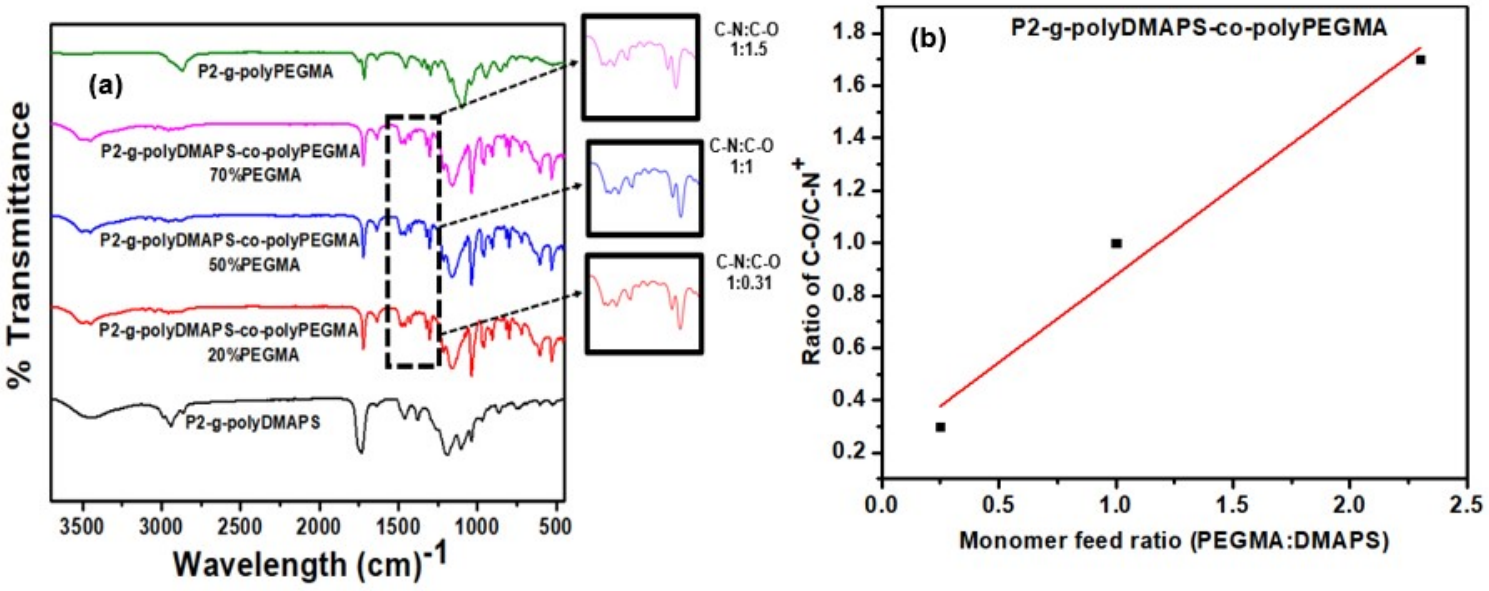


Figure S7. FTIR spectra of P2 blend (P1:PLA=5:95) surface modified with (a) polyDMAPS, polyPEGMA and polyDMAPS-*co*-polyPEGMA at various PEGMA content, Mn of PEGMA =950, polymerization time: 48h (5 samples) (b) Ratio of characteristic peaks (C-O/C-N^+) for PEGMA and DMAPS in the copolymer with respect to their corresponding monomer feed ratio.

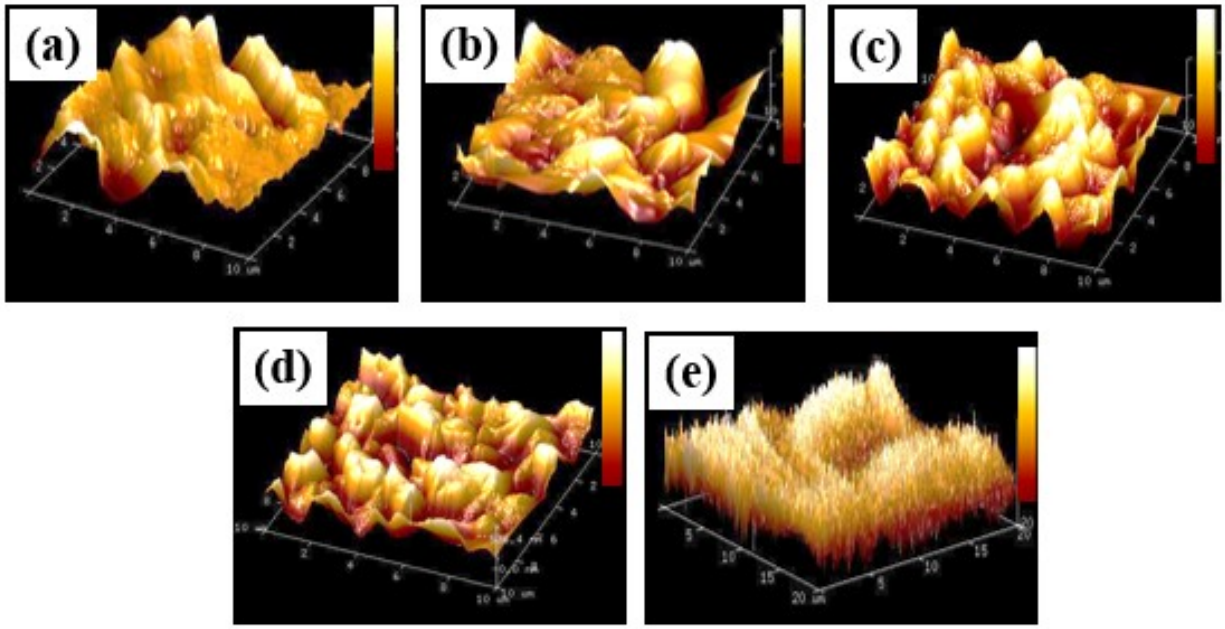


Figure S8. AFM topography images obtained for (a) pure PLA, Ra= 14nm (b) Scaffold P2 (P1/PLA=5/95) Ra=20nm, (c) P2-Br, Ra=30nm (d) P2-g-polyDMAPS (12h) Ra=100nm and (e) P2-g-polyDMAPS (24h) Ra=167nm (Scale bar = 20 micron). Information in bracket for (d), (e), and (f) represent polymerization time. (Scale bar = 10 micron)

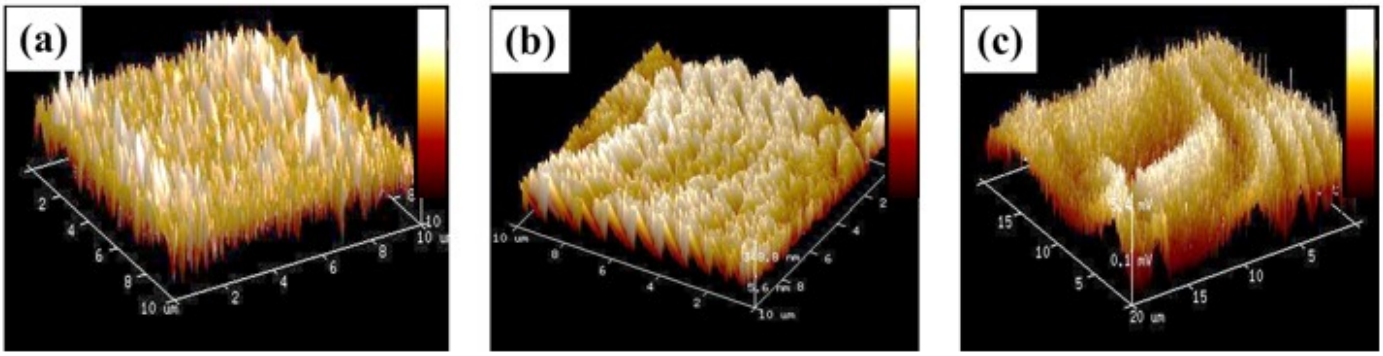


Figure S9. AFM topography images obtained for P2-g-polyDMAPS surface at various blend compositions, PLA:P1 (a) 50:50; (b) 75:25; and (c) 95:5 for maximum time of polymerization (48 h), respectively. Scale bar = 10 micron for a and b, and for c = 20 micron

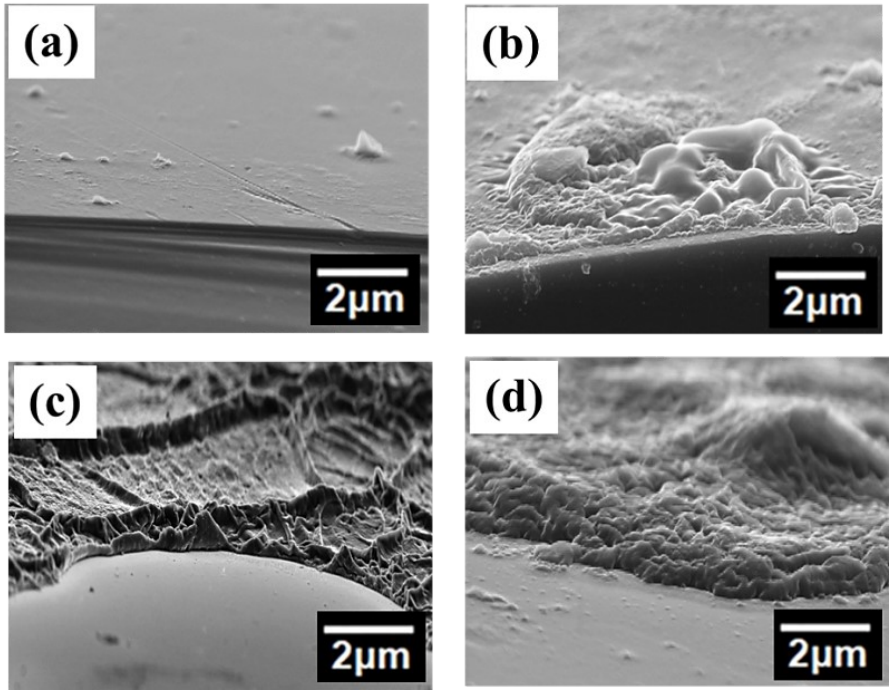


Figure S10. SEM images obtained for (a) Scaffold P2(P1/ PLA=5/95), (b) P2-g-polyDMAPS (12h) and (c) P2-g-polyDMAPS (24h) (d) P2-g-polyDMAPS (48h) surfaces. Information in bracket for b,c and d represent polymerization time. (4 samples)

<u>Thickness calculation from 'grafting yield' values</u>

Volume = Mass/Density,

$\pi r^2 h$ = Mass/Density

h = (Mass/per unit area) / Density = Grafting yield (g/cm^2)/ Density (g/cm^3)

h (Thickness of the polymer) = Grafting yield (G.Y.)/Density (~1 g/cm^3);

G.Y. = (Wa-Wb)/A; where Wa and Wb represent the weight of the dry films before and after polymer grafting onto the surface, respectively, and A is the area of the surface measured.
Using G.Y. measured from weighing balance, we can find out the thickness of the film.

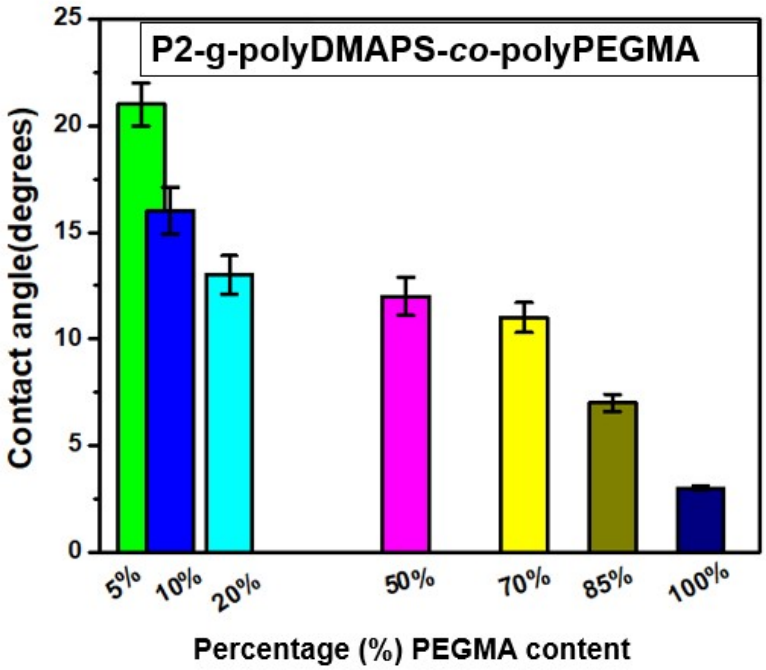


Figure S11. Variation of water contact angle after brush modifications of Scaffold P2 (P1/PLA=5/95) with copolymer brush, i.e., polyDMAPS-*co*-polyPEGMA with change in **percentage of PEGMA** (Mn of PEGMA=950, n=21) content, polymerized for 48h. All data points are shown as average + standard deviation (error bar). Note: Measurement was carried out on surfaces attached with thickest brush (i.e., polymerized for maximum time period).

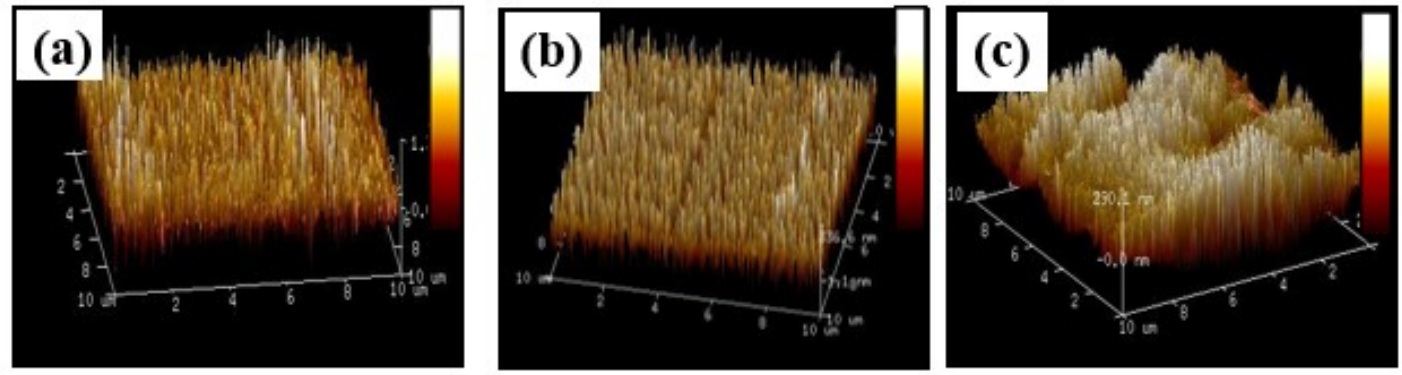


Fig. S12 AFM topography images obtained for brush modified Scaffold P2 surface with varying content of PEGMA: (a) Scaffold P2-g-polyDMAPS-*co*-polyPEGMA, (20%PEGMA), (b) Scaffold P2-g-

polyDMAPS-*co*-polyPEGMA, (50%PEGMA) and (c) ScaffoldP2-g-polyPEGMA, (100%PEGMA) (Scale bar = 10 micron).

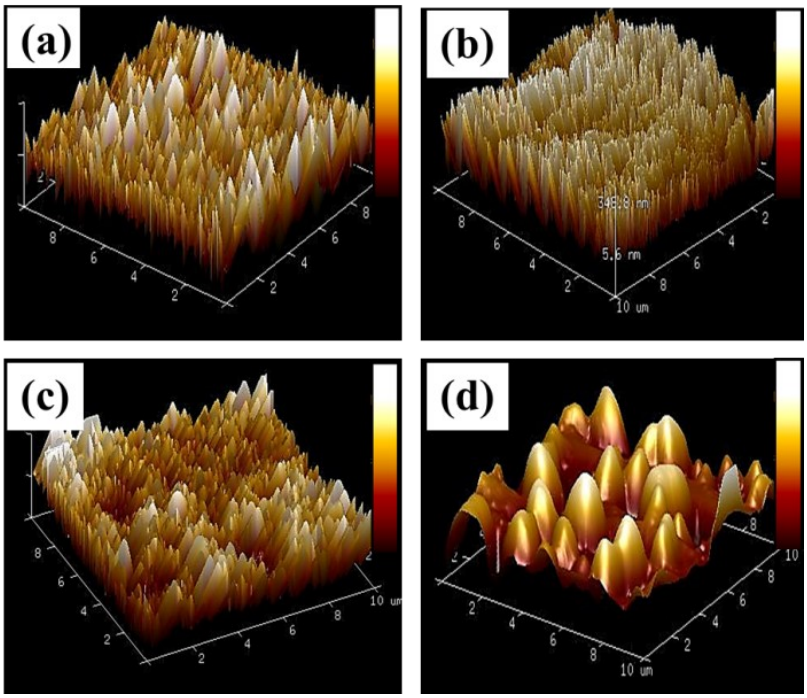


Figure S13. AFM topography images obtained for polyDMAPS-*co*-polyPEGMA (50:50 composition) on the surface of polymer P2 (PLA:P1=95/5) for 48 hours of polymerization. (a) polyDMAPS-*co*-polyPEGMA, (Mn of PEGMA =144), (b) polyDMAPS-*co*-polyPEGMA, (Mn of PEGMA =300), (c) polyDMAPS-*co*-polyPEGMA, (Mn of PEGMA =500), (d) polyDMAPS-*co*-polyHEMA (50:50 brush composition)

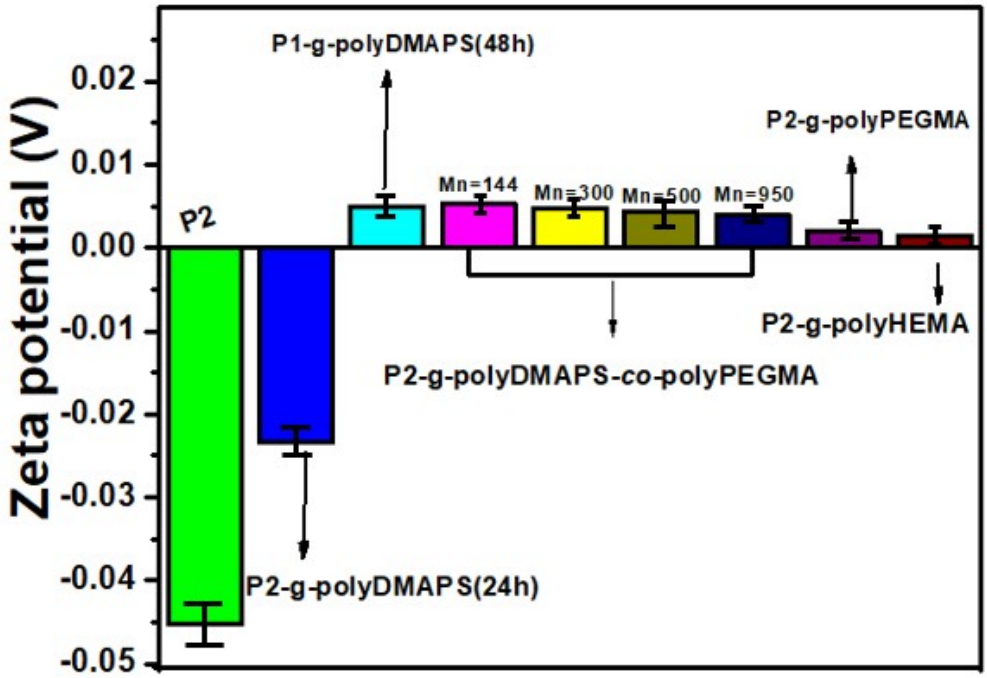


Figure S14. Surface charge evaluation (zeta potential) for polymer blend P2 (P1:PLA=5:95) surface and brush modified surfaces at pH 7. Mn represents the molecular weight for PEGMA monomer.

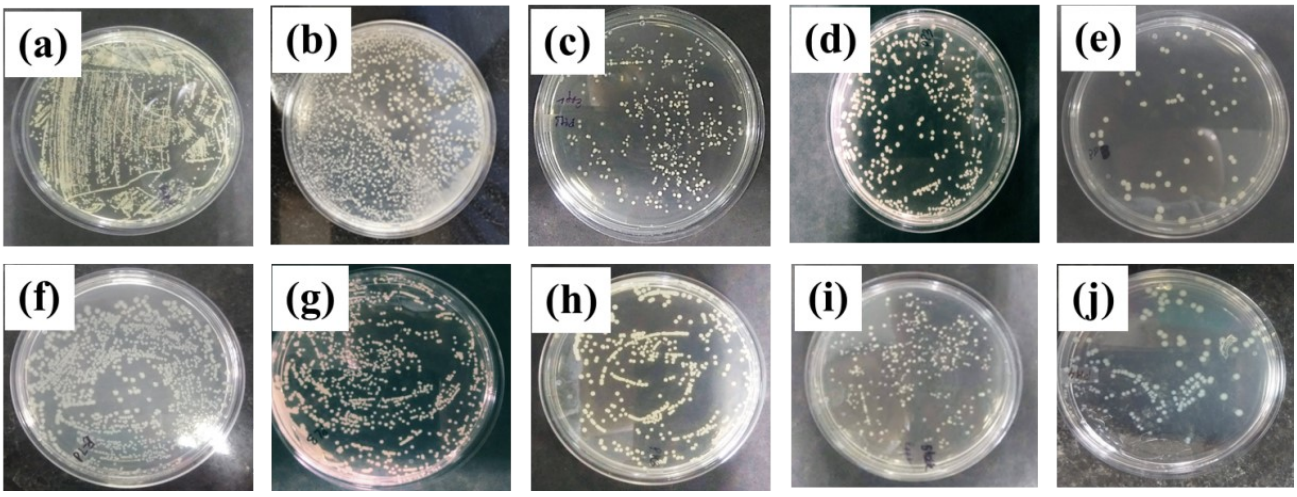


Figure S15. Images of bacterial growth for (a) & (f) Polymer P (b) & (g) P1-g-polyDMAPS (6h), (c) & (h) P1-g-polyDMAPS (12h), (d) & (i) P1-g-polyDMAPS (24h) and (e) & (j) P1-g-polyDMAPS (48h) surfaces against *E. coli* (5 samples) and *S. aureus,* (5 samples) respectively. Information in bracket represent polymerization time for each brush.

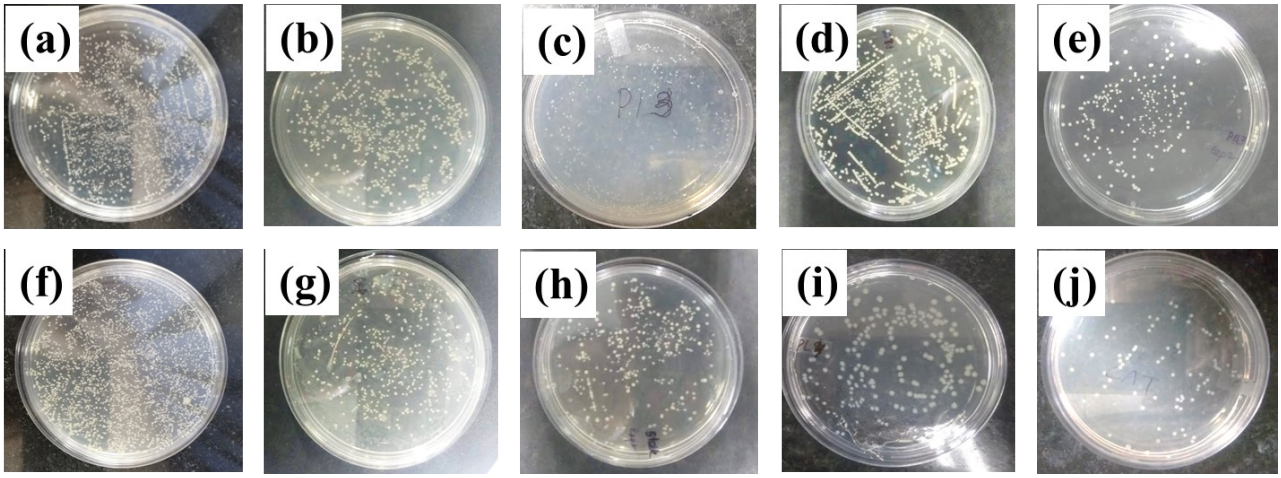


Figure S16. Images of bacterial growth for (a) & (e) Scaffold P2 (P1/PLA=5/95) surface, (b) & (g) P1-g-polyDMAPS (6h), (c) & (h) P1-g-polyDMAPS (12h), (d) & (i) P1-g-polyDMAPS (24h) and (e) & (j) P1-g-polyDMAPS (48h) surfaces against *E. coli* (5 samples) and *S. aureus,* (5 samples) respectively. Information in bracket represent polymerization time for each brush.

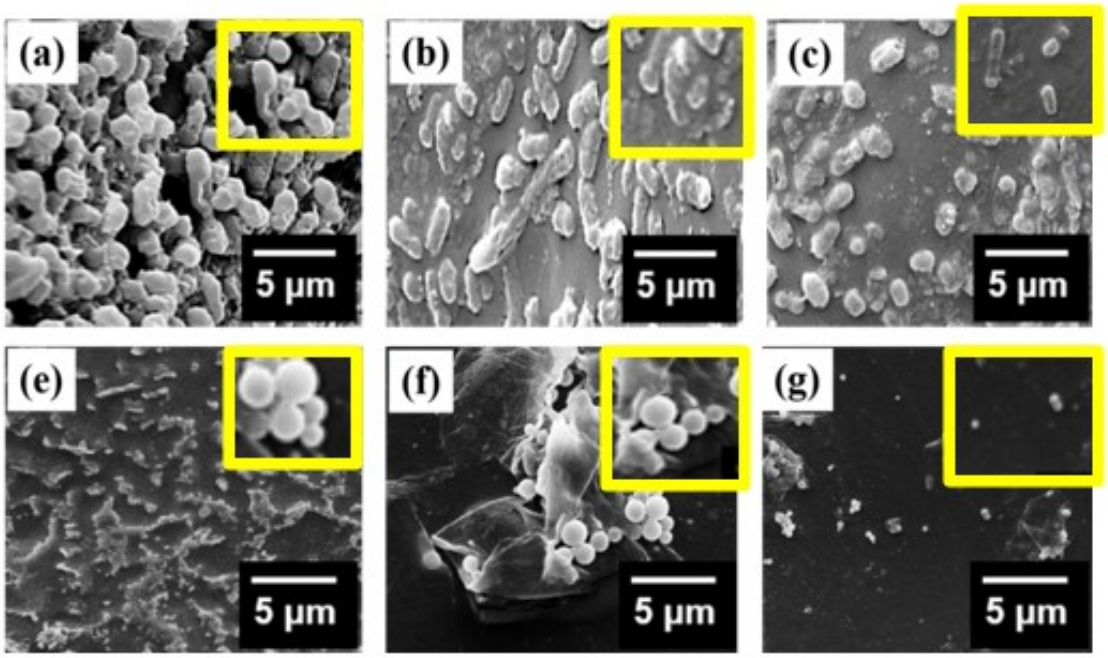


Figure S17. SEM images of *E. coli* bacteria and *S. Aureus* obtained for (a) and (e) Scaffold P2 surface, (b) and (f) P2-g-polyDMAPS (24 h) and (c) and (g) P2-g-polyDMAPS (48 h). Information in bracket for (b), (c) and (d) represent polymerization time. Zoom in images are shown as inset.

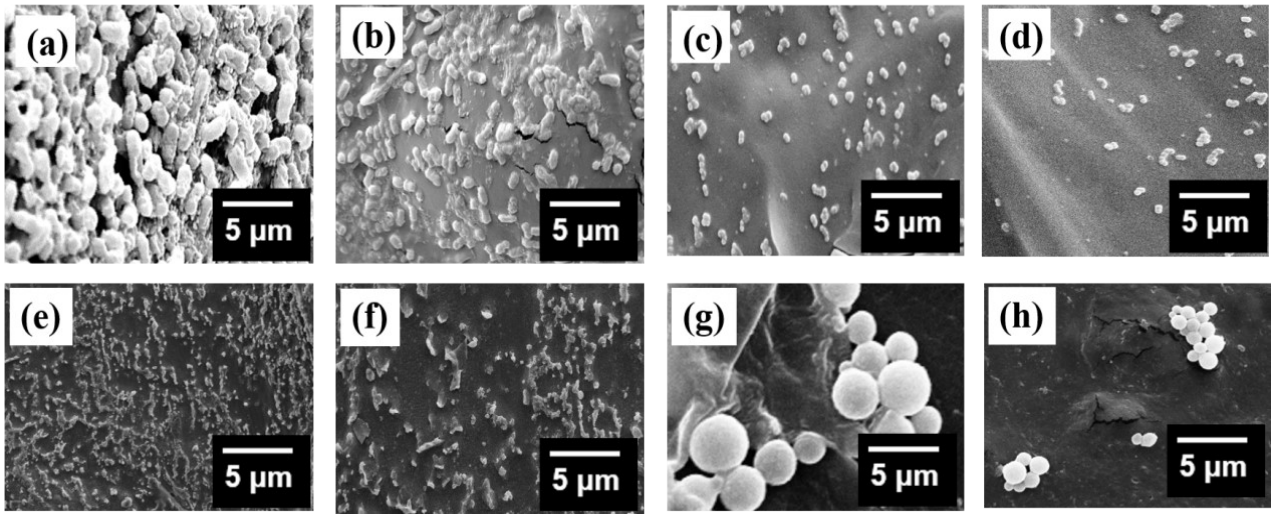


Figure S18. SEM images of *E. coli* bacteria obtained for (a) Scaffold P2 (50:50) surface, (b) Scaffold-g-polyDMAPS (12h) and (c) Scaffold-g-polyDMAPS (24h) (d) Scaffold-g-polyDMAPS (48h) and (e), (f), (g) and (h) for *S.Aureus* for the same substrates. Information in bracket for b,c and d represent polymerization time.

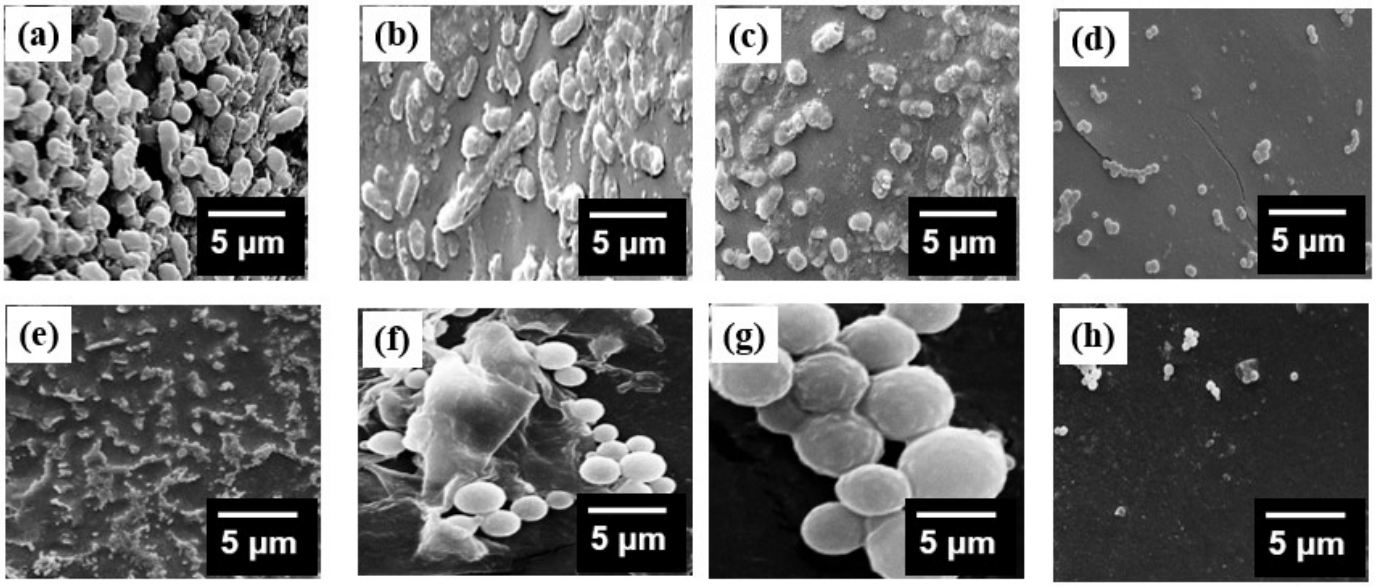


Figure S19. SEM images of *E.coli* and *S. Aureus* bacteria obtained for (a) and (e) Scaffold P2(5:95) surface, (b) and (f) Scaffold-g-polyDMAPS (12h) and (c) and (g) Scaffold-g-polyDMAPS (24h) (d) and (h) Scaffold-g-polyDMAPS (48h), respectively. Information in bracket represent polymerization time.

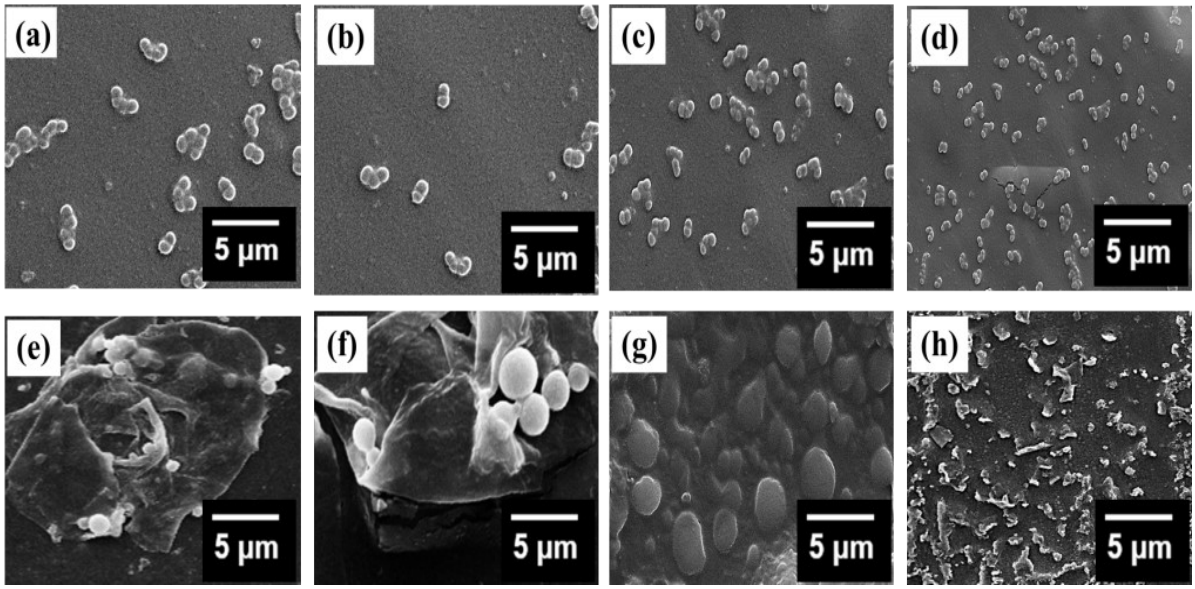


Figure S20. SEM images of *E. coli* and *S. Aureus* bacteria obtained for P2-g-polyDMAPS-*co*-polyPEGMA (50:50) surface (a) and (e) Mn of PEGMA =300, (b) and (f) Mn of PEGMA =500, (c) and (g) Mn of PEGMA =144; polymerized for 48 hours, (d) and (h) P2-g-*co*-polyHEMA (50:50) polymerized for 6 hours of polymerization, respectively

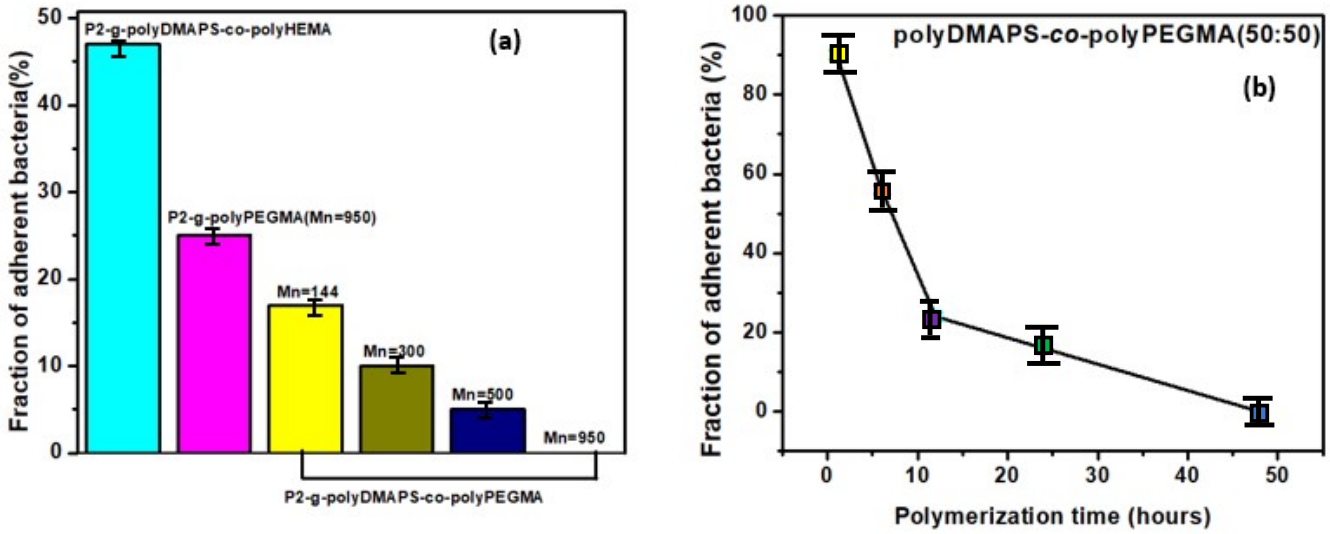


Figure S21. (a) Variation of bacterial growth on Scaffold P2 surface modified with polyDMAPS-*co*-polyPEGMA brush (50:50) using various Mn of PEGMA monomer (polymerization time: 48h) and **(b)** Variation of bacterial growth on Scaffold P2 surface modified with polyDMAPS-*co*-polyPEGMA brush with Mn of PEGMA =950, polymerized for different time period. Line is added for guiding the eye. All data points are shown as average + standard deviation represented by error bar ($p \leq 0.05$).

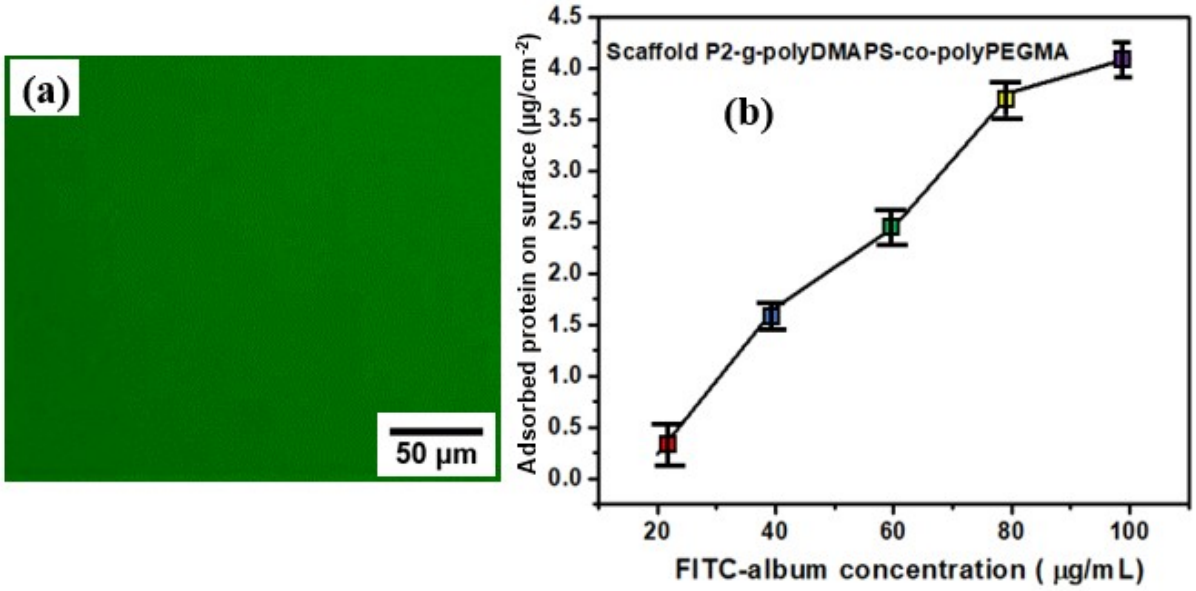


Figure S22. Fluorescent micrograph of (a) Scaffold P2 (b) Protein adsorption data on brush modified Scaffold dipped at various protein solutions of varying protein concentrations. Note: polymerization time was kept at 48 h for brush modified scaffold. All data points are shown as average + standard deviation represented by error bar ($p \leq 0.05$).

Table S1. Monomer feed ratios for copolymerization of DMPAS and PEGMA to produce polyDMAPS-*co*-polyPEGMA via SITRP onto the polyester surface

Monomer 1	**Monomer 2**	**Monomer 1: Monomer 2 ratio for copolymerization (PEGMA: DMAPS)**
PEGMA with different Mn 144 300 500 950	DMAPS	50:50
PEGMA (Mn=950)	DMAPS	5:95 10:90 20:80 50:50 70:30 85:15 100:0

Table S2. Variation of static water contact angle with polymerization time for polyDMAPS brush tethered on the blend surface (PLA: P1) of various blend compositions

Type of surfaces	Reaction time (h)	Water contact angle (±2º) for PLA: P1=P2		
		50:50	**75:25**	**95:5**
PLA	-	82.4	86	89
P2	0.5	43	49.5	52
P2-Br	12	55	62	69
P2-g-polyDMAPS (**6h**)	6	44	50	55
P2-g-polyDMAPS (**12h**)	12	25	28.5	33
P2-g-polyDMAPS (**24h**)	24	20.1	23.8	26
P2-g-polyDMAPS (**48h**)		10.5	15	17

Table S3. Ratio of C-N^+/C-O for polyDMAPS-*co*-polyPEGMA with different PEGMA content calculated from FTIR shown in Figure S6

S.No	**Ratio of C-N^+/C-O from FTIR**	**Monomer feed ratio (DMAPS:PEGMA)**
1	**1:0**	**100:0**
2	**1:1.5**	**30:70**
3	**1:1**	**50:50**
4	**1:0.31**	**80:20**

5	**0:1**	**0:100**

Table S4. Comparison of average hardness H (MPa) and reduced modulus Er (GPa) for various surfaces

S.No	Sample Name	Average H (MPa)	Average Er (GPa)
1	5_95_ 0h (polyDMAPS)	43	2.8
2	5_95_ 24h (polyDMAPS)	10.8	1.8
3	5_95_ 48h (polyDMAPS)	6.5	1.4
4	P2-g-polyDMAPS-co-polyHEMA	0.057	0.82
5	P2-g-polyPEGMA (Mn=950, PEGMA)	0.017	0.31
6	P2-g-polyDMAPS-*co*-polyPEGMA (Mn=144)	0.048	0.40
7	P2-g-polyDMAPS-*co*-polyPEGMA (Mn=500)	0.024	0.35
8	P2-g-polyDMAPS-*co*-polyPEGMA (Mn=950) **50:50 polymer brush combination**	0.018	0.29

Table S5. Comparison of adherent bacterial growth percentage (%) on blend surface P2 at various blend compositions, modified with brushes polymerized for varying time against *E. coli*

S. No	Sample Name	Compositions (P1: PLA) =P2			
		100:0	**50:50**	**25:75**	**5:95**
1	P2	100	100	100	100
2	P2-g-polyDMAPS (6h)	79	83	88	93
3	P2-g-polyDMAPS (12h)	62.3	68	71	77.9
4	P2-g-polyDMAPS (24h)	44	50	54	60
5	P2-g-polyDMAPS (48h)	10.6	11	12.4	13.2

Table S6. Comparison of adherent bacterial growth percentage (%) on blend surface P2 at various blend compositions, modified with brushes polymerized for varying time against *S. aureus*

S. No	Sample Name	Compositions (P1: PLA) =P2			
		100:0	**50:50**	**25:75**	**5:95**
1	P2	100	100	100	100
2	P2-g-polyDMAPS (6h)	74	80	88	92
3	P2-g-polyDMAPS (12h)	58	67.4	70	76
4	P2-g-polyDMAPS (24h)	41	46.3	52	58
5	P2-g-polyDMAPS (48h)	9.9	10.4	11.8	12.6