Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

SUPPORTING INFORMATION

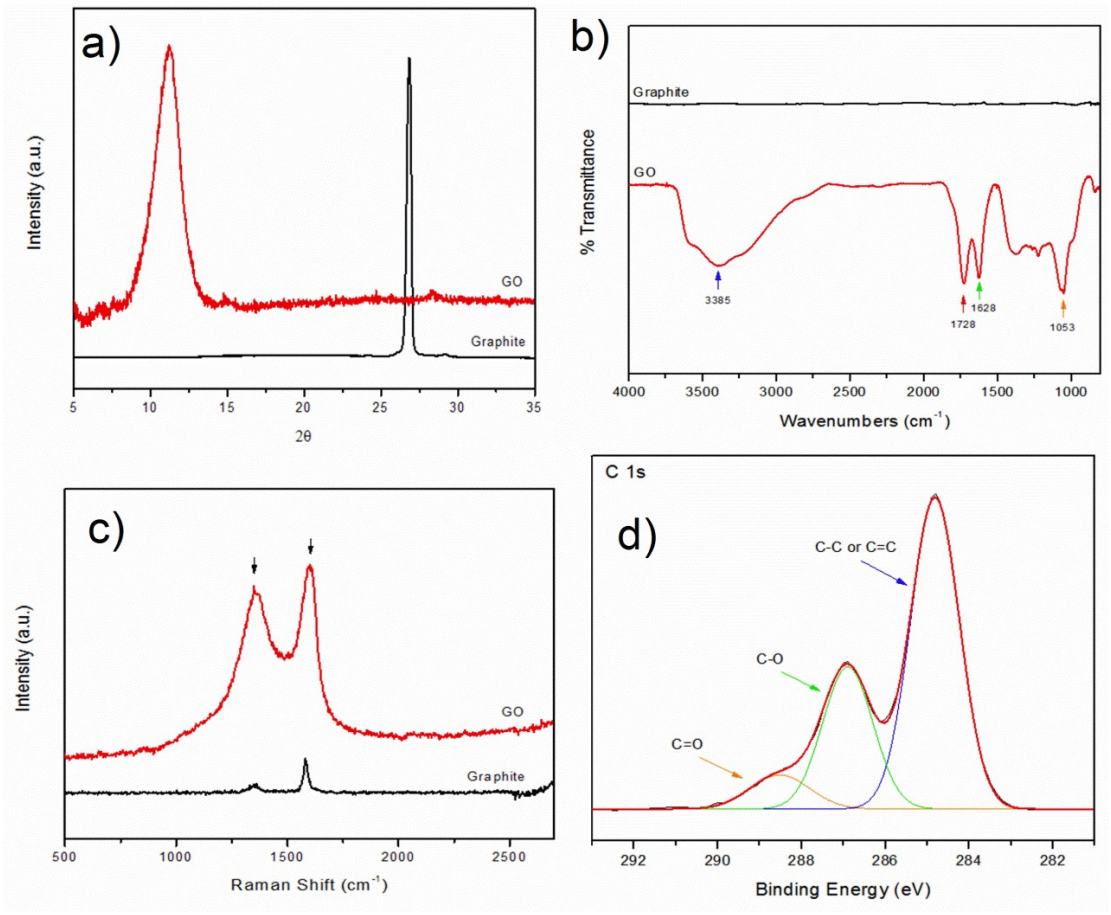
Designing Polymeric Adhesives for Antimicrobial Materials: Poly(ethylene imine) Polymer, Graphene, Graphene Oxide and Molybdenum Trioxide - A biomimetic Approach

Hang Ngoc Nguyen[a], Enrico T. Nadres[a], Bryan Alamani[b], Debora Frigi Rodrigues[a*]

[a]Department of Civil and Environmental Engineering, University of Houston, Houston, TX 77204 – 4003 (U.S.A.)

[b]Department of Chemical Engineering, University of the Philippines, Diliman, Quezon City, The Philippines

*Corresponding Author's Email: dfrigirodrigues@uh.edu Phone: +1-713-743-1495



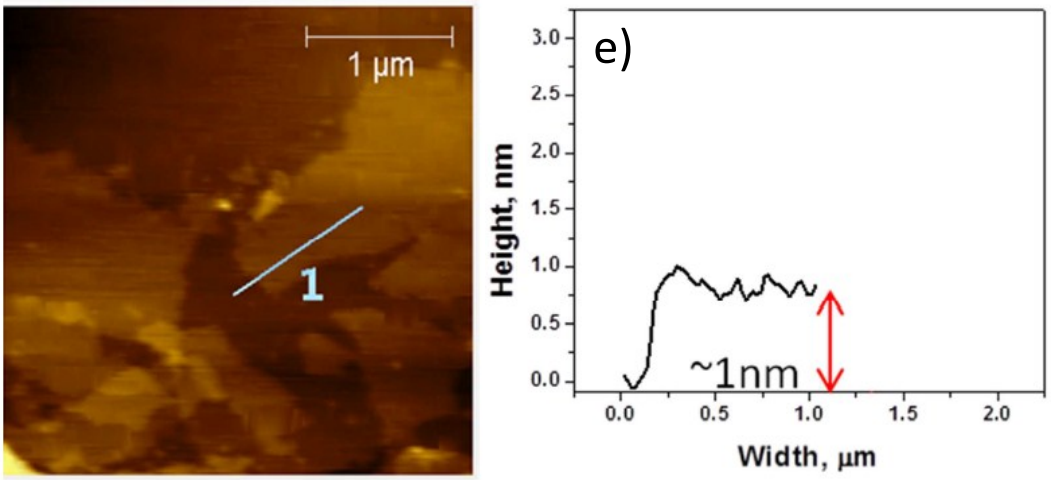
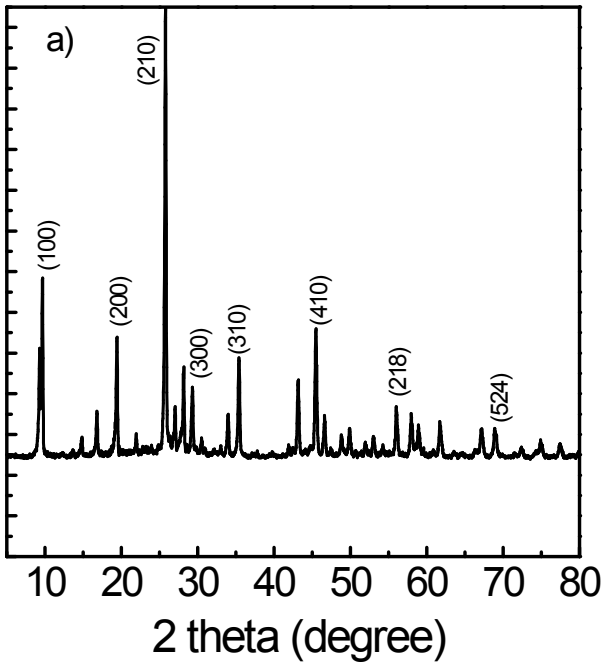
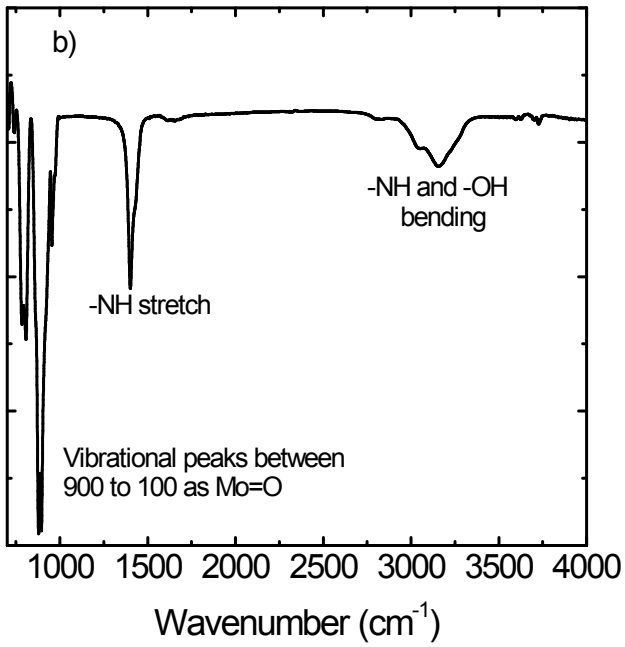
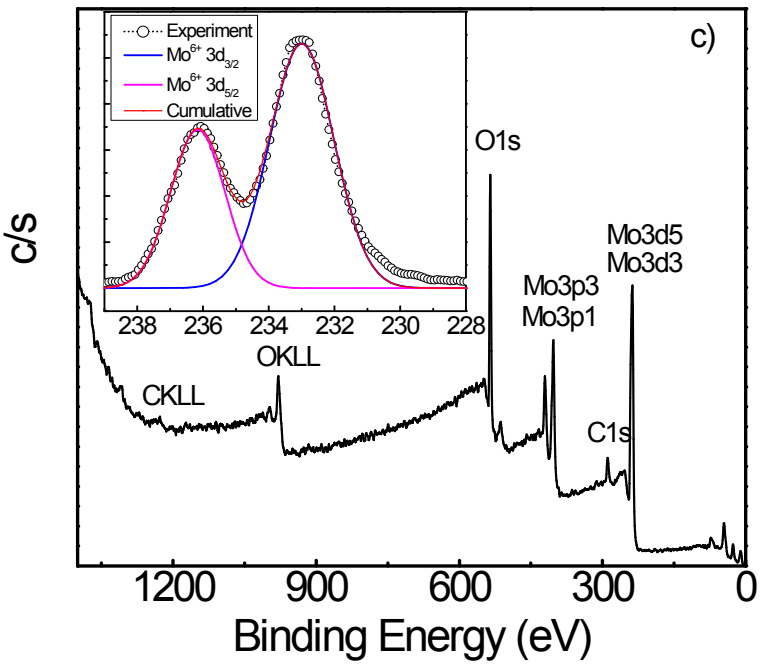


Fig. S1: a) XRD spectrum showing the peak 2 theta at 11.25 (representative of GO peak); b) FTIR with characteristic peaks of GO functional groups (-C-OH, -C=O, -COOH and -C-O); c) Raman spectrum showing 1350 (cm^{-1}) for functional group in GO; d) XPS with GO bonding for the functional groups and e) AFM analysis of GO showing the height ~ 1nm.







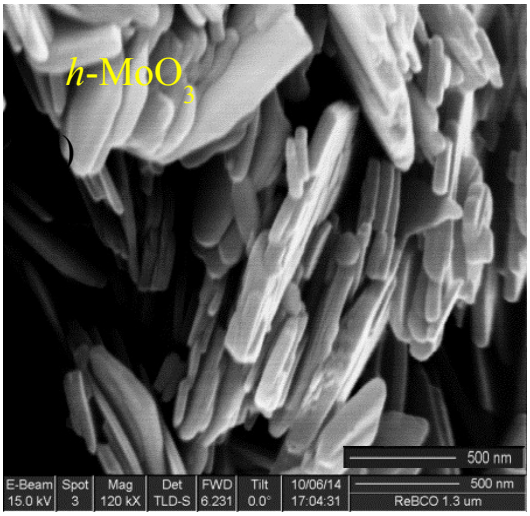
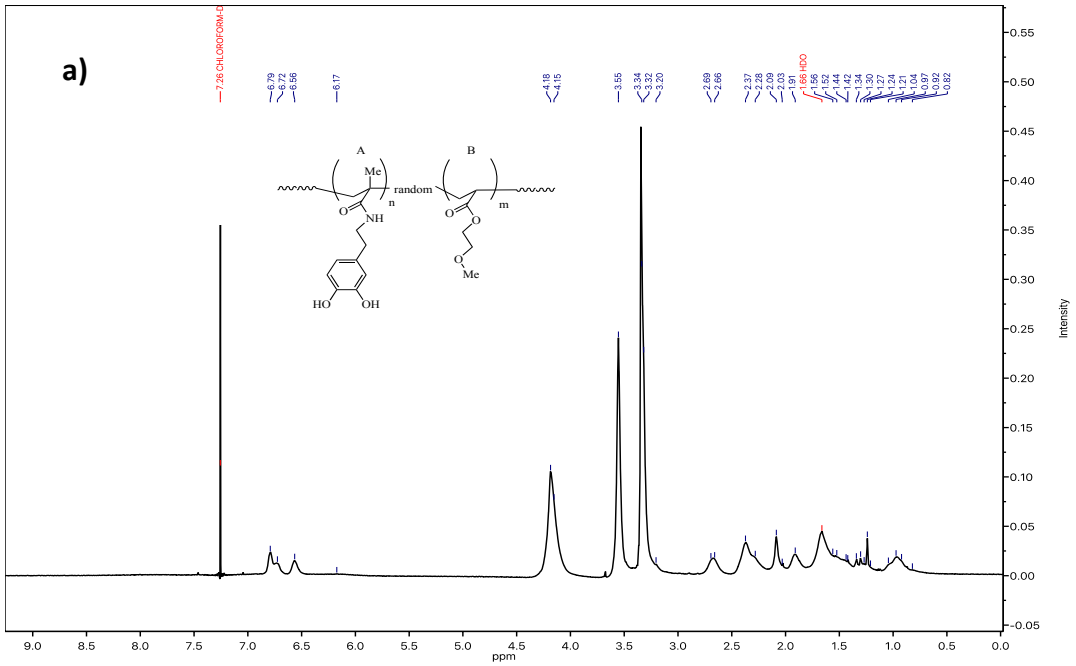
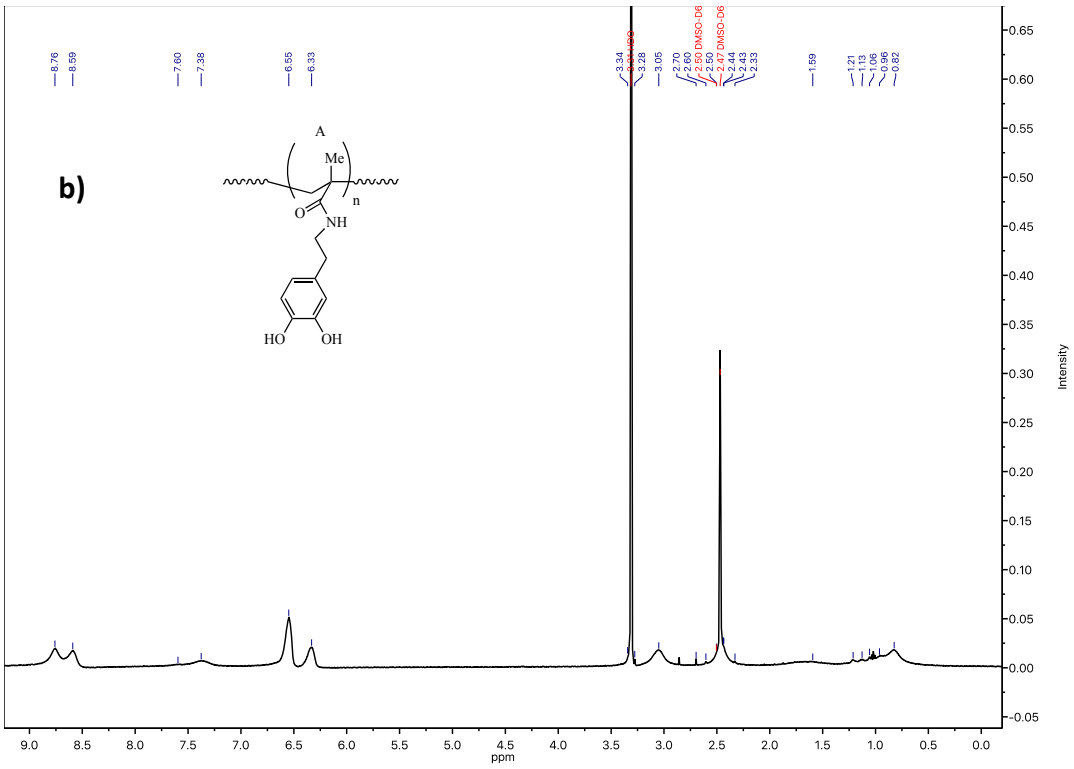


Fig. S2: (a) X-ray diffraction (XRD) spectra of *h*-MoO_3 the spectra confirmed the successful synthesis of both MoO_3 structure with the characteristic peaks[1] (b) FTIR spectra of MoO_3 (*h*-MoO_3) with the spectra matched where Mo in the previous publication in MoO_3 [2] and (c) X-ray photoelectron spectroscopy (XPS) spectrum and (d) Scanning Electron Microscope (SEM) image of *h*-MoO_3


a)
7.26 CHLOROFORM-D
6.79
6.72
6.66
6.17
4.18
4.15
3.55
3.34
3.32
3.20
2.69
2.66
2.37
2.28
2.09
2.03
1.91
1.81 HDO
1.52
1.44
1.42
1.34
1.30
1.27
1.24
1.21
1.04
0.97
0.92
0.82
A
B
Me
random
n
m
O
NH
HO
OH
Intensity
ppm


b)
8.76
8.59
7.60
7.38
6.55
6.33
3.34 H2O
3.28
3.05
2.70
2.69
2.50 DMSO-D6
2.50
2.47 DMSO-D6
2.44
2.43
2.33
1.59
1.21
1.13
1.06
0.96
0.82
A
Me
n
O
NH
HO
OH
Intensity
ppm

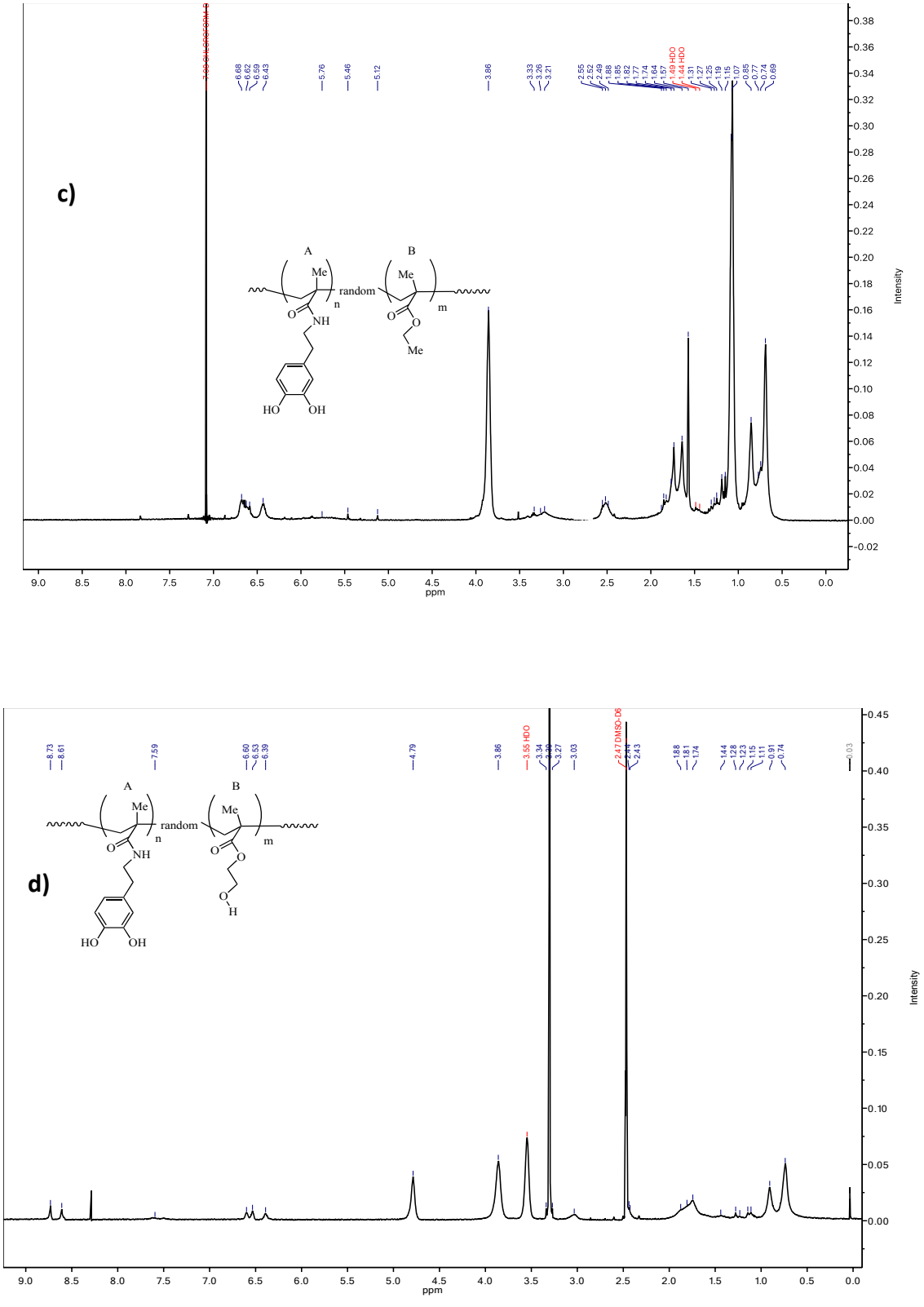
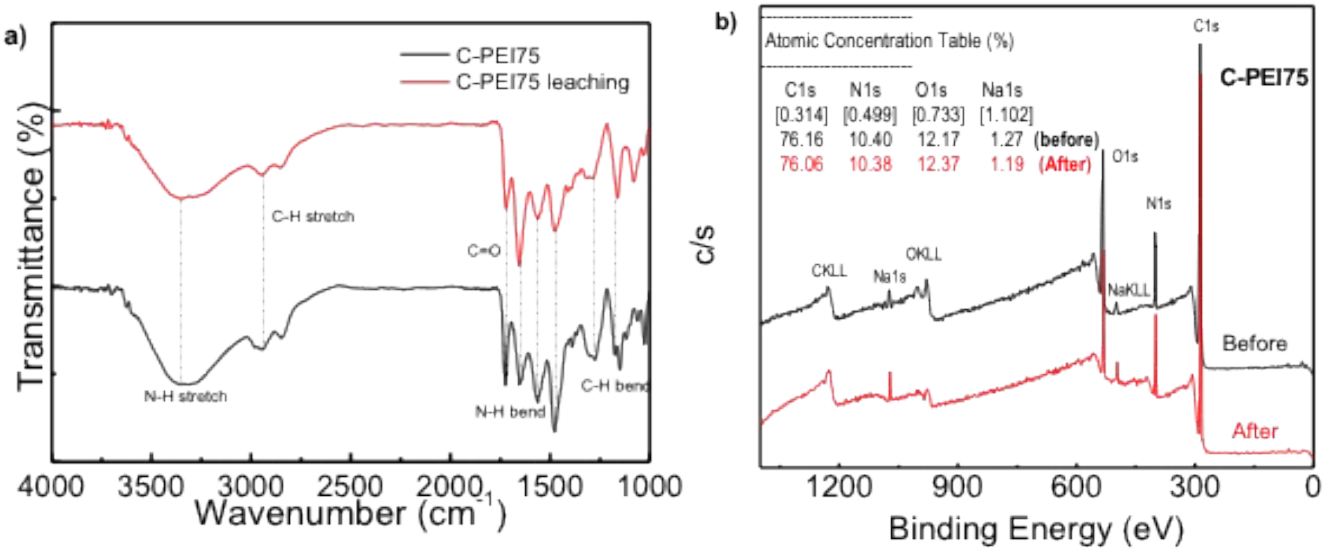


Fig. S3. Spectra NMR (a) polymer A, (b) polymer B, (c) polymer C, (d) polymer D


a)
C-PEI75
C-PEI75 leaching
C-H stretch
C=O
N-H stretch
N-H bend
C-H bend
Transmittance (%)
4000 3500 3000 2500 2000 1500 1000
Wavenumber (cm-1)
b)
Atomic Concentration Table (%)
C1s N1s O1s Na1s
[0.314] [0.499] [0.733] [1.102]
76.16 10.40 12.17 1.27 (before)
76.06 10.38 12.37 1.19 (After)
C-PEI75
C1s
O1s
N1s
CKLL
Na1s
OKLL
NaKLL
Before
After
c/s
1200 900 600 300 0
Binding Energy (eV)

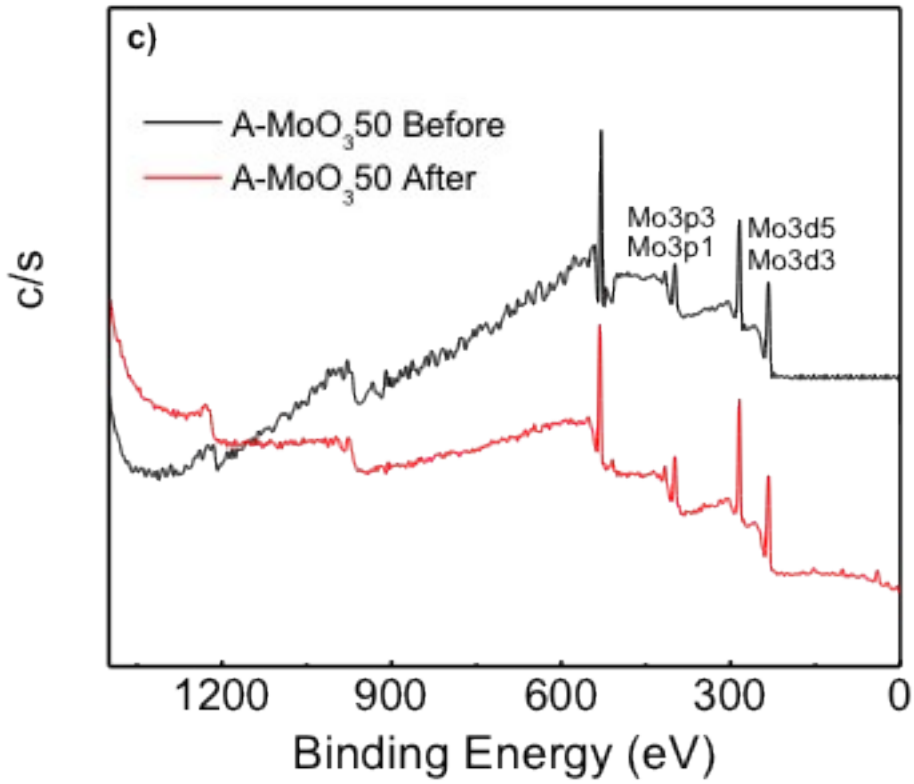

c)
A-MoO3 50 Before
A-MoO3 50 After
Mo3p3
Mo3p1
Mo3d5
Mo3d3
c/s
1200 900 600 300 0
Binding Energy (eV)

Fig. S4: FTIR of C-PEI75 (a), XPS spectrum of C-PEI75 (b) and XPS of A-$MoO_3$50 (c) before and after leaching process showing there was no significant changes of the samples in term of compositions.

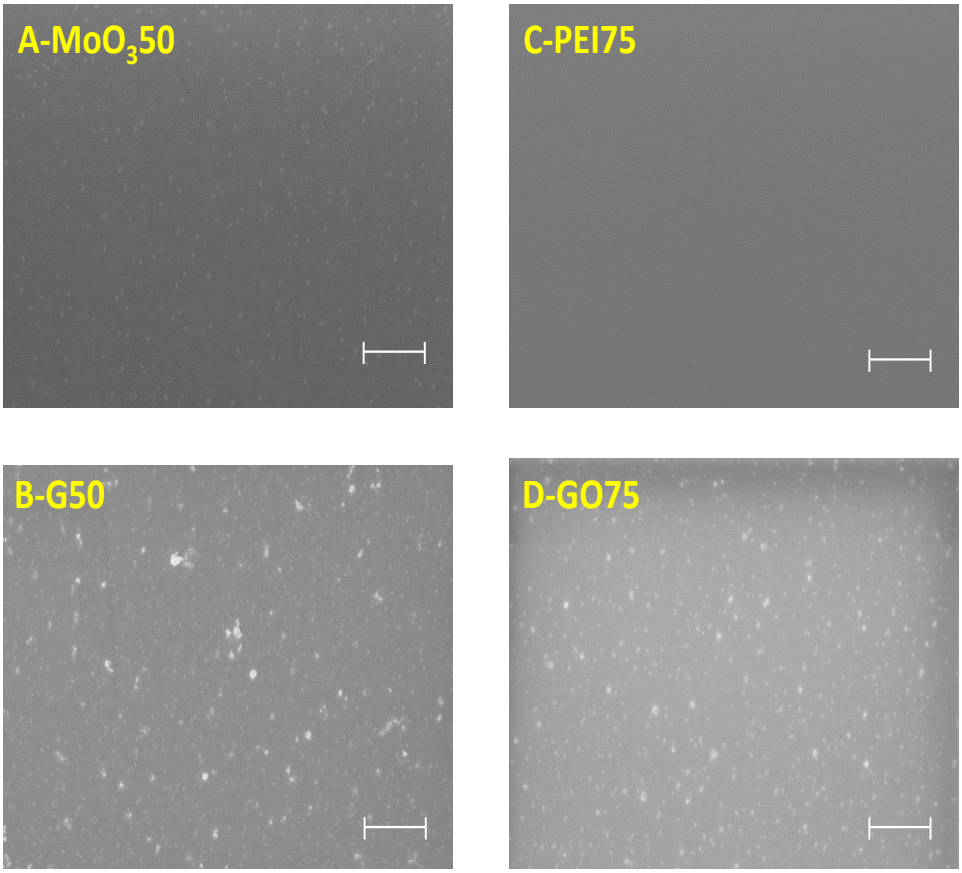


Fig. S5: SEM images of the antimicrobial coatings after 24h leaching. Scale bar 1 µm.

Antimicrobial and anti-biofilm tests for other bacteria

Bacterial suspension preparation

Antimicrobial experiment was carried out using Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Streptococcus pyogenes* and *Staphylococcus epidermidis*). The 16h growth culture was prepared freshly each time from a single colony on a tryptic soy agar plate (TSA) inoculated in tryptic soy broth (TSB, Oxoid, U.S.A.) for *P. aeruginosa* and nutrient broth #1 for *S. pyogenes* and *S. epidermidis*. All the growth was conducted at 35^{o}C with shaking at 150 rpm (ThermoFisher, U.S.A). The growth culture was centrifuged at 10,000 rpm for 5 minutes, and then rinsed twice using phosphate buffered saline (PBS, pH = 7.4, Sigma Aldrich, U.S.A.) to completely remove TSB. The pellets were then re-suspended in PBS at 0.05 optical density of 600nm (OD_{600}) which were 5 x 10^4 colony forming units per milliliter (CFU/mL).

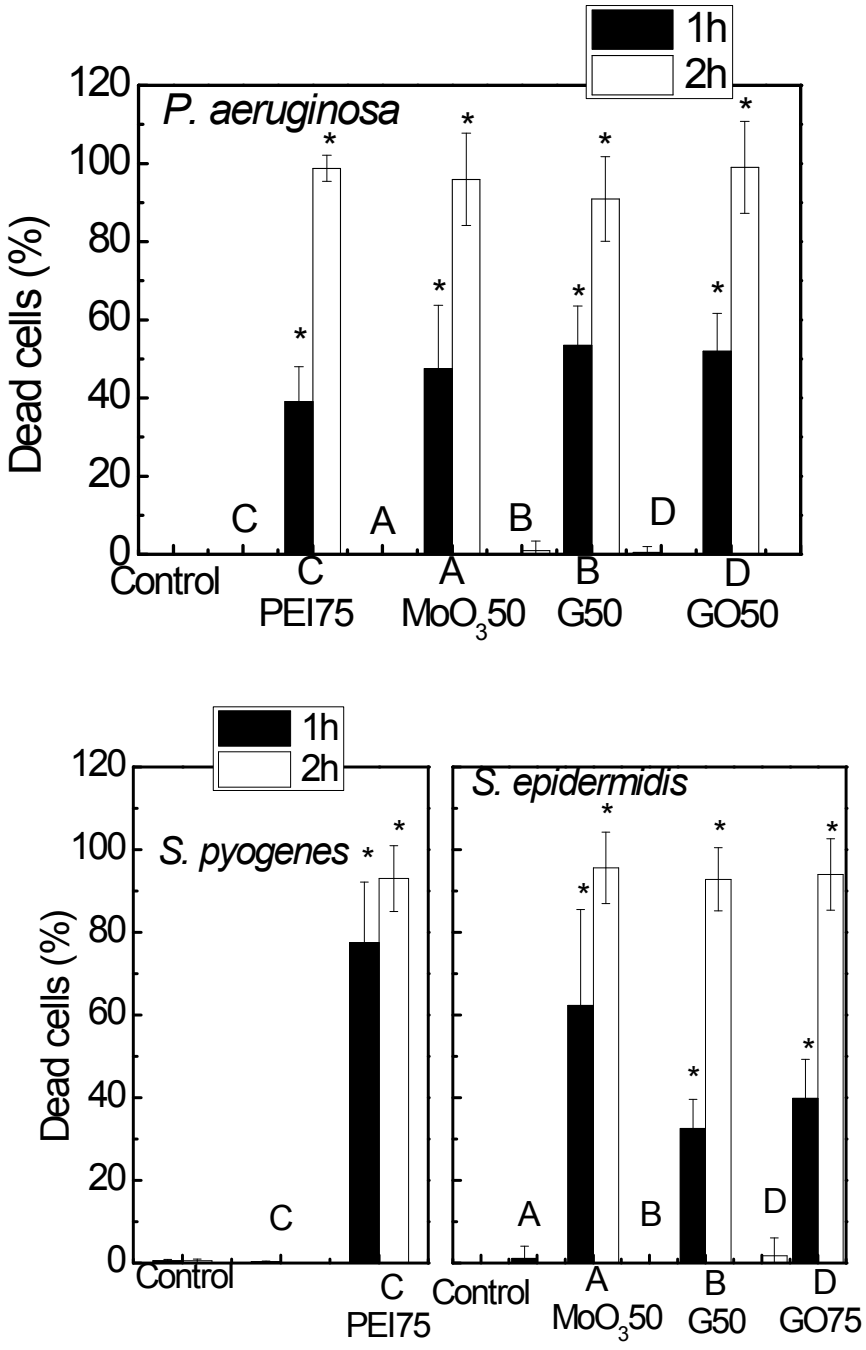


Fig. S6: Live and dead assay for antimicrobial properties of the coatings expressed in percentage of dead cells for other microorganisms (*P. aeruginosa*, *S. pyogenes* and *S. epidermidis*). The results of both 1 and 2 h interaction between microorganisms and coated surface were shown. The control with only glass slide and he adhesive only were showing zero or less than 2% of dead cells. (*) indicates statistically significant different in comparison to the control (uncoated glass slide) and adhesive only.

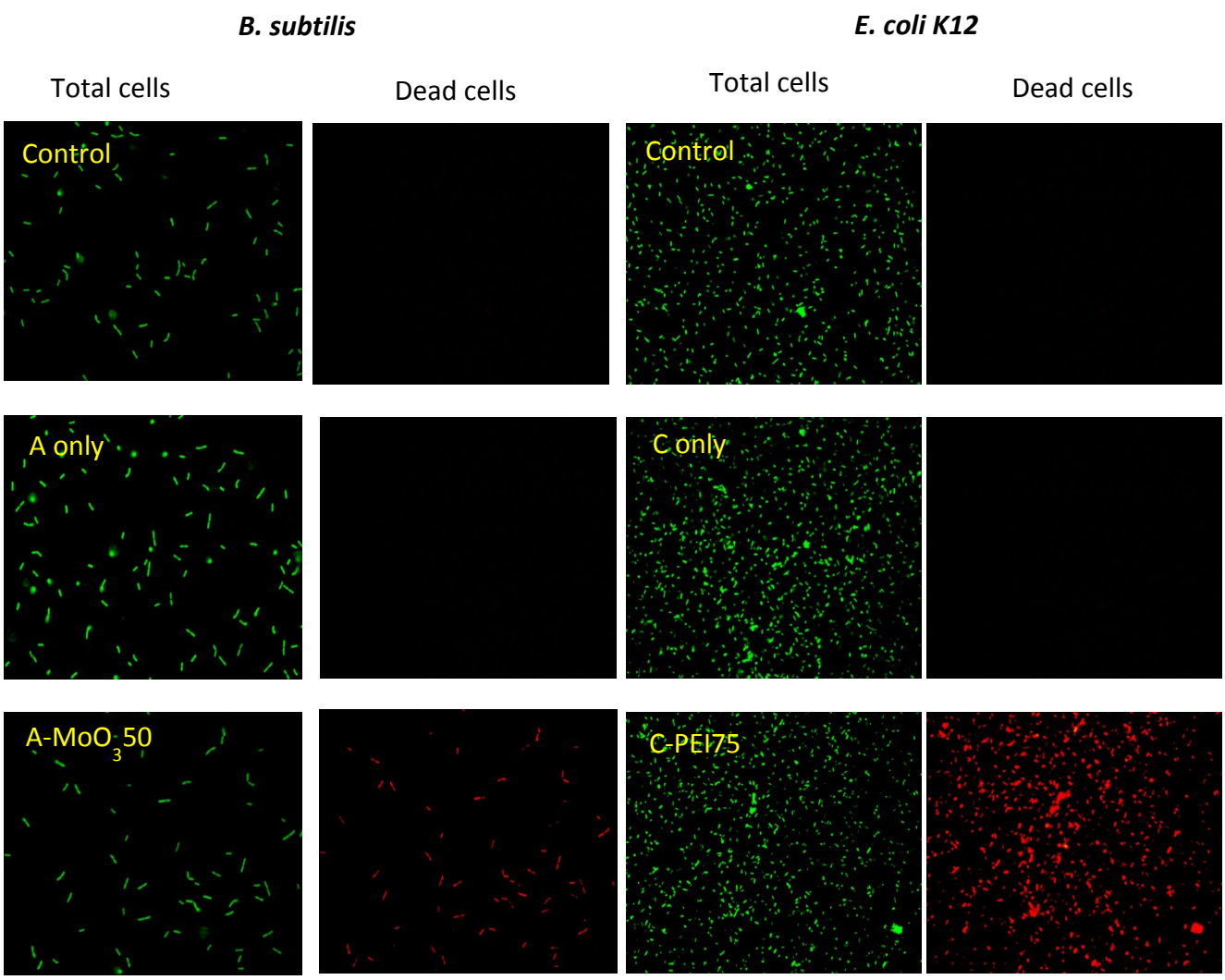
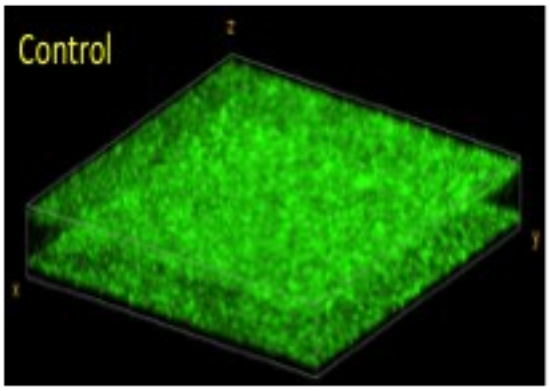
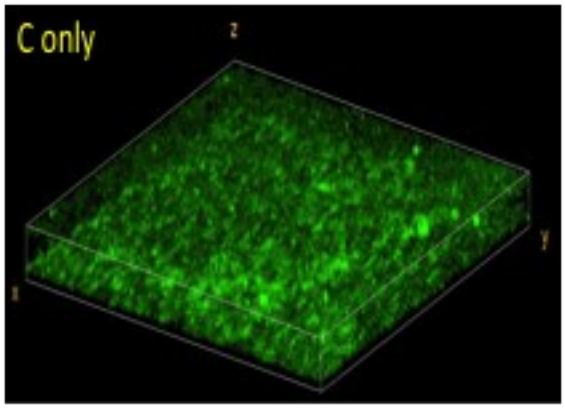


Fig. S7: Images of live and dead assay for antimicrobial properties of the coatings showing total cells (green) and dead cells (red). The images showed *E. coli* (C-PEI75) and *B. subtilis* (A-$MoO_3$50)

	Biomass ($\mu m^3/\mu m^2$)	Thickness (μm)
Glass	23.74 ± 3.86	29.00 ± 0.00
C	12.43 ± 2.47	16.00 ± 2.74
C-PEI75	7.70 ± 0.63	9.00 ± 0.00





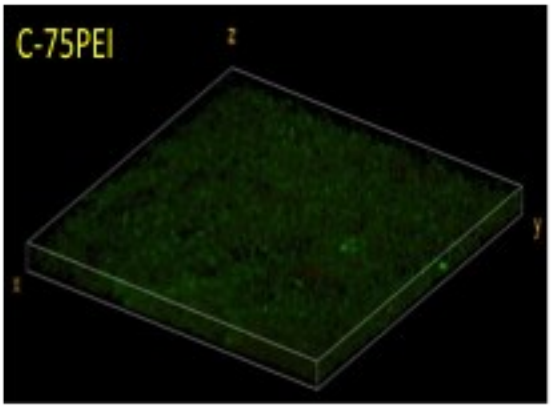


Fig. S8: The total biomass of biofilm of *E. coli* with 72h incubation and confocal images of the biofilm (72h growth) on the adhesive C and PEI 75% coated surface.

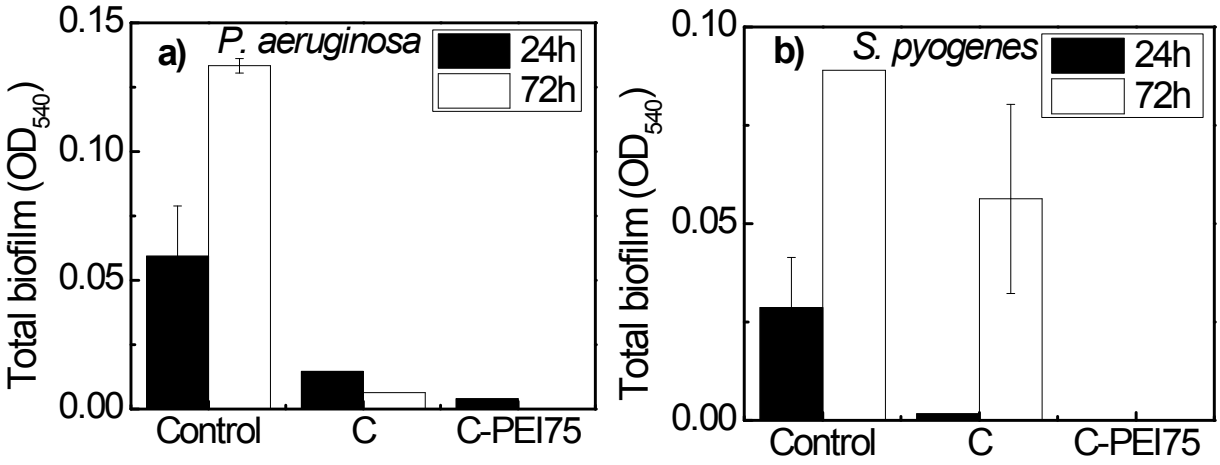


Fig. S9: The total biofilm forming on the surface of coatings using crystal violet assay for adhesive and PEI coatings *P. aeruginosa* (a) and *S. pyogenes* (b)

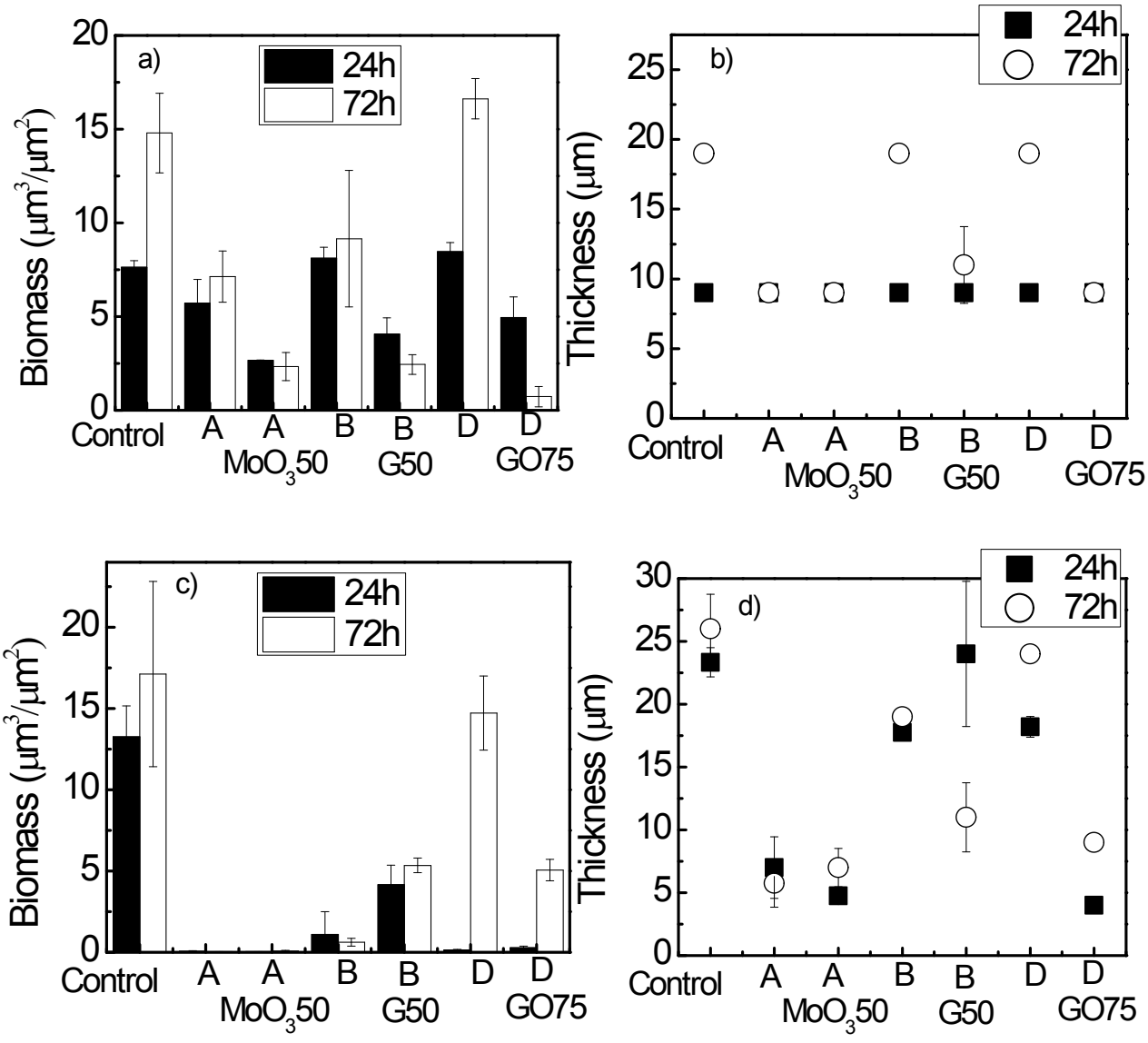


Fig. S10: Biomass and thickness results on coatings of adhesive and nanoparticles namely: adhesive A with MoO_3 50% (A-$MoO_3$50), adhesive B with graphene 50% (B-G50) and adhesive D with graphene oxide 75% (D-GO75) in 24 h and 72h for both *P. aeruginosa* (a), (b) and *S. epidermidis* (c), (d). Column graph is for total biomass with 24h (solid black) and 72h (solid white). The point graph is for the thickness of the biofilm with 24h (solid square black) and 27h (open circle).

X-ray photoelectron spectroscopy

XPS measurement was performed using PHI 5700, which was equipped with a monochromatic Al KαX-ray source (hν = 1486.7 eV) incident at 90^{o} relative to the axis of a hemispherical energy analyzer. Spectrometer measurement was performed in both high and low resolutions with pass energies of 23.5 and 187.85 eV, respectively. A photoelectron take off angle of 45^{o} from the surface, and analyzer spot diameter of 1.1 mm were used. The survey spectra was carried out at energies from 1 to 1400 eV. All spectra were collected at room temperature with a base pressure of 1 x 10^{-8} torr. Electron binding energies were calibrated with respect to the C1s line at 284.8 eV.

The coated slides were further characterized to confirm the successful coatings. Fourier Transform Infrared Spectroscopy Nicolet iS10, which was equipped with Nicolet Smart ATR (FTIR), was used to identify the functional groups present on the surface of the coatings after polymerization. Background of the spectra was subtracted from carbon dioxide and moisture in the environment before correcting the baseline. In addition, X-ray photoelectron spectroscopy (XPS) measurements were performed on the coatings using PHI 5700 X-ray photoelectron spectrometer. The spectra had the baseline corrected and analyzed using Multipak V7.0.1 version. XPS measurement confirmed the presence of atomic bonds that are present on the coated surface.

Further characterization of surface coated with composite of Polymer C and PEI

FTIR analyses of the coatings made from composite of Polymer C and PEI revealed peaks that are a combination of the signals from the parent compounds Polymer C and PEI (Fig. S11).

Further characterization of surface coated with composite of Polymer A and *h*-MoO_3

As expected, the XPS spectra of the samples containing polymer has carbon, oxygen and nitrogen signals. The XPS spectra of the samples coated with composite of Polymer A and *h*-MoO_3 showed signal for molybdenum. An increase in C–O bond indicated an increased oxidation of the Polymer C in the presence of *h*-MoO_3 (Fig. S12). This could be due to oxidation of the polymer as h-MoO3 is known to induce hydroxyl radical formation upon exposure to light.

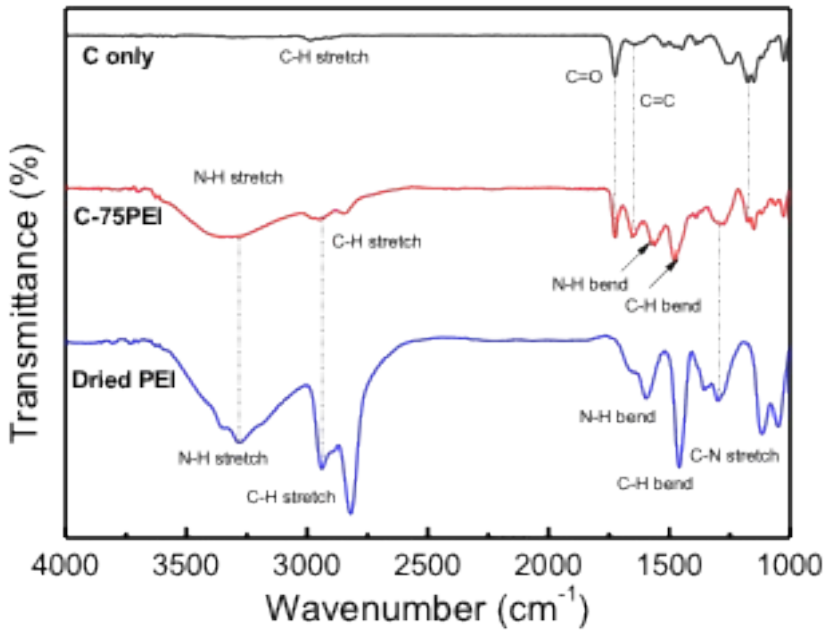


Fig. S11: FTIR spectra of successful coating of dried PEI, C and C-PEI75 showing the essential functional groups of PEI and adhesive on the surface of the coating.

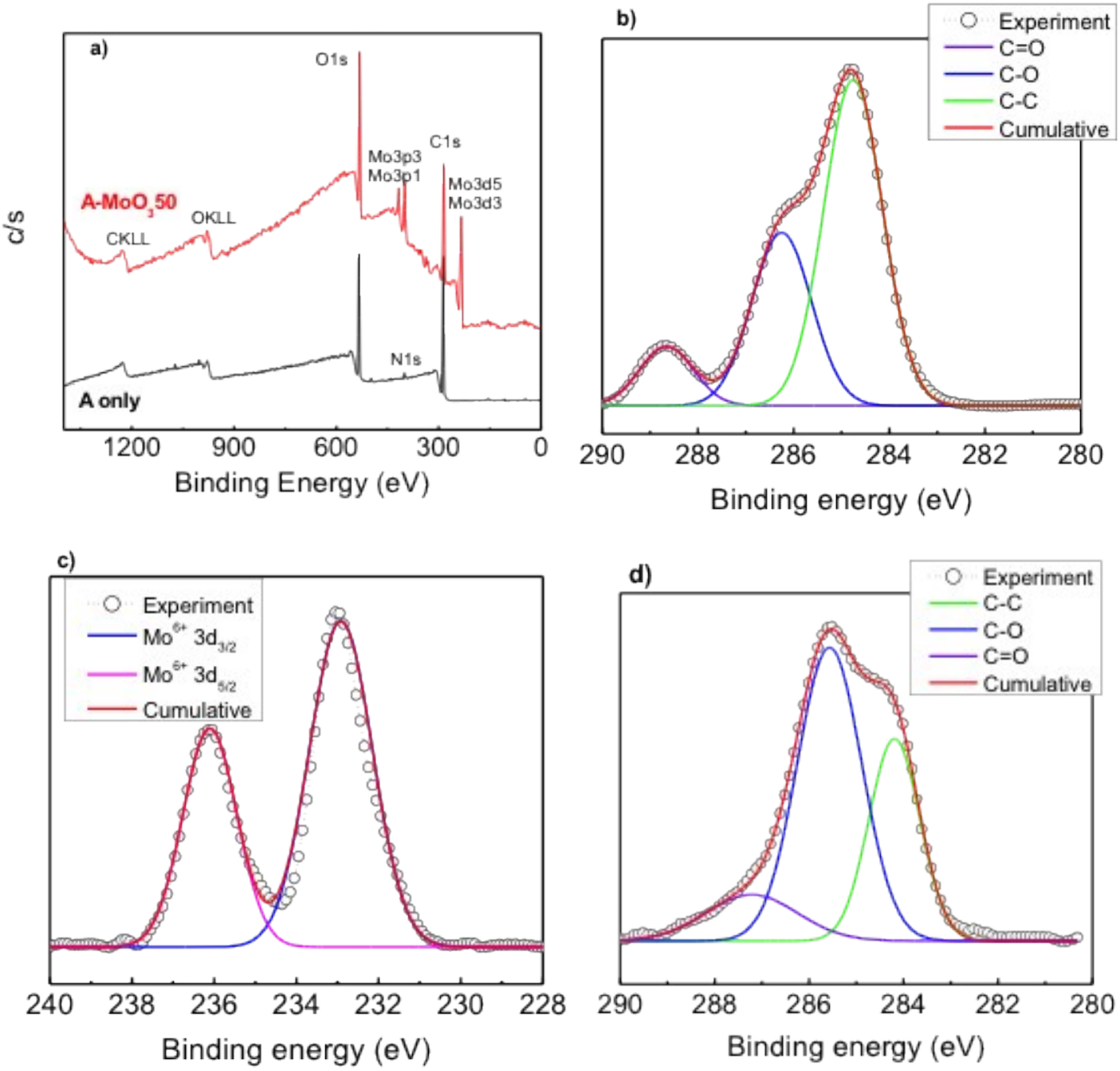

a)
O1s
C1s
Mo3p3
Mo3p1
Mo3d5
Mo3d3
A-MoO3 50
OKLL
CKLL
N1s
A only
C/s
1200
900
600
300
0
Binding Energy (eV)
b)
Experiment
C=O
C-O
C-C
Cumulative
290
288
286
284
282
280
Binding energy (eV)
c)
Experiment
Mo6+ 3d3/2
Mo6+ 3d5/2
Cumulative
240
238
236
234
232
230
228
Binding energy (eV)
d)
Experiment
C-C
C-O
C=O
Cumulative
290
288
286
284
282
280
Binding energy (eV)

Fig. S12: XPS spectra of the coatings: adhesive A only and A-$MoO_3$50 (adhesive A with 50% *h*-MoO_3) which showing the presence of Mo on the coated surface indicating the presence of MoO_3 and adhesive (a). The high resolution of C1s for adhesive A only (b), Mo^{6+} (c) and C1s of A-$MoO_3$50 (d) were expressed in this figure.

Table S1: Thickness of the coatings before and after leaching for 7 days at 37 ^{o}C

Coatings	Thickness (nm)*	Standard deviation
A only	69.74	0.3
A-$MoO_3$50	69.46	0.11
A-$MoO_3$50 leached	69.48	0.31
B only	68.56	1.08
B-G50	68.79	0.19
B-G50 leached	68.97	0.34
D only	72.06	1.29
D-GO75	69.4	0.21
D-GO75 leached	69.4	0.2
C only	70.18	0.3
C-PEI75	69.39	0.15
C-PEI75 leached	69.29	0.44

* thickness was determined using ellipsometry (Gaertner, model LSE – USB, U.S.A.)

References

1. A. Chithambararaj and A. C. Bose, *Journal of Alloys and Compounds*, 2011, **509**, 8105-8110.
2. A. Chithambararaj, N. Rajeswari Yogamalar and A. C. Bose, *Crystal Growth & Design*, 2016, **16**, 1984-1995.