Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B. This journal is © The Royal Society of Chemistry 2022

Supporting Information

Photoinduced micropatterning on biodegradable aliphatic polyester surface for anchoring dual brushes and its application in bacteria and cell patterning

Shaifali Dhingra¹, Vidit Gaur², Jayanta Bhattacharya² and Sampa Saha¹*

Department of Materials Science and Engineering, Indian Institute of Technology Delhi

Centre for Biomedical Engineering, Indian Institute of Technology Delhi

*Corresponding author: Sampa Saha, Email: ssaha@mse.iitd.ac.in

Experimental section:

Synthesis of Monomer (Dimethyl 2,3-*O*-isopropylidentartarate) and polymer Poly(hexamethylene 2,3-*O*-isopropylidentartarate) (P):

The monomer, Dimethyl 2,3 O-isopropylidentartarate and also polymer P, Poly(hexamethylene 2,3-O-isopropylidentartarate) were synthesized and characterized by following a reported method described in our earlier research work [18].

Evaluation of antibacterial activity

Antibacterial activity for different samples (film coated on glass) was evaluated by following our previously published procedures[18, 34, 35].

Analysis of Statistical data

The statistical analysis was evaluated by using the t-test and ANNOVA analysis possessing a significance level of (p) \leq 0.05 of the bacteria growth data acquired from the different samples. All the data obtained from the antibacterial activity measurement for both the bacteria were expressed in \pm standard deviation.

SEM analysis of bacteria

For SEM imaging. the bacterial fixing procedure was adopted by following a literature reported method[18]. After incubating the samples individually with different bacterial cultures (both *E. coli* and *S. Aureus*), the bacterial residues left on the samples' surface were fixed with 2% glutaraldehyde solution in PBS buffer and the treated samples were placed on a glass slide. Subsequently, the samples were dried for about 3 h and then kept in the fridge for 12 to 14 h for proper fixation. The fixed cells of bacteria were

thoroughly washed for 4 to 5 times with the solution of absolute ethanol in distilled water possessing various concentrations like 0, 20, 40, 60, 80 and 100% of ethanol. At last, the samples were dried fully for 3 h at room temperature and then mounted for SEM imaging.

Surface charge measurements

The surface charge of the brush modified polymer surface was evaluated by the streaming current measurement carried out by SurrPass3 Electrokinetic Analyzer (Anton Parr GmbH, Graz, Austria) using the adjustable Gap cell. All the polymeric films were soaked in a KCl solution (0.001mol/L) for 24 hours before mounting into the measuring cell. The solution was adjusted to pH 7 and also the streaming potential or streaming current was measured with electrodes placed at both sides of the sample. The zeta potential(ζ) was then calculated according to Helmholtz-Smoluchowski equation:

$$\zeta = \frac{\text{d-Istr}}{\text{d-}\Delta p} X \frac{\acute{\eta}}{\text{er x } \epsilon 0} X \frac{L}{A}$$

where I_{str} corresponds to the streaming current, Δp refers to the hydrodynamic pressure difference, η is the viscosity, ϵ_r and ϵ_o are the dielectric permittivity of liquid and vacuum respectively. L denotes the length of the streaming channel and A is the cross section of the streaming channel.

_

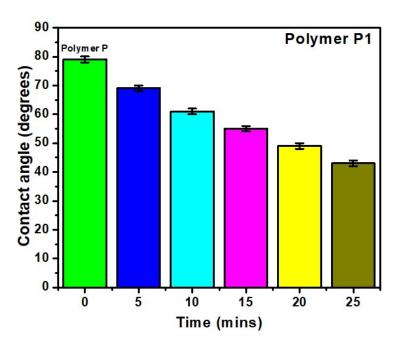
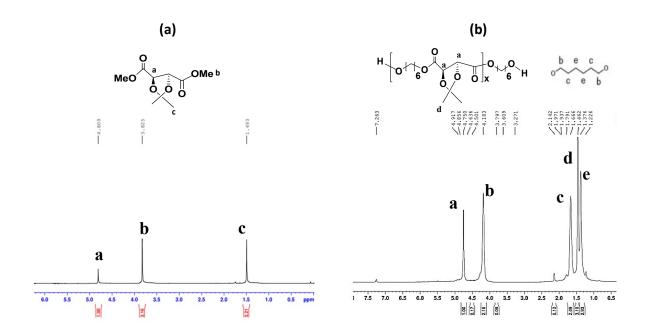


Figure S1. Variation of water contact angle acquired from polymer P substrate after exposing to UV radiation for various time intervals. All data points are shown as average + standard deviation (error bar).



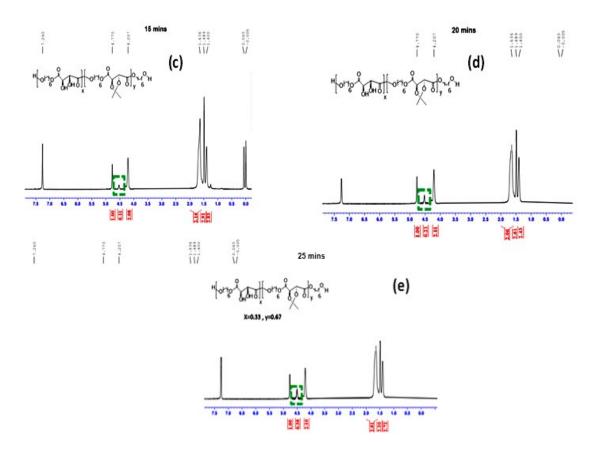


Figure S2. Representative NMR spectra for (a) Monomer (Di-methyl 2,3-O-isopropylidenetartarate) (b) Polymer P (c) Polymer P1 taken after exposed to UV radiation for 15 mins (d) Polymer P1 taken after exposed to UV radiation for 20 mins and (e) Polymer P1 taken after exposed to UV radiation for 25 mins

<u>Determination of released HCI upon UV irradiation:</u>

Experimental description:

50 mg of polymer P dispersed in 10 mL of water was exposed to UV radiation (395 nm) and then amount of released HCl upon UV radiation was estimated by detecting chloride ions with the help of a conductometer (model number: Fischer SMP350) having chloride ion detector. The released chloride ion concentration was plotted against exposure time. Moreover, the released chloride ion concentration at 25 min was closely matched with the HCl concentration (0.000165 g/mL) obtained from standard acid-base titration using phenolphthalein as an indicator.

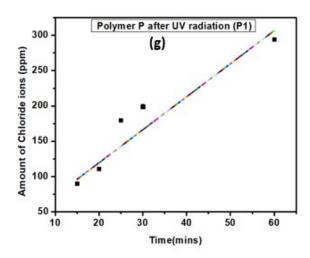


Figure S2f: Evolution of released chloride ion with respect to UV exposure time.

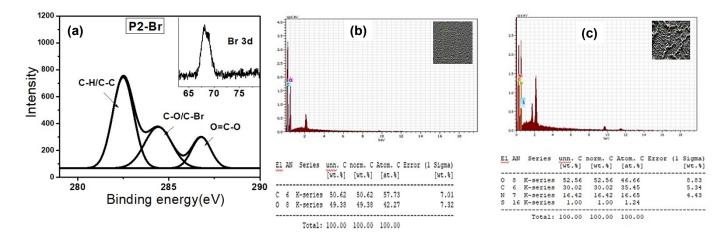


Figure S3. (a) Representative deconvoluted XPS spectrum for P2-Br (taken from boundary region of the pattern) after second initiator immobilization step; EDX spectrum with composition derived from patterned **(b)** P2-g-polyPEGMA brush (middle area of square pattern) **(c)** P2-g-polyDMAPS brush (boundary area of square pattern). All samples were polymerized for 24 h.

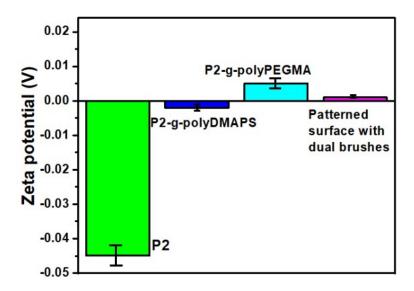


Figure S4. Evaluation of surface charge (zeta potential) for neat polymer blend P2 (P:PLA=5:95) surface and brush modified non-patterned (individual) and patterned surfaces measured at pH 7. All data points are shown as average + standard deviation (error bar).

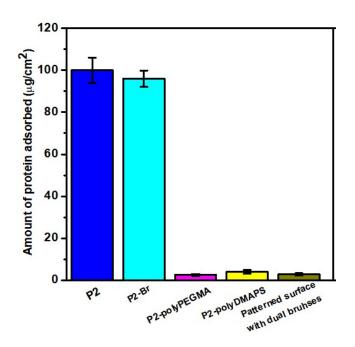


Figure S5. Quantitative analysis for protein adsorption on various surfaces using BCA. All data points are shown as average + standard deviation (error bar).

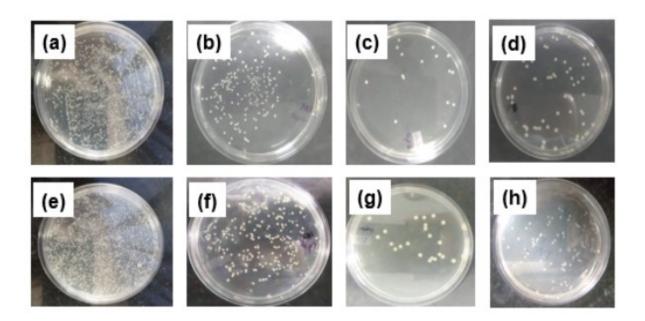


Figure S6. Representative images of bacterial growth on various surfaces **(a) & (e)** Polymer P1 **(b) & (f)** P1-g-polyPEGMA (24 h), **(c) & (g)** Patterned surface modified with dual brushes (24 h for each brush) **(d) & (h)** P1-g-polyDMAPS (24 h) against *E. coli* and *S. aureus,* respectively. Information in bracket represent polymerization time for each brush. 4 samples from each category were taken for the study.

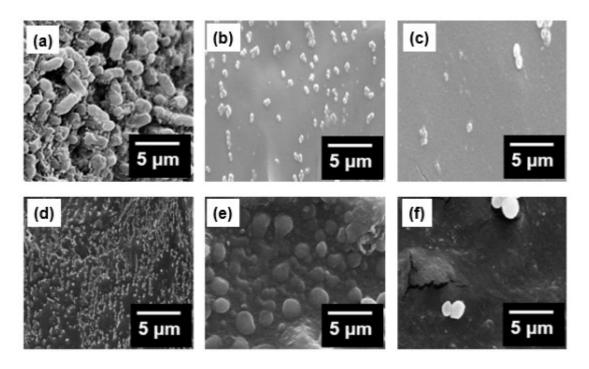


Figure S7. Representative SEM images of *E. coli* and *S. aureus* adhered on various surfaces (a) and (d) Polymer P (b) and (e) P1-g-polyPEGMA (c) and (f) P1-g-polyDMAPS, respectively. All samples were polymerized for 24 h.

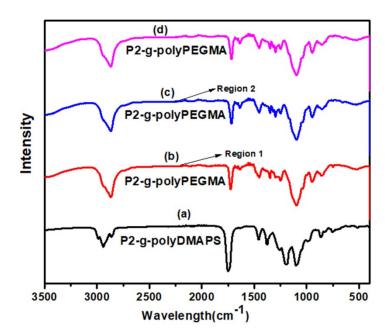


Figure S8 Representative ATR-FTIR spectra of (a) polyDMAPS brush after first step of ATRP grown in the middle region of the pattern (b) polyPEGMA brush covered middle region of the pattern acquired after second step of ATRP using PEGMA (**Region 1**, **middle area**) (c) polyPEGMA brush covered boundary region of the pattern acquired after second step of ATRP using PEGMA (**Region 2**, **boundary area**) (d) homopolymer brush of polyPEGMA onto the blend P2 (P:PLA=5:95) surface (reference spectrum)

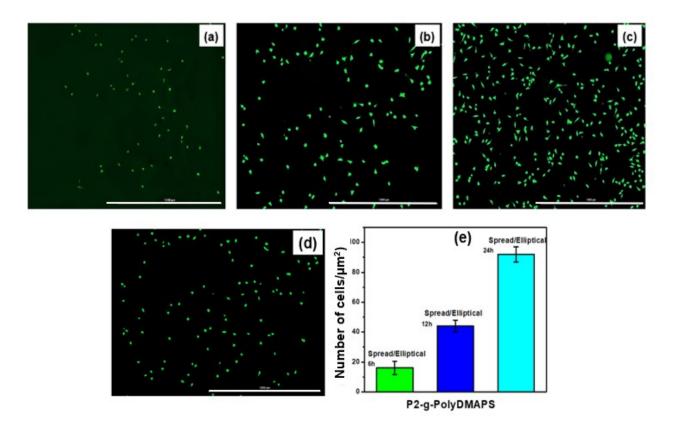


Figure S9. Fluorescent images acquired after *in vitro* culture of L929 cells on various surfaces (a) P2-g-polyDMAPS brush (6h), (b) P2-g-polyDMAPS brush (12h) and (c) P2-g-polyDMAPS brush (24h); Information in the bracket represents the polymerization time. (d) Glass surface (e) Number of adherent L929 cells per unit area on polyDMAPS brush modified surfaces obtained at various polymerization times such as 6h, 12h and 24h. Scale bar: 100 μm.

Table S1: Assignments of characteristic peaks acquired from Raman spectra shown in Figure 4a.

S.No	Peaks	Assignments
1	1034 cm ⁻¹	sulfonate group of poly- DMAPS
2	1757 cm ⁻¹	C=O stretching peak for polyDMAPS
3	1458 cm ⁻¹	C-N ⁺ stretching peak for polyDMAPS
4	1440 to 1520 cm ⁻¹	(O–C–C) CH ₂ -scissoring character for polyPEGMA
5	830 and 871 cm ⁻¹	Modes have a mixed character of CH ₂ -rocking (polyPEGMA)
6	120 and 1520 cm ⁻¹	C–H-stretching region for polyPEGMA
7	1289 cm ⁻¹	C-O stretching peak for polyPEGMA
8	1744 cm ⁻¹	C=O stretching peak for polyPEGMA