Supporting Information

Favorable Antibacterial, Antibiofilm, Anti-adhesion to Cells, and Biocompatible Polyurethane by Facile Surface Functionalization

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S1. Synthesis of PHMG

A three-necked round-bottomed flask equipped with a mechanical stirrer and vacuum system was charged with hexamethylenediamine (1 mol) and guanidine hydrochloride (1.1 mol). The mixture reacted at 110 °C for 60 min. During the reaction, by-product ammonia was neutralized by bubbling through aqueous HCl. After one hour, the temperature was increased to 190 °C. The gas formation was significantly increased at 190 °C. The reaction was continued on the condition of removing ammonia by vacuum system for 4 h. Afterward, the membrane pump was connected to the reactor, and the pressure was carefully pulled down to 250 m bar and held for 60 minutes. Finally, the PHMG as a viscous liquid was prepared by cooling.

S2. Spectroscopic, mechanical, and thermal analysis

^{1}H-NMR spectra were recorded on a Bruker ARX300 spectrometer at room temperature using DMSO-d6 as solvents, and MestReNova software was used for quantitative evaluation. Digilab spectrometer (model Excalibur FTS-3000 series, USA) equipped with an ATR unit was used for recording FTIR spectra at a resolution of 0.5 cm^{-1} and signal-averaged over 16 scans. Thermogravimetric analysis (TGA) was performed on a Netzsch instrument (model TG 209 F1 Libra, Germany) at a heating rate of 10 °C/min under an N_2 atmosphere. The Scanning electron microscopy (SEM) micrographs were taken on a Zeiss LEO 1530 GEMINI. The acceleration voltage was set to 3 kV, and the sample was sputter-coated with a 1.3 nm platinum layer.

The tensile strength, elongation-at-break, and modulus of samples were carried out using a tensile tester with a 50 mm/min crosshead speed. Films were cut into bars of each 50 mm length and 5 mm width. The test was performed at room temperature, and the data were analyzed by the software testXpert II from Zwick / Roell company. For each sample, three specimens were tested.

A drop shape analyzer (Krüss, model DSA25E, Germany) was used to measure water's contact angle on the sample sheet surfaces using 2.0 μL drop volume. The images were analyzed through Advance drop shape software (version 1.3.1.0) using Sessile drop orientation and circle fitting mode. An average of five measurements is reported.

S3.1 Protocol of QualiScreen Test

First, in 80 mL of an autoclaved nutrient solution consisting of 0.4 g peptone, 0.6 g meat extract, and 80 mL Millipore® water, a thawed E.coli (DMS 1077) or B. subtilis Cryo-Stock culture composed of 0.5 mL of a 30% glycerol solution and 0.5 mL bacterial suspension is poured. Then incubate at 37 °C for 4-6 hours.

The optical density of the bacterial suspension is determined using a photometer at a wavelength of 600 nm. The bacterial suspension is diluted with a 20% Nutrient-Broth solution (in PBS) until an optical density of 0.125 can be measured on the photometer. This optical density of 0.125 at a wavelength of 600 nm can be assigned to a $1x10^8$ CFU/mL bacterial concentration. 1 mL of this solution is diluted with 20% Nutrient-Broth solution until a bacterial concentration of $1x10^6$ CFU/mL is obtained. This working solution should be used directly so that the bacteria cannot multiply during this time.

The samples to be tested are placed in a 96 well plate (one granule per well). Then 200 µl of the bacterial suspension is added to each well. The plate is incubated at 37 °C for 1 hour. The samples are then transferred to a new 96 well plate with sterile tweezers and washed with 200 µl PBS for 10 min at RT while shaking. Transfer the samples with sterile forceps to a new 96 well plate and cover each plate with 200 µl 1% Nutrient-Broth solution (in PBS) and incubate for 18 h at 37 °C. After the incubation period, remove the granules with sterile forceps and add 50 µl Nutrient-Broth solution (30 g/L) to each well. The photometric measurement is carried out at 37 °C and a wavelength of 600 nm throughout 48 h while shaking.

S3.2 Antibacterial activity of samples by colony-forming count method

Antibacterial activity was tested according to ASTM E 2180-07 (colony forming count method) using the bacterial concentration of 2×10^8 CFU/ml and 1 cm×1 cm sample size. The detailed procedure is shown in literature: RSC Adv 2014, 4, 62046.

S4. Biocompatibility study

MTT assay was carried out on the sterilized PU films which were cut to cover the bottom of wells of a 96-well culture plate. The 5×10^3 L929 fibroblast cells were incubated at 37 °C for 45 min on the samples. The film/cell construct was then provided with more culture medium for 24 h at 37 °C. The percentage of relative cell viability was calculated according to the following equation:

$$\textbf{cell viability}\% = \frac{\mathbf{OD_{sample}} - \mathbf{OD_{positive\ control}}}{\mathbf{OD_{negative\ control}}}$$

OD = optical density, the standard TCP was considered a negative control, and the film with no cells was tested as a positive control.

S5. FTIR and ^{1}HNMR characterization of UPU3 sample

The FTIR and ^{1}HNMR of UPU3 as a representative sample are presented in Figure S1 a and b. In FTIR, the area of the peak assigned as follows: 3300 cm-1 (broad, stretching vibration N-H groups), 1730 and 1705 cm-1 (the non-hydrogen bonded and hydrogen-bonded stretching vibration of -NH-CO-O), 1760 cm^{-1}(stretching vibration of the uretdione C(O)), 1530 cm^{-1}(the stretching vibration (C-N) and the out of plane bending vibration (N-H), 1050-1150 cm^{-1} (the stretching vibration peak of the ether C-O of PHMG), 3050 cm-1 (the stretching vibration, aromatic C-H from MDI). As shown in the ^{1}HNMR spectrum of UPU3 (Figure 1 b), NH urethane groups' signal was observed at 9.5 ppm. Protons of aromatic groups originated from MDI, and methylene groups of HDI-Uretdione were also observed at 7-7.5 and 1.3 ppm. The signal related to the central methylene group of MDI structure was at 3.75 ppm. The methylene group attached to the ether group (C-O) and central methylene group of PTMEG structure was detected at 3.3 and 1.5 ppm, respectively.

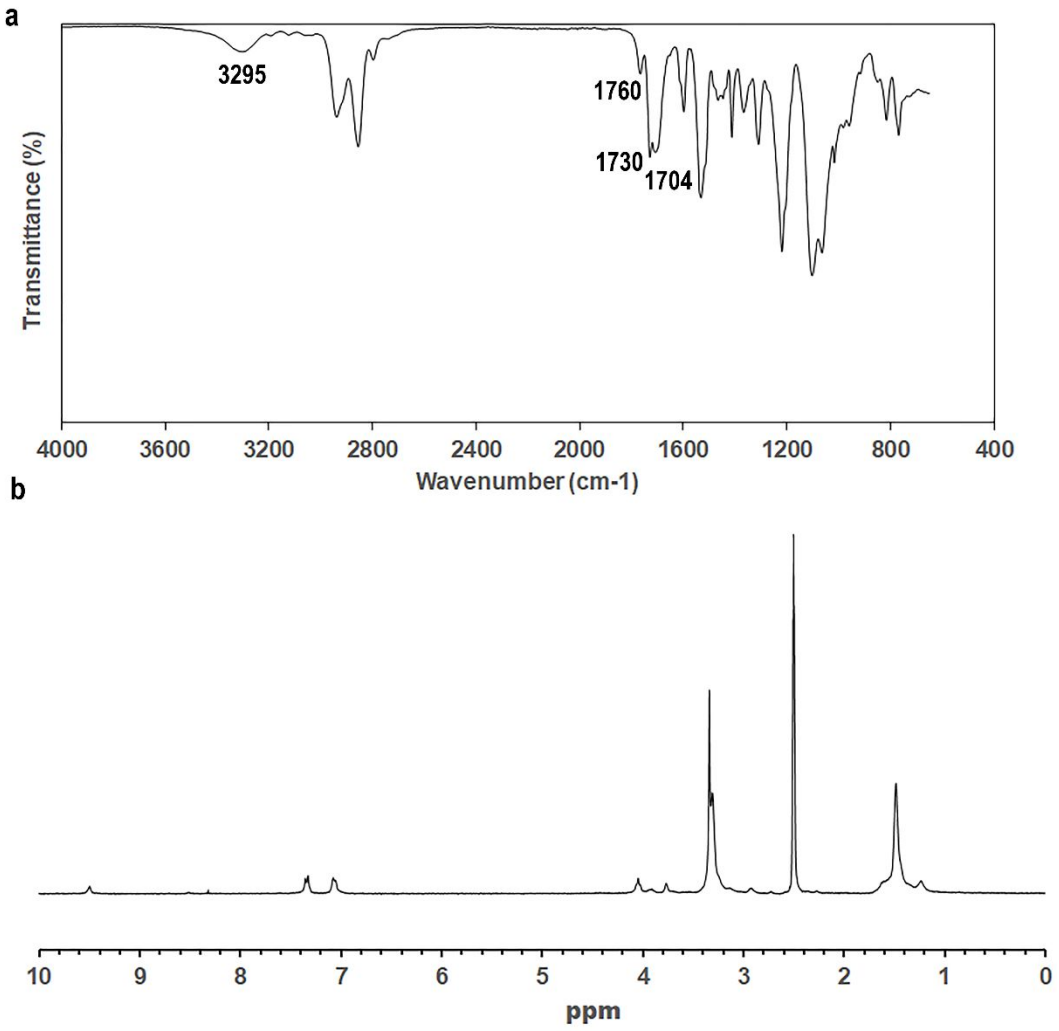


Figure S1: a) FTIR spectra and b) ^{1}HNMR spectra of UPU3 sample.

S6. Spectroscopic and thermal characterization of MPU blends

The structural characterization of prepared blends (MPUs) was evaluated with the ATR-FTIR technique, and the spectrum data of pristine Texin PU and MPU2 as representative samples are shown in Figure S2. The stretching vibration of urethane N-H groups of pure Texin PU was noticed at about 3310 cm^{-1}. The hydrogen-bonded and non-hydrogen bonded urethane (NH-CO-O-) carbonyl groups were detected as separate peaks at 1728 and 1702 cm^{-1}. The stretching vibration of C-N and the out of plane bending vibration of N-H urethane groups were combined and observed at 1530 cm^{-1}. The stretching vibration peak of the ether C-O group was detected at 1000-1150 cm^{-1}. The same pattern was seen for the FTIR spectrum of MPU2 samples, and a new peak related to the stretching vibration of the uretdione carbonyl group appeared at 1760 cm^{-1}.

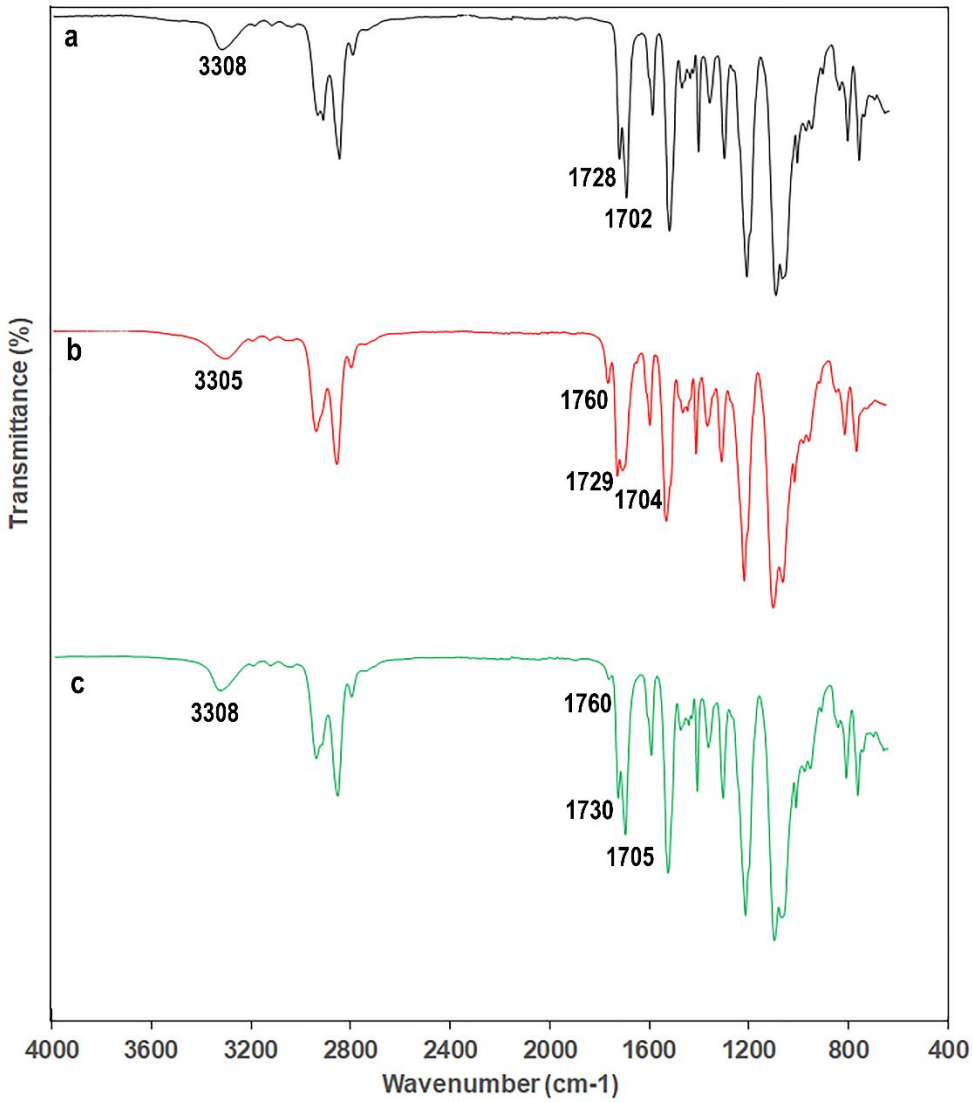


Figure S2: FTIR spectra of a) Texin PU, b) UPU3, and c) MPU2 samples

TGA and DTG thermograms of MPU1-3 and Texin PU are shown in Figure S3. The pristine Texin PU showed the three steps thermal degradation pattern. The degradation of labial urethane linkages was noticed as a first weight loss at around 310 °C. The second weight loss at 360 °C is due to the thermal deterioration of aliphatic groups of polymers' backbones. The third degradation step was noticed at 450 °C; this significant weight loss could be attributed to the degradation of PTMEG

ether bonds and the aromatic structure of Texin PU. The same pattern without substantial changes in thermal properties and patterns were observed for the MPU1-3 blend samples.

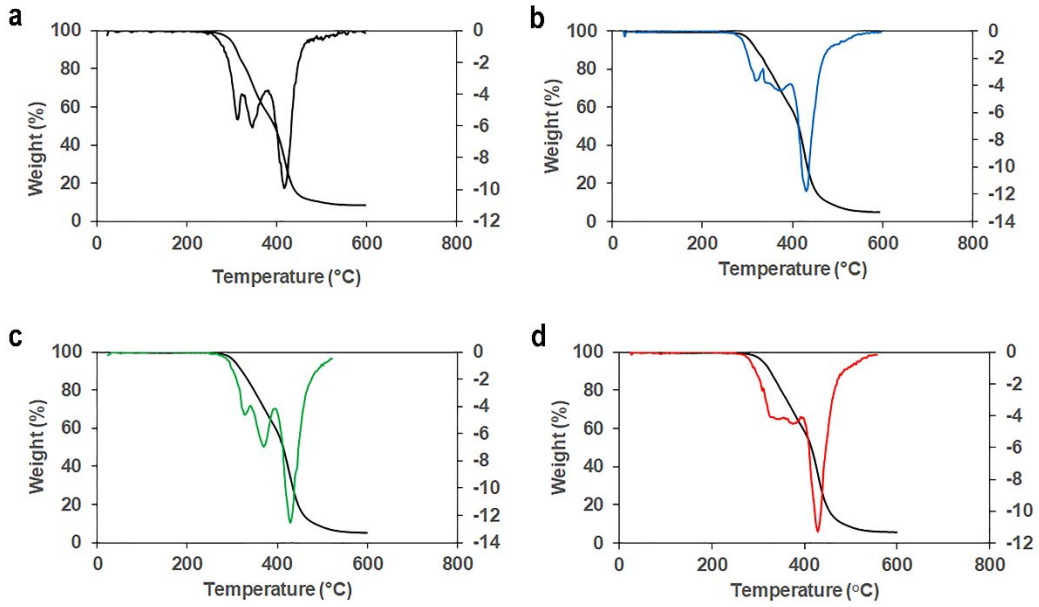


Figure S3: TGA and DTG curves of a) Texin PU, b) MPU1, c) MPU2, and d) MPU3.

S7. PHMG characterization

As shown in Figure S4, the characteristic peak of the guanidine group of PHMG was noticed at 1615 cm^{-1}. The symmetric and symmetric stretching of NH_2 was detected at 3150 and 3285 $cm^{-1,}$ respectively. The signal of methylene groups linked to the guanidinium moieties appeared at 3.15 ppm according to the ^{1}HNMR of PHMG in D_2O and DMSO. The signals at about 1.4 and 1.5 ppm belonged to the central methylene group of hexamethylene structure of PHMG. The protons of guanidinium moieties appeared at 2.1 and 8.5 ppm in DMSO, while these signals did not detect in D_2O solvent due to rapid proton exchange. The molecular weight of synthesized PHMG was determined around 5760 g/mol.

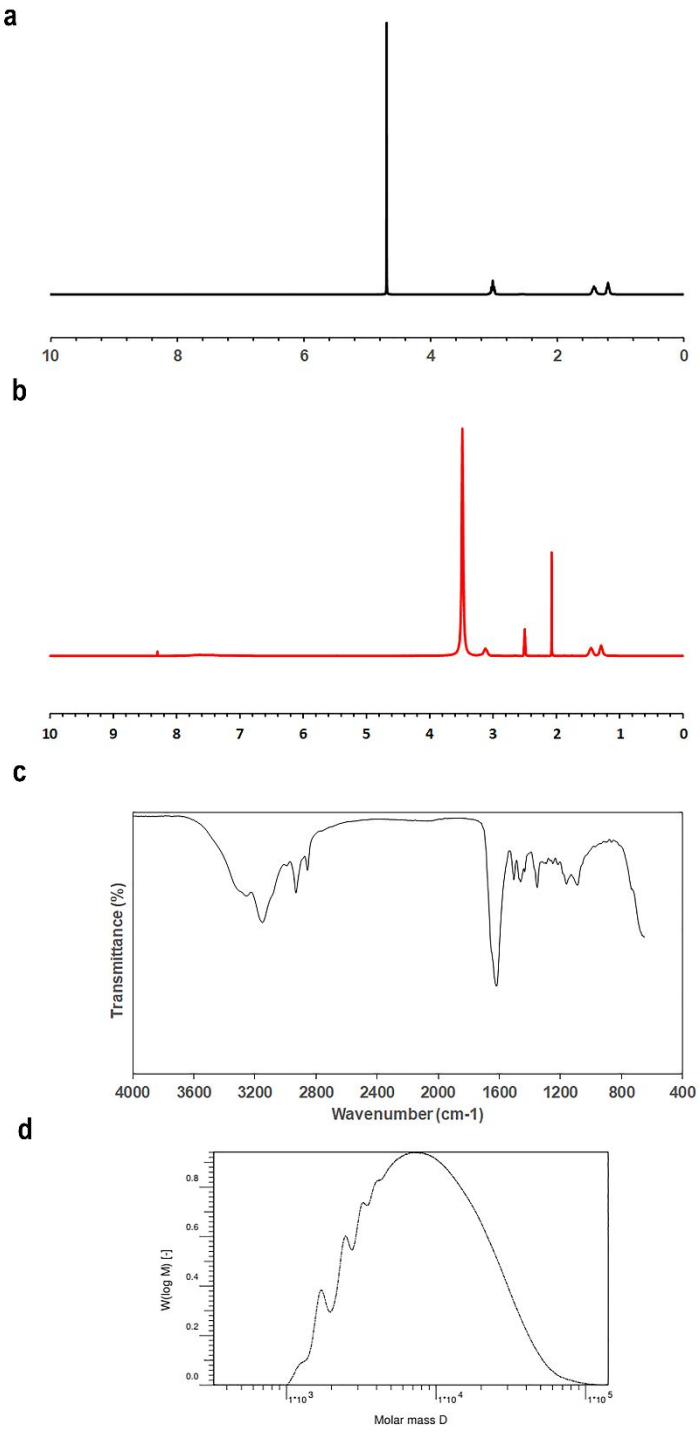

a
10
8
6
4
2
0
b
10
9
8
7
6
5
4
3
2
1
0
c
Transmittance (%)
4000
3600
3200
2800
2400
2000
1600
1200
800
400
Wavenumber (cm-1)
d
W(log M) [-]
0.0
0.2
0.4
0.6
0.8
1*10^3
1*10^4
1*10^5
Molar mass D

Figure S4: a) ^{1}HNMR spectra of PHMG in D_2O b) ^{1}HNMR spectra of PHMG in DMSO,

c) FTIR, and d) GPC trace of PHMG

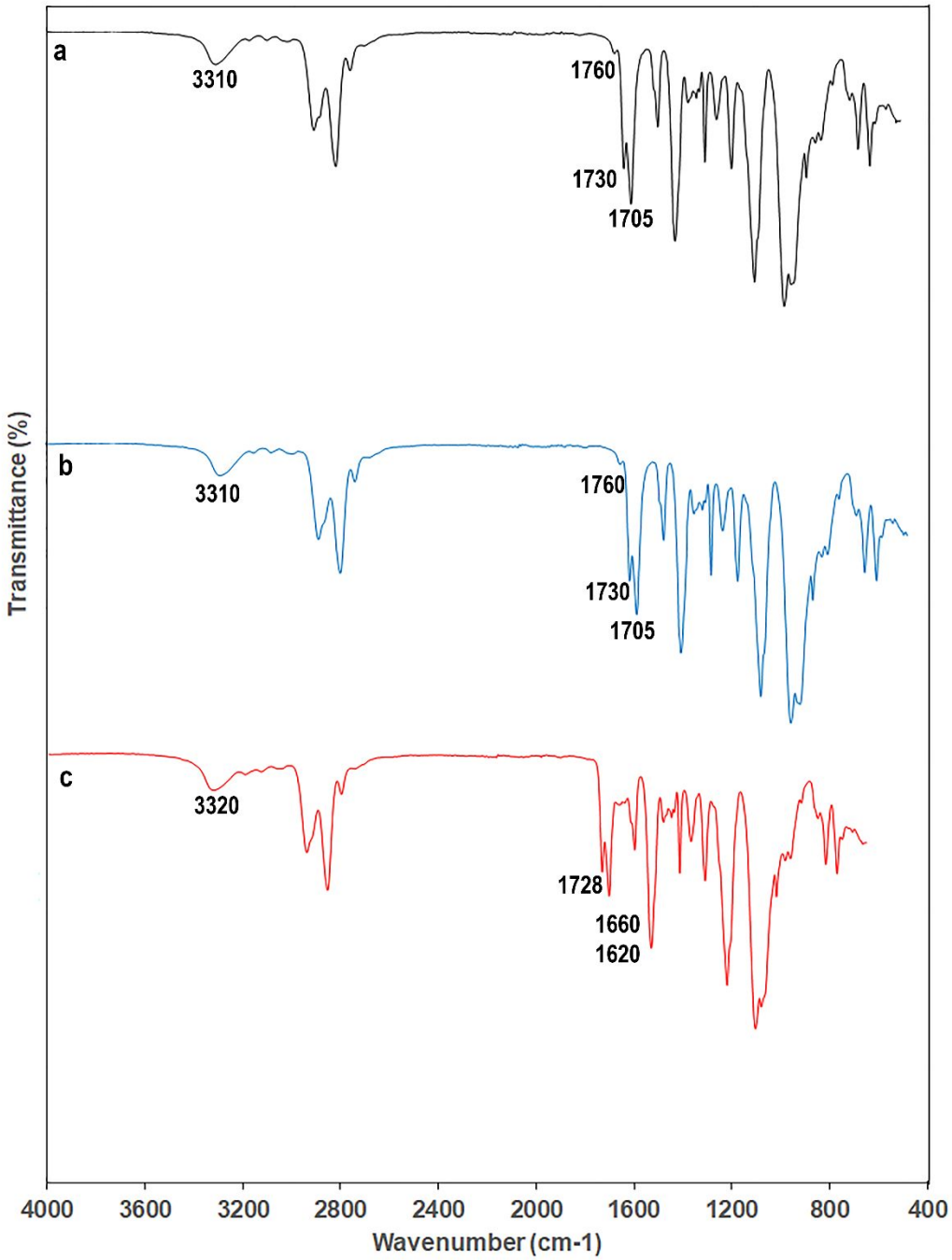


Figure S5: FTIR spectra of a) bare MPU2 and b) PHMG-MPU2 after 1 h and c) PHMG-MPU2 after 4 h reaction.

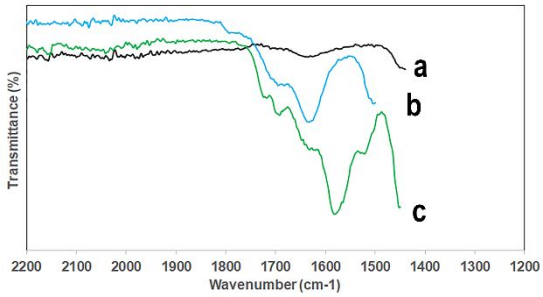
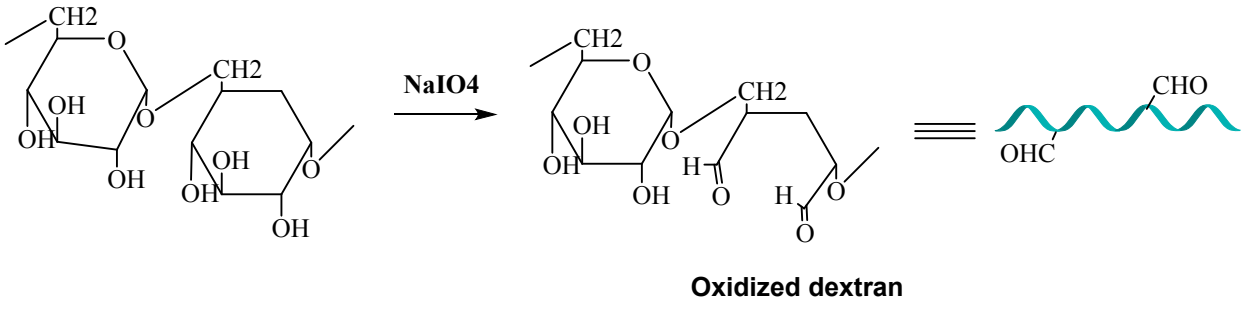


Figure S6: FTIR spectra of oxidized dextran with different % of oxidation a)10%, b) 20% and c) 30%.

As shown in Figure S4, the aldehyde group peak was observed at 1720 cm^{-1} in the IR spectrum of dextran with an oxidization degree of 30%, and other peaks remained intact. However, the aldehyde group peak was not detected on the oxidized dextran by 10% and pure dextran. The 30% oxidized dextran was used for surface modification. The synthetic pathway for oxidized dextran is illustrated in Scheme S1.



Scheme S1: Schematic illustration of dextran oxidization.

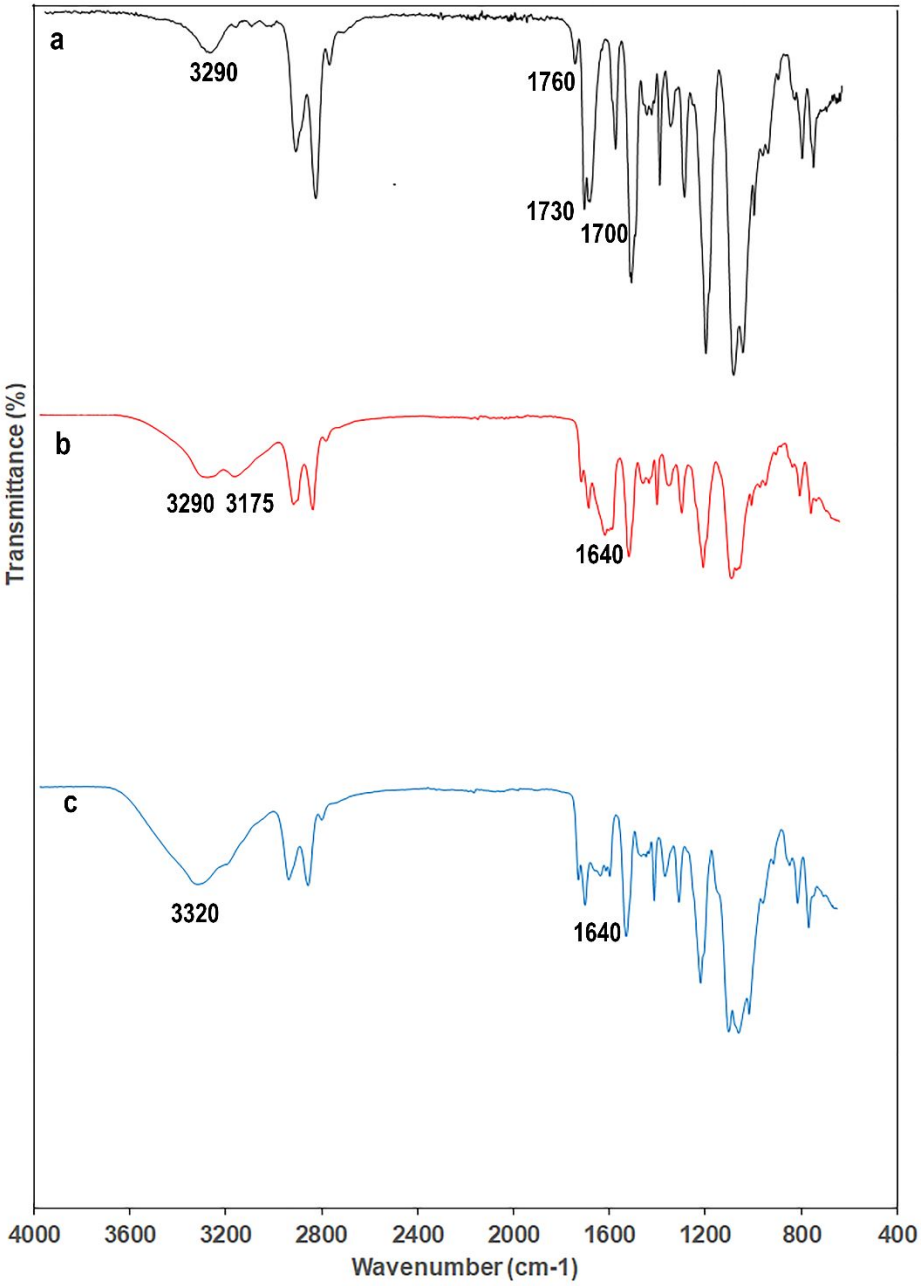


Figure S7. ATR-FTIR spectra of a) bare MPU (70/30) and b) PHMG-MPU (70/30), and c) Dex-PHMG-MPU (70/30).

The indirect proof was gained by following the schemed surface functionalization on the blend consisting of 70% of UPU3 and 30% Texin PU. The mixture's structural characterization

consisting of a high UPU3 counterpart (70% UPU3 and 30% TPU) that offers the high reactive sites for functionalization was evaluated by ATR-FTIR spectra, as shown in Figure S7 a-c. As expected, the intensity of stretching vibration of the uretdione carbonyl group was intensified within higher content of UPU3 polymer (Figure S7 a). The appearance of the stretching vibration of the guanidinium NH group at 3157 cm^{-1} and the stretching vibration of the C=NH and generated urea carbonyl group a broad peak at 1640-1680 cm^{-1} confirmed the successful grafting of PHMG to the MPU70/30 blend surface. Upon modification with dextran, the NH and OH groups peak was merged and significantly broadened at around 3320 cm^{-1}. The increased intensity and broadening of C-O bonds' stretching vibration peaks at 1015-1100 cm^{-1} confirmed successful immobilization of the dextran layer on PHMG-MPU films via reaction of the PHMG free amine with oxidized dextran.

Table S1: The element composition, EWA, and contact angel of prepared samples [a)]

Samples Name	[N]/[O]	[O]/[C]	Contact angel (°)	EWA (%)
MPU2	0.037	0.345	109 ± 2[a]	1.20±0.1[a]
PHMG-MPU2	0.071	0.268	95 ± 2 [b]	1.30±0.1[a]
Dex-PHMG-MPU2	0.078	0.346	84 ± 2[c]	1.40±0.2[a]

a) $p \geq 0.05$

Table S2: Antibacterial activity of PUs.

Sample	*E. coli*	*B. subtilis*
Texin PU	0	0
PHMG-MPU1	71.3	75.9
PHMG-MPU2	100	100
Dex-PHMG-MPU2	100	100