## Electronic Supplementary Information (ESI)

# Silver-Doped Laser-Induced Graphene for Potent Surface Antibacterial Activity and Antibiofilm Action

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# A. Experimental Section

## 1. Materials and Methods:

PES polymer flakes (E 6020P, 75 kD) were obtained from BASF, Germany. Sodium chloride (NaCl, 99%), sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_2O$, > 99%), monobasic potassium phosphate ($KH_2PO_4$, 99%) and silver nitrate ($AgNO_3$) were purchased from Merck, Israel. Dichloromethane (DCM), dimethyl sulphoxide (DMSO), Syto 9 and propidium iodide were purchased from Sigma-Aldrich, Israel. Ethanol (anhydrous, 99.5%) was obtained from Macron Fine Chemicals. Deionized (DI) water was obtained from a Milli-Q ultrapure water purification system (Millipore, Billerica, MA, USA) and used unless otherwise specified. A 10.6 μm $CO_2$ pulse laser, 50 W, 2.0 in. lens kit (VLS 3.50) from Universal Laser Systems was used.

## 2. Ag@LIG Preparation:

Ag-PES polymer sheets were prepared as follows. $AgNO_3$ (0.34 mg) was dissolved in 0.2 mL DMSO while stirring at rt. This solution was added to 19.8 mL of DCM, and this was added to PES polymer flakes (2.5 g) with stirring. The dissolved polymer solution was added to a glass petri dish (inner diameter 11.5 cm) and covered with an inverted funnel to slow the evaporation of the solvent. After evaporation and drying, the prepared thin film (thickness ~150 μm) was used for Ag@LIG preparation. For this, laser scribing using a Universal Laser Systems laser platform (VLS3.50), equipped with a 10.6 μm $CO_2$ pulsed laser (50 W) was used. An image density of 1000 pulses per inch (DPI) in both axis, a scanning speed of 25 cm/s and laser duty cycle of 2.5% were used. A nozzle provided with the instrument was used to blow air toward the laser spot, while the general atmosphere within the laser platform was still air at ambient pressure.

### 3. Raman Spectroscopy:

The Raman system comprised a Horiba LabRam HR evolution micro-Raman system, equipped with a Synapse Open Electrode CCD detector air-cooled to -60°C. The excitation source was a 532 nm laser with a power on the sample of 0.5 mW. The laser was focused on LIG and Ag@LIG sheets with an x50 objective to a spot of about 2 μm. The measurements were taken with a 600 g $mm^{-1}$ grating and a 100 μm confocal microscope hole. Typical exposure time was 30 seconds.

### 4. X-ray Photoelectron Spectroscopy (XPS):

The XPS spectrometer ESCALAB 250 (Thermo Fisher Scientific, Waltham, MA) with an ultrahigh vacuum ($10^{-9}$ bar), installed with an AlKα X-ray source (beam size: 500 μm) and a monochromator, was used. The signals from $C_{1s}$, $O_{1s}$, $S_{2p}$ and $Ag_{3d}$ were detected by fixing different separated elements to the experimental data. The broad-spectrum survey spectra with pass energy (PE) of 150 eV and the high-energy resolution spectra (narrow spectrum of surface chemical functionality) with a PE of 20 eV were recorded. Prior to the measurements, the Ag@LIG sample was dried completely overnight in vacuum at rt.

### 5. XRD Measurements:

XRD diffraction patterns were recorded on Rigaku Smart Lab (Model- Rigaku D/Max ultima II) using Cu-Kα radiation as X-ray source (λ= 1.5405 Å) at rt. The voltage and current for the measurement were kept 45 kV and 100 mA, respectively. Approx. 25 mg of scribed Ag@LIG powder was dried inside a desiccator at 37°C with vacuum prior starting the measurement and analysis.

**6. Scanning Electron Microscopy (SEM) Imaging:**

The Ag@LIG sheet (0.5 cm × 0.25 cm) samples were mounted (vertically for the cross-section view) on the circular aluminum stub using carbon tape. The samples were not coated with gold because the Ag@LIG samples were conductive. The surface analysis and elemental mapping (EDS) was performed using a FEI Quanta 400 ESEM operating at 5 kV.

**7. Transmission Electron Microscopy (TEM) Imaging:**

TEM Tecnai T12 200 kV TEM (FEI, TWIN Electron Optics) electron microscope with 120 kV input voltage was used. LIG and Ag@LIG were scraped from the substrate, dissolved in DI water and sonicated for 6 hours. TEM grids were prepared by placing 2 μL diluted and sonicated sample solutions on a carbon coated copper grid and the solution was evaporated at room temperature. Silver nanoparticles were isolated from the graphene sheets using 10000 rpm centrifugation, the pellet was dispersed in DI water and drop casted on the TEM grids as before.

**8. Zeta Potential:**

Electrokinetic Analyzer (SurPASS, Anton Paar Gmbh) was used to measure the zeta potential on the Ag@LIG surface. Two identical Ag@LIG sheets (2 cm × 1 cm) were used for the measurements. Here, 0.1 mM KCl was used as an electrolyte, and 0.1 N NaOH and 0.1 N HCl were used to control the pH.

**9. UV Vis Absorption Spectroscopy:**

A small amount of black LIG powder was scraped from the Ag@LIG surface and dissolved in DI water. This solution of Ag@LIG was added to a quartz cuvette (1 cm path length) and the absorption spectrum was measured using a UV-1800 SHIMADZU spectrophotometer instrument.

**10. Inductively Coupled Plasma (ICP Measurement):**

For ICP analysis of leached ions, PES, Ag-PES sheets, or Ag@LIG sheets (100 $cm^2$) were incubated with aqueous PBS buffer (5 mL) for 3, 6, 12, 18 and 24 h. After each time point, 2 mL samples were collected and analyzed using ICP for leached silver content and compared to calibration standards prepared from stock standards over the concentration range of 0.05–5 ppm. Calibration standards were freshly prepared prior to analysis. Silver signal was monitored at $^{107}Ag$ and $^{109}Ag$, and data are reported for $^{107}Ag$. For ICP analysis, the sample preparation was done by VARIAN SPS 3 sample preparation system and the leached $Ag^+$ ions were calculated by using VARIAN 720-ES, ICP Optical Emission Spectrometer.

**11. Biofilm Growth for SEM analysis:**

*P. aeruginosa* (PAO1) wild type bacteria were cultured in Luria−Bertani (LB) broth at 30°C and harvested at mid-exponential phase (12-18 h), as verified by measuring the optical density at 600 nm. Cultures were centrifuged at 4000 rpm for 15 min; Cells were washed additionally three times with phosphate buffered saline and further diluted to an $OD_{600}$ of 0.1, in LB broth. Biofilm growth experiments were performed in a custom-made flow cell as reported earlier[1]. Briefly, the Ag-PES polymer substrate and Ag@LIG, (1 × 2 cm) were attached with double-sided tape to a glass slide and placed separately in two flow cells with vertical alignment. Inoculation of *P. aeruginosa* cultures in the flow cell was performed with the bacterial suspension ($OD_{600nm}$ 0.1 in LB, 50 mL) at a flow rate of 2.5 mL $min^{-1}$. Thereafter, sterile nutrient medium (10% LB) was flowed at 2.0 mL $min^{-1}$ (cross-flow rate of 0.44 cm $min^{-1}$) through the flow cell for 36 h.

SEM Analysis: Immediately after the biofilm experiment, the samples were washed with 0.9% sterile saline solution, fixed with 2% paraformaldehyde and 2.5% glutaraldehyde in 0.2 M Sorenson's buffer (pH 7.2), and stored for 3 h. Afterward, the samples were carefully dehydrated

by immersion in a series of water/ethanol solutions (50, 70, 80, 90, and 100% ethanol), and samples were stored in a desiccator until SEM analysis. The high-resolution SEM (FEI Quanta 400 ESEM operating at 5 kV) images were recorded after gold sputter coating.

**12. Biofilm growth analysis using confocal laser scanning microscopy (CLSM):**

A two-channel flow-through system with internal recirculation mimicking the flow conditions of a spiral-wound module, operating in continuous mode at a linear flow velocity of about 0.16 m/s as described detail in our previous work.[2,3] Briefly, the samples (2 cm x 1 cm) PES (control), PES-LIG, Ag-PES, and Ag@LIG were placed in the flow cells with surfaces facing upwards. The feed consisted of sterile LB medium diluted in place (1:250) with distilled water to a total organic carbon concentration of approx. 20 mg/L. The system was inoculated with *P. aeruginosa* to a concentration of $10^4$ CFU/mL and the experiment was performed for a duration of 48 h. At the end of each experiment all the specimens were removed, gently washed with a sterile saline solution, and taken for visualization and analysis by CLSM. Images were taken from at least three random areas on the surface of duplicate samples and the images presented were representative of the average. The results are reported as number of bacterial cells attached per 100 μm$^2$. *Confocal & image analysis:* CLSM imaging was performed using a Carl Zeiss CLSM (LSM 510 META) with a × 40 oil-dipping objective. To visualize bacterial viability and attachment, a dead/live fluorescence kit (5 μM Syto 9 and 30 μM propidium iodide) was used and image analysis for biofilm quantification and calculation of the theoretical number of bacteria (*TNb*) within the 3-D structure of the biofilm (Imaris 7.6.5, Bitplane) were performed as previously reported.[2,3] All z-sections were processed and flattened into a single overlaying layer using Image J. For each analysis, the optimal threshold value was used. The size of each image processed was 500 × 500 μm.

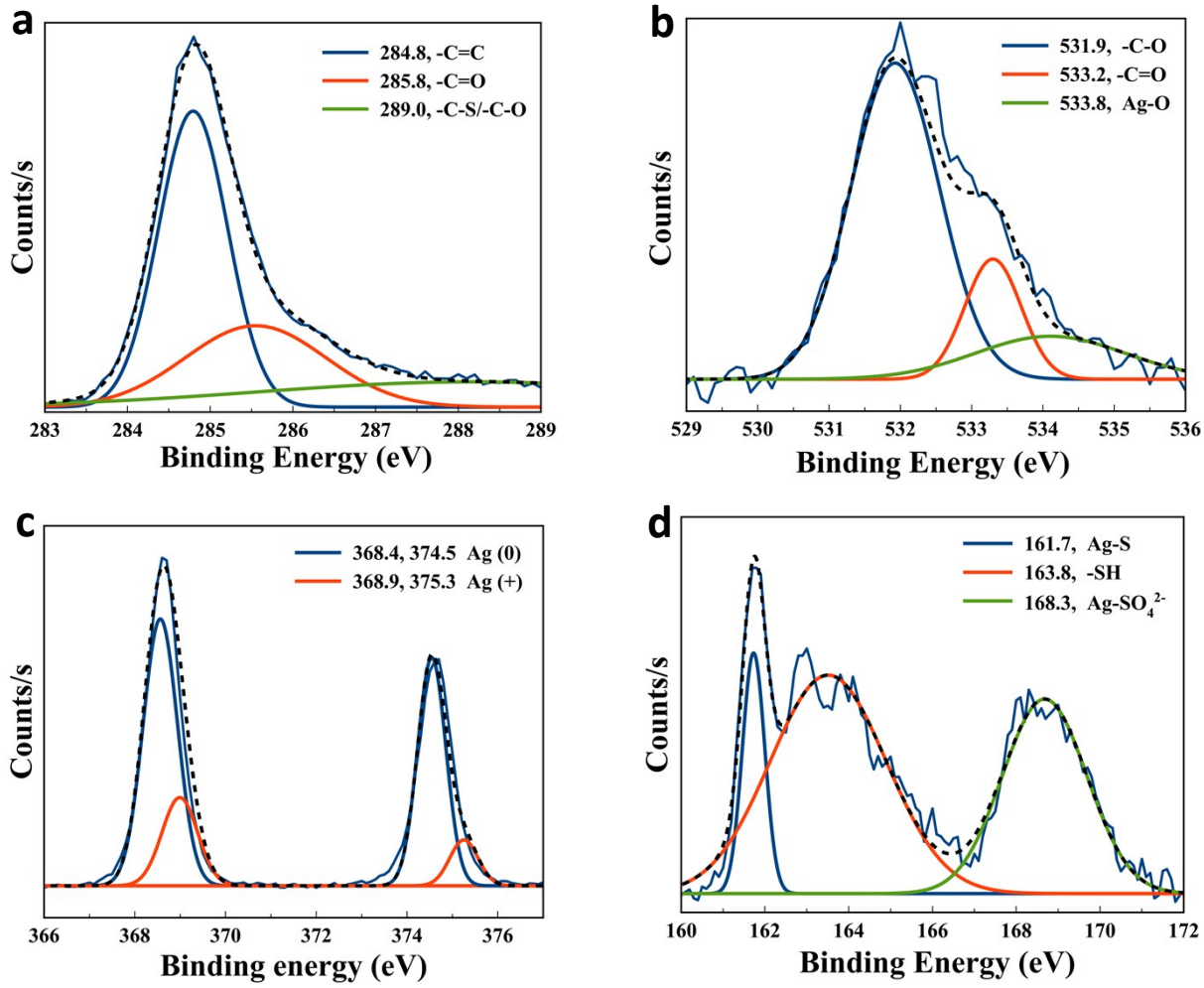
**13. Antibacterial Activity:**

Bacterial growth inhibition tests were performed[4] to check the viability of *P. aeruginosa* bacteria in the presence of Ag@LIG sheets. *P. aeruginosa* PAO1 WT strain that was grown overnight ($OD_{600}$ of 1.193) was inoculated (10 $\mu$L) into autoclaved 50 mL LB BACTO™ (10 g/L Tryptone, 5 g/L yeast extract, 5 g/L NaCl) in 250 mL autoclaved Erlenmeyers. Ag@LIG sheets of variable surface areas were added and incubated in the Erlenmeyers. The bacteria were incubated for 24 h at 37°C±1.0 ºC at 180 rpm. A microplate reader (TECAN, infinite M200) was used and absorbances were measured ($OD_{600}$) using 200 µL of the incubated medium.

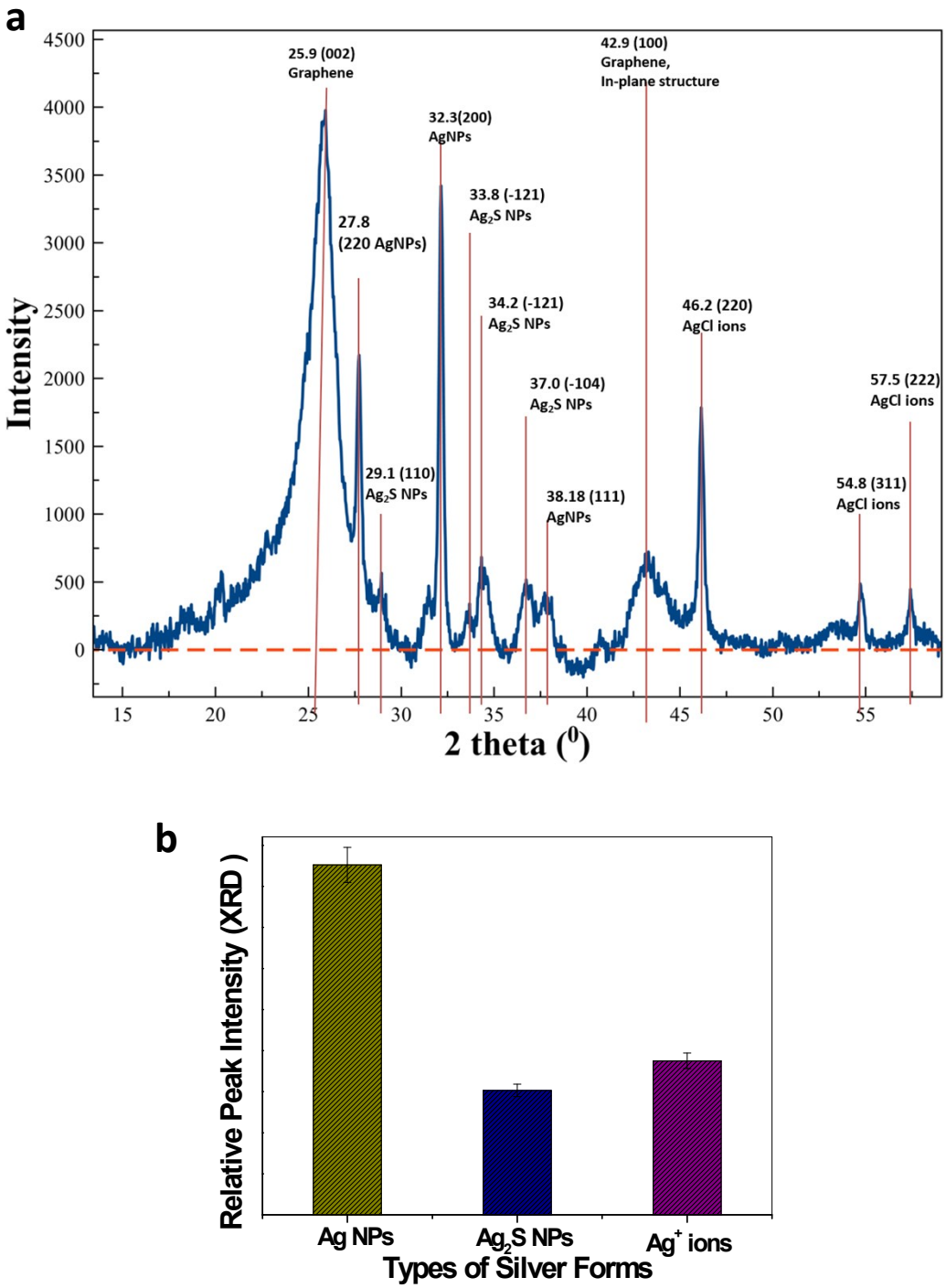
**14. Contact Killing Assay:**

This method was performed similarly as previously described.[4] Briefly, a fresh single colony of *P. aeruginosa* (PAO1) was inoculated in 10 mL of LB broth and incubated overnight at 30°C on a shaker at 150 rpm. The resulting culture was re-diluted in 5 mL LB broth (a 1:100 ratio) and was allowed to grow for 6 h to the exponential growth phase ($OD_{600} \approx 0.4$). The cells were diluted with a sterile PBS buffer solution (pH 7.4) to ~4000 CFU/mL. Ag@LIG sheets (2 cm × 1 cm) were taped with a double-sided adhesive tape to a sterile glass slide and placed inside a sterile Petri dish. 100 µL of the diluted bacterial culture was placed on top of the Ag@LIG sheets surface and evenly spread on it by covering it with a microscope coverslip. The Ag@LIG sheets were then incubated for 3 h at room temperature, the coverslips were gently removed, and the LIG sheets, together with the coverslips, were washed with 0.5 mL of sterile PBS by continuous aspiration. The wash solution was collected, and PBS was added to make a total volume of 1 mL. The plate-count method was used to quantify the viable CFUs with sterile LB agar incubated for 24 h at 30°C.

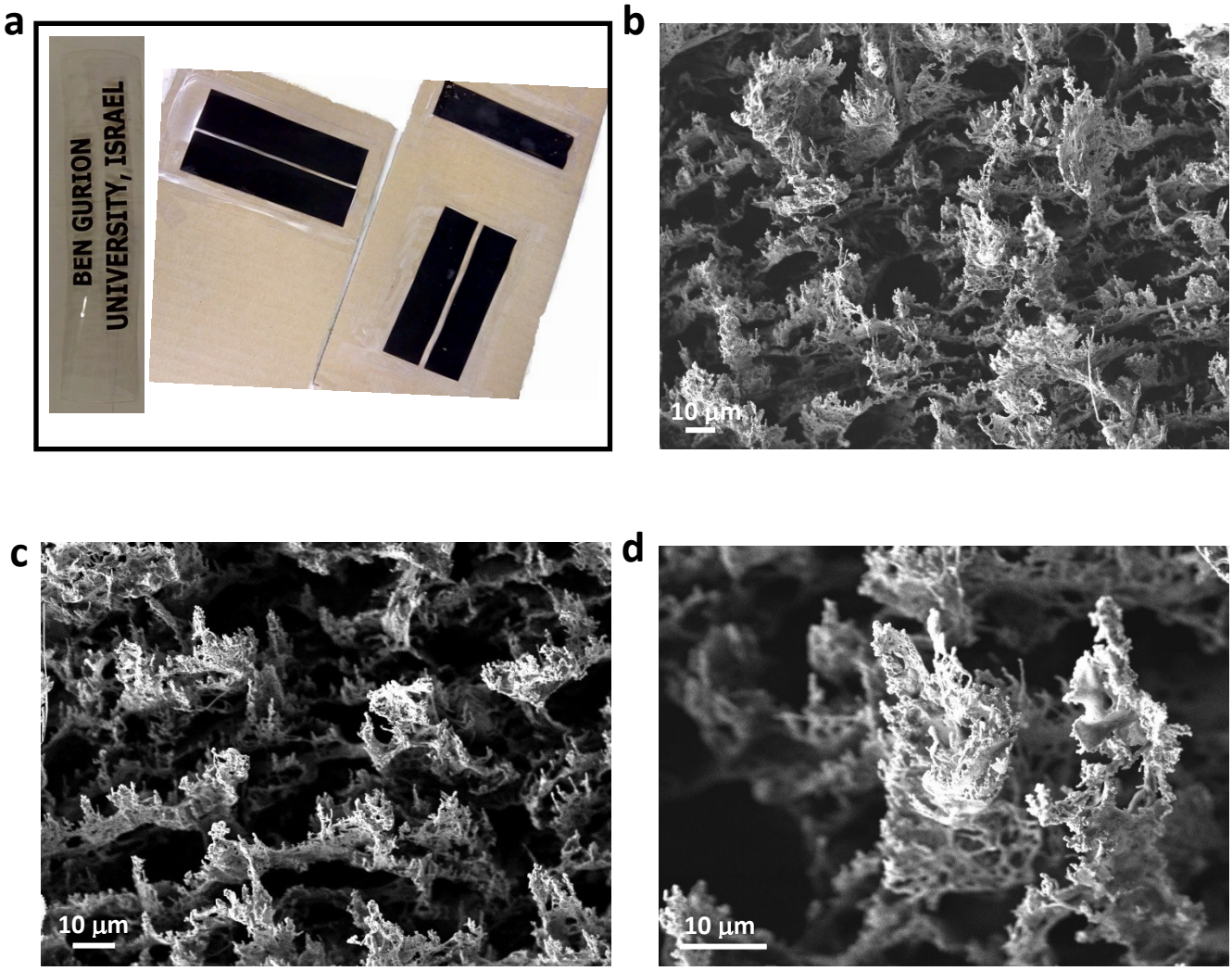
# B. Supplementary Figures (S1-S11)



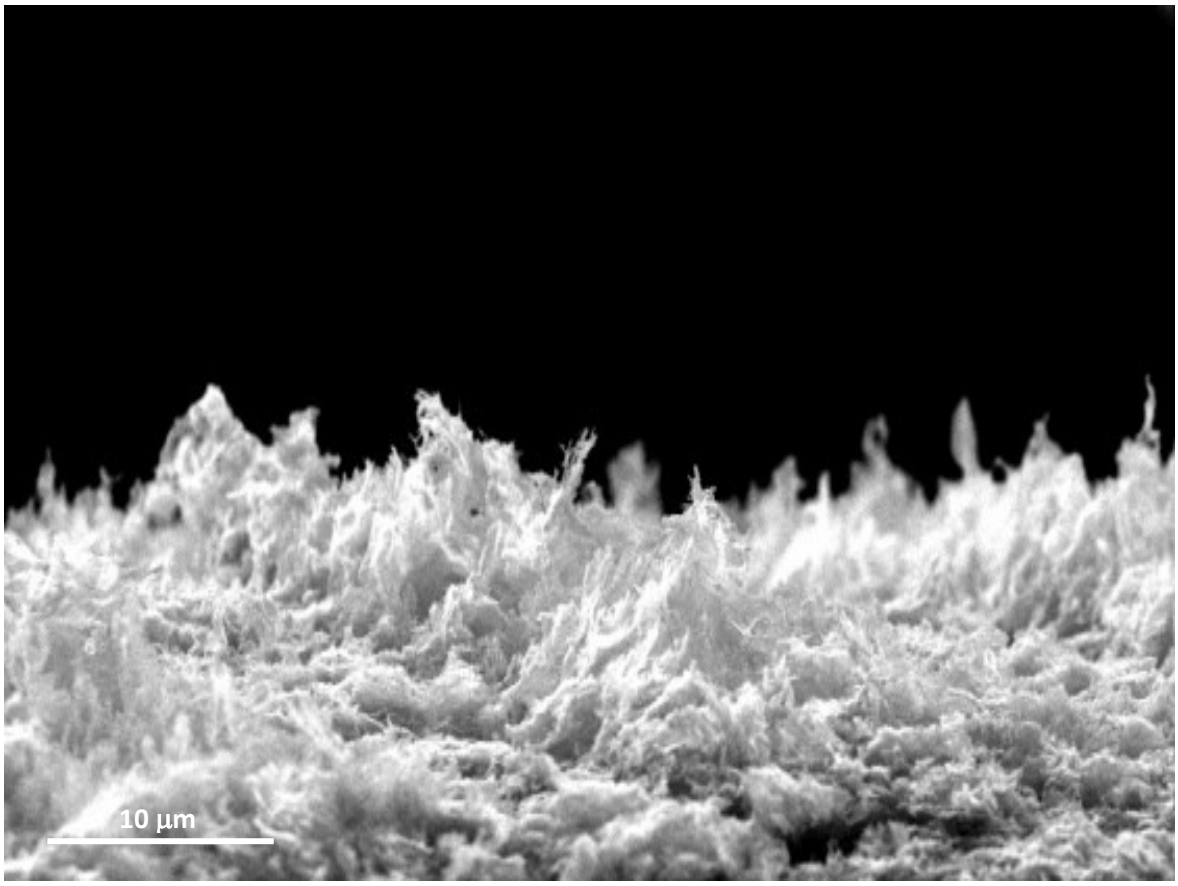

**Supplementary figure S1:** (a-d) Narrow spectrum high-resolution XPS measurements of Ag@LIG (a) C1s deconvolution (b) O1s deconvolution (c) Ag3d deconvolution and (d) S2p deconvolution. The presence of silver nanoparticles in the graphitic surface confirmed by the Ag3d signals observed in spectra (c).



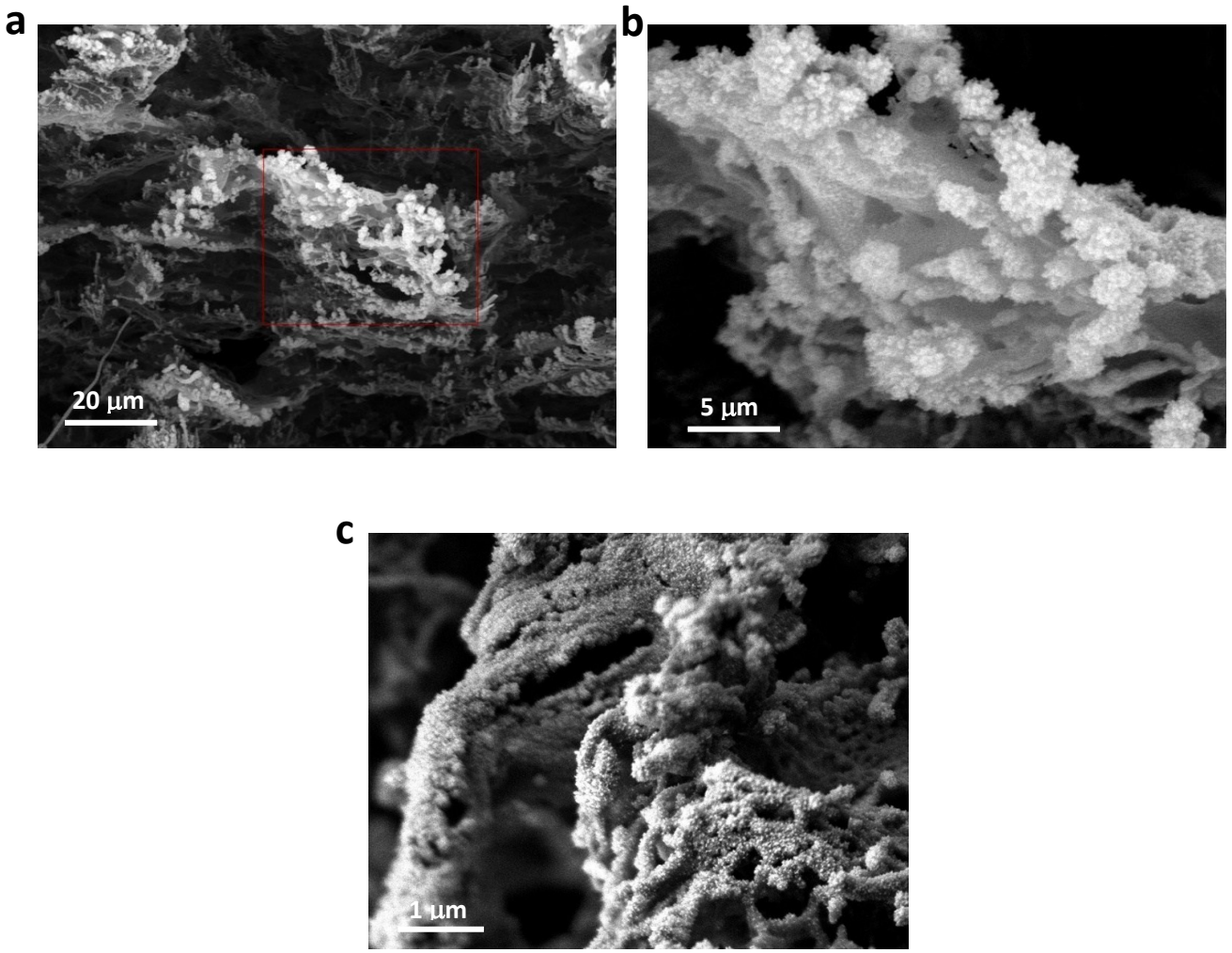

**Supplementary figure S2:** (a) Full range XRD spectrum of Ag@LIG. The X-ray diffraction (XRD) pattern of Ag@LIG showed peaks at 25.9° (2θ) for the 002 plane[5] and a second peak at 42.9° (2θ) for 100 plane, and confirms the presence of graphitic structures. The peaks at 27.8° (220), 32.3° (200), 38.18° (111) are due to the silver nanoparticles, 29.1° (110), 33.8° (-121), 34.2° (-121), 37.0° (-104) for the $Ag_2S$ nanoparticles and 46.2° (220), 54.8° (311), 57.5° (222) for the AgCl ions/nanoparticles and (b) XRD peak analysis shows 75.5% silver nanoparticles and 24.5% silver ions.



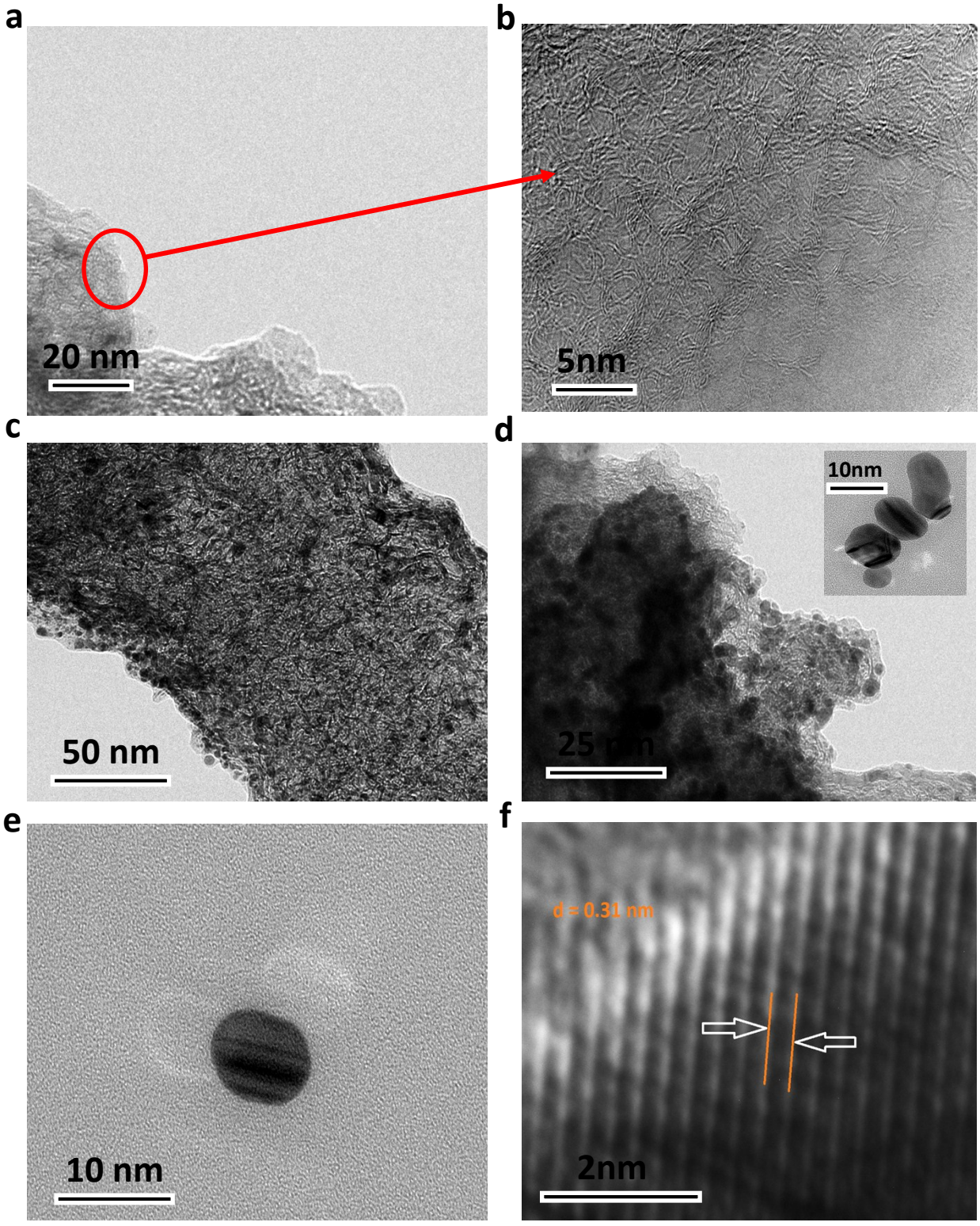

**Supplementary figure S3:** (a) Sample photographs of Ag@LIG made on Ag-PES polymer substrate. Scanning electron microscopy images (b-d) of synthesized Ag@LIG, which show the rough and fibrous brush shape structure.



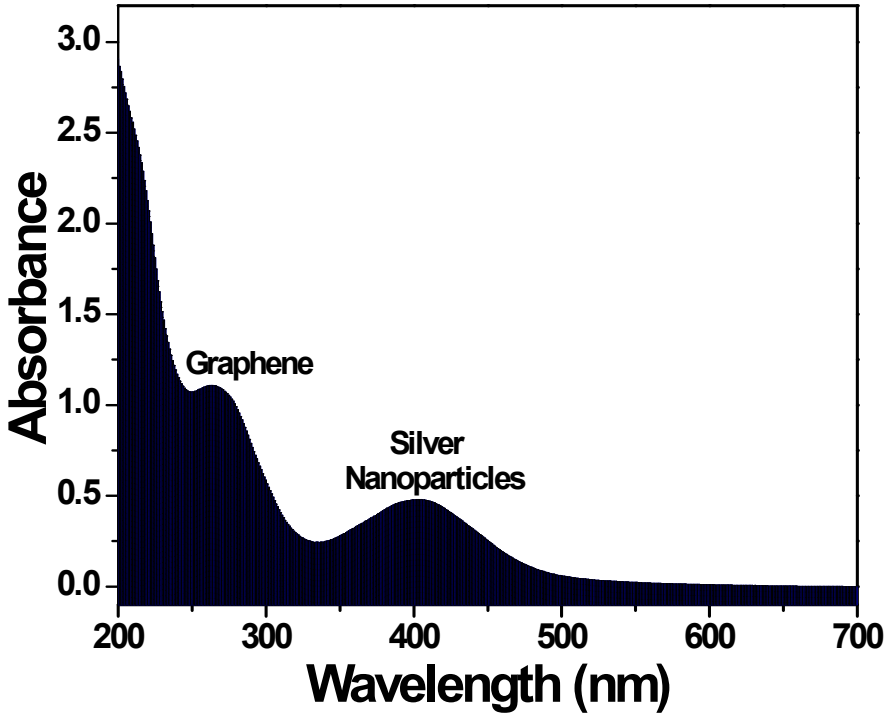

**Supplementary figure S4:** Scanning electron microscopy image shows the cross-section view of the synthesized Ag@LIG. The average thickness of the Ag@LIG was measured to be ~30 μm.



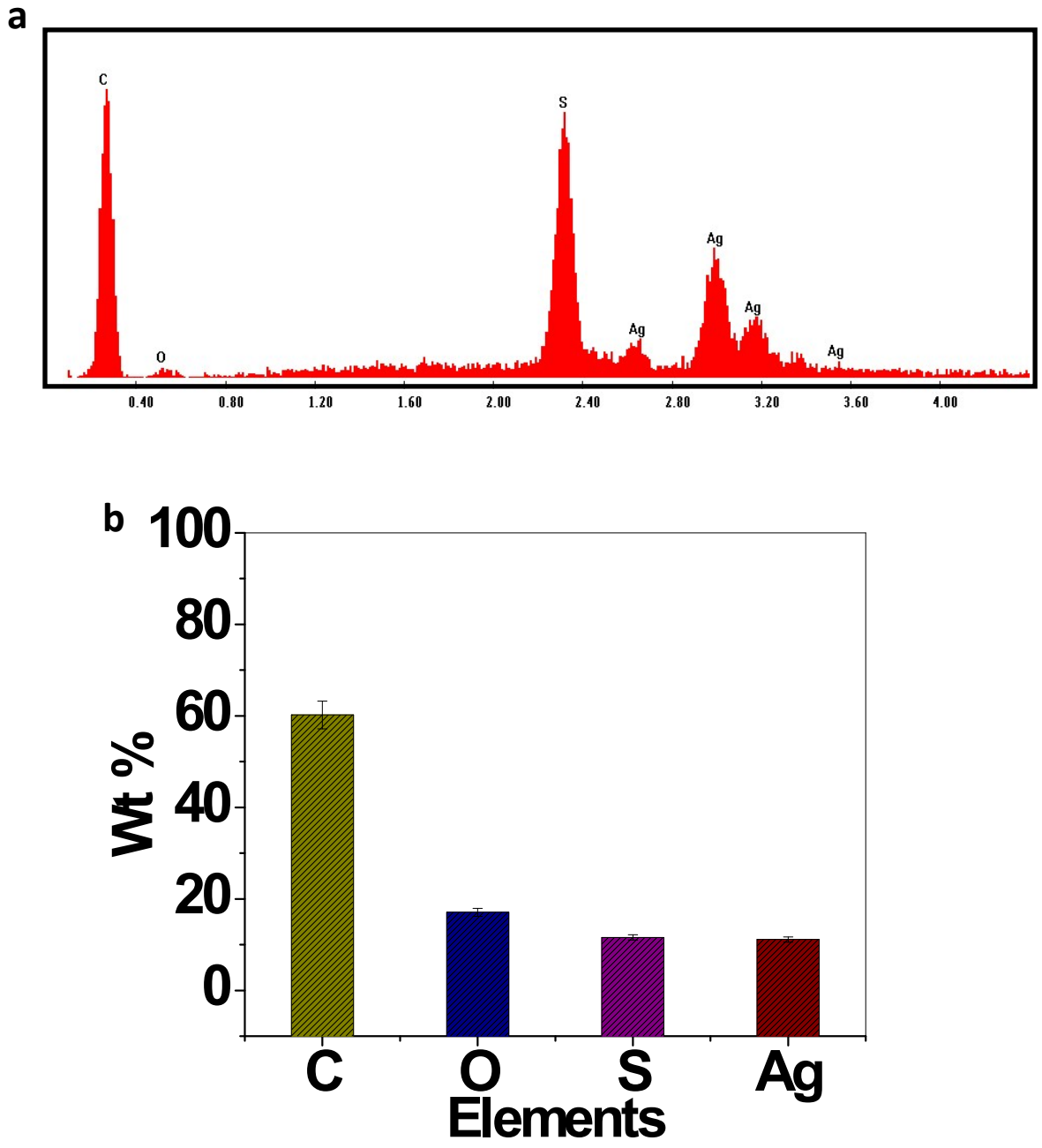

**Supplementary figure S5:** Scanning electron microscopy images of Ag@LIG showing silver nanoparticles embedded into the LIG surface. (a-b) low magnification and (c) SEM image showing silver nanoparticles estimated to be ~5-10 nm.



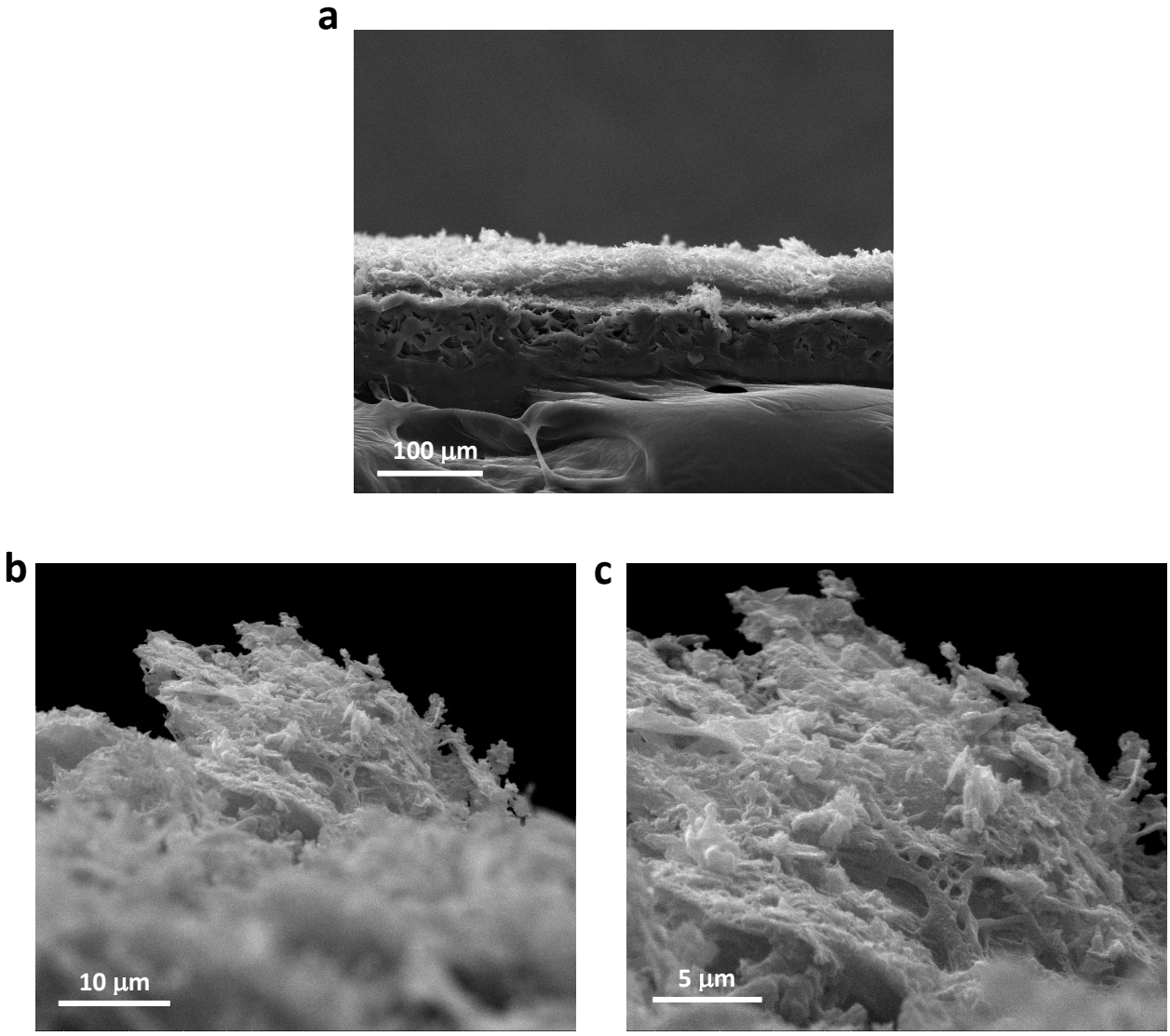

**Supplementary figure S6:** (a) TEM image of LIG only; (b) HRTEM of LIG; (c, d) TEM image of Ag@LIG showing the silver nanoparticles embedded in the graphene structure; d (inset) showing the silver nanoparticle morphology; (e) separated single silver nanoparticle via centrifugation; (f) the set of lattice fringes with spacing of 0.31 nm, which corresponds to the largest interplanar distance of $Ag^0$.



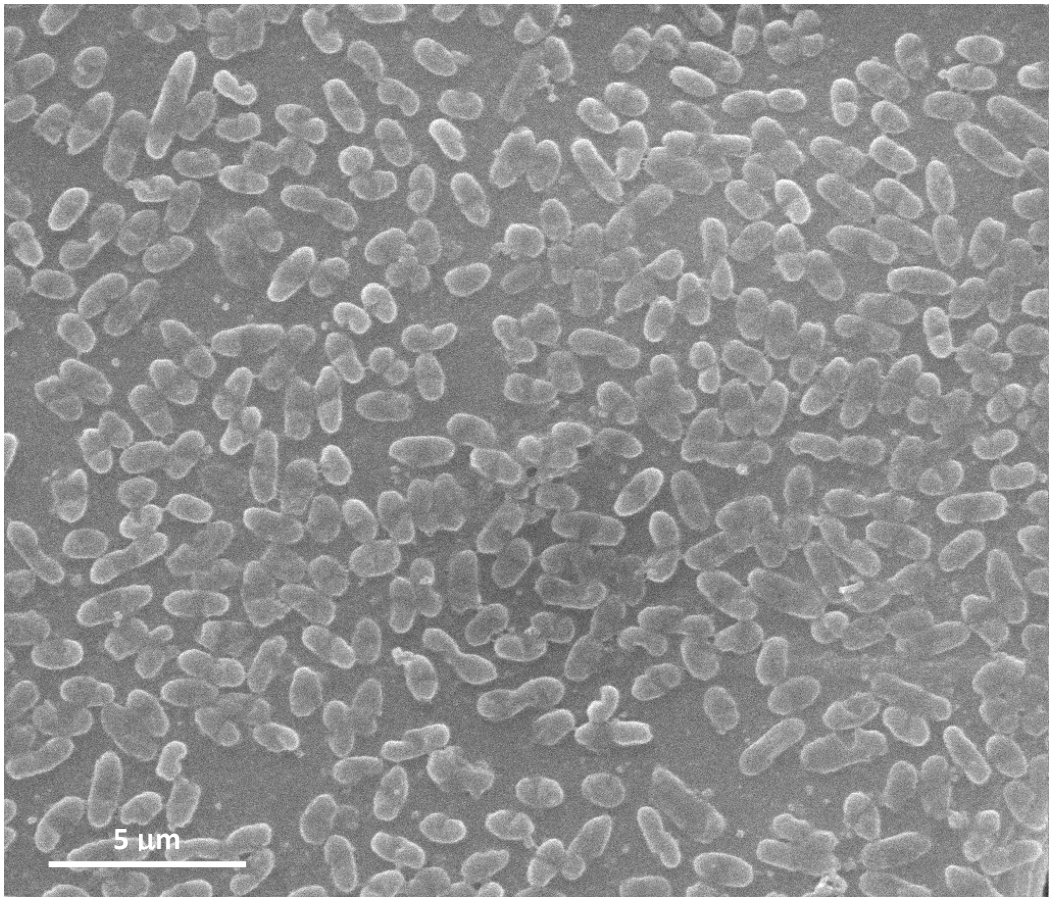

**Supplementary figure S7:** UV-Vis absorption spectra of Ag@LIG powder dissolved in DI also confirms the presence of silver nanoparticles on the LIG surface.



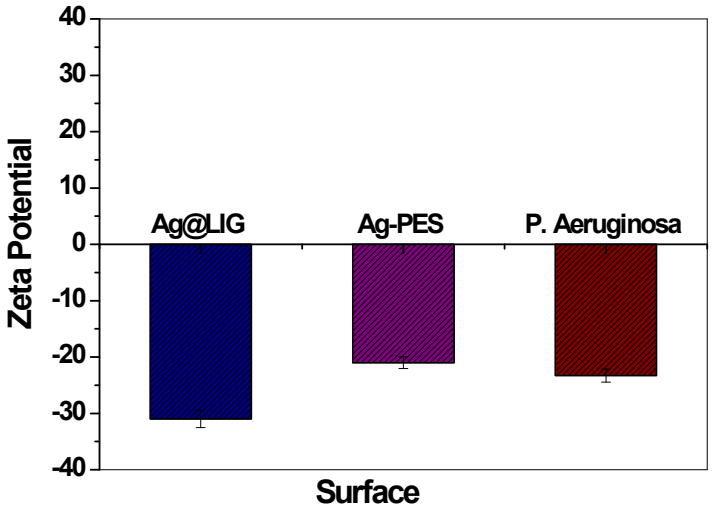

**Supplementary figure S8:** (a) EDS elemental analysis for the different constituents (%) for Ag@LIG. (b) % of carbon (C), oxygen (O), sulfur (S), silver (Ag). Scanning was performed using the areas that visibly contained silver nanoparticles.




**Supplementary figure S9:** Cross sectional SEM images of Ag@LIG after performing the antibiofouling activity test. (a) low magnification, (b-c) higher magnification.




**Supplementary figure S10:** Bacterial growth on the Ag-PES polymer surface.




**Supplementary figure S11:** Zeta potential of Ag-PES and Ag@LIG compared to *P. aeruginosa.*

# C. Supplementary table

| S. No. | Element | Peak BE | FWHM eV | Atomic % |
|---|---|---|---|---|
| 1 | $C_{1s}$ | 284.82 | 1.12 | 60.20 |
| 2 | $O_{1s}$ | 532.02 | 2.14 | 17.07 |
| 3 | $S_{2p}$ | 161.77 | 0.89 | 11.59 |
| 4 | $Ag_{3d5}$ | 368.62 | 0.85 | 11.13 |

**Supplementary table T1:** Atomic % of the elements and their respective binding energy obtained from the narrow spectrum analysis by X-ray photoelectron spectroscopy (XPS) of Ag@LIG.

## References:


1. S. P. Singh, Y. Li, J. Zhang, J. M. Tour and C. J. Arnusch, *ACS Nano*, 2018, **12**, 289-297.
2. C. Thamaraiselvan, A. Ronen, S. Lerman, M. Balaish, Y. Ein-Eli and C. G. Dosoretz, *Water Research*, 2018, **129**, 143-153.
3. C. Thamaraiselvan, Y. Carmiel, G. Eliad, C. N. Sukenik, R. Semiat and C. G. Dosoretz, *Journal of Membrane Science*, 2019, **573**, 511-519.
4. N. Shtreimer Kandiyote, G. Mohanraj, C. Mao, R. Kasher and C. J. Arnusch, *Langmuir*, 2018, **34**, 11147-11155.
5. L. Xia, D. Li, J. Long, F. Huang, L. Yang, Y. Guo, Z. Jia, J. Xiao and H. Liu, *Carbon*, 2019, **145**, 47-52.