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Supporting Information

On the Antibacterial Mechanism of Graphene Oxide (GO) Langmuir-Blodgett Films

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Experimental Section

Materials: All reagents used in the synthesis of graphene oxide (GO) were purchased from Sigma-Aldrich and were used as received.

Characterization: UV-Vis spectra of the GO solutions and films were recorded in Agilent 8453 spectrometer. For ATR-FTIR, the spectra were collected on a Digital FTS 7000 equipped with a HgCdTe detector from 4000 cm^{-1} to 600 cm^{-1} wavenumbers. All spectra were taken with a nominal spectral resolution of 4 cm^{-1} in absorbance mode. The measurements were obtained under ambient and dry conditions. On the other hand, XPS measurements were conducted on a PHI 5700 x-ray photoelectron spectrometer equipped with monochromatic Al Kα x-ray source ($h\nu$ = 1486.7 eV) incident at 90° relative to the axis of a hemispherical energy analyzer. Low and high resolution spectra were collected with pass energies of 23.5 and 187.85 eV, respectively, a photoelectron take off angle of 45° from the surface, and an analyzer spot diameter of 1.1 mm.

AFM imaging was performed under ambient conditions with PicoSPM II (Picoplus, Molecular Imaging – Agilent Technologies) with a scan rate of 1.0 to 1.5 line/s. Commercially available tapping mode tips (TAP300, Silicon AFM Probes, Ted Pella, Inc.) were used on cantilevers with a resonance frequency in the range of 290-410 kHz. All AFM topographic images (AAC tapping mode) were processed using Gwyddion 2.19 software.

SEM analysis was performed in field emission scanning electron microscopy (FE-SEM) using a JSM 6330F JEOL instrument operating at 15 kV.

Substrate Treatment: Poly(ethylene terephthalate) (PET) substrate was sonicated thrice in isopropanol for 5 min each. Afterwhich, the films were dried with compressed nitrogen gas. The PET substrates were then treated by pure oxygen plasma for 15 seconds to make the surface hydrophilic. [1, 2] A parallel experiment was performed on quartz substrates for spectroscopy purposes.

Synthesis of GO: The synthesis of GO was carried out by chemical exfoliation of graphite using modified Hummers and Offeman's method [3]. Briefly, 3 g of graphite was stirred with 3 g of $NaNO_3$ in 138 mL H_2SO_4 in an ice bath. Then, 18 g of $KMnO_4$ was slowly added and shortly after, the reaction mixture was transferred to 35 ± 5 ^{0}C oil bath for an hour followed by the

addition of 240 mL H_2O. The reaction mixture was stirred in a 90 ± 5 ^{0}C oil bath for 30 minutes, followed by the addition of 600mL H_20 and 18mL H_2O_2. After series of washing with H_2O, the final sediment was re-dispersed in methanol and ultrasonicated for 30 min to fully exfoliate the GO. After which, the methanol was evaporated and the GO was stored in the vacuum oven until further use.

Langmuir-Blodgett Assembly: A KSV-2000 system (KSV Instruments, Finland) was used to investigate the surface behavior of GO and to control the monolayer formation. Prior to spreading the GO on the water subphase, the LB trough (width 150 mm, area 78 000 mm^2) and the Teflon-coated barriers were cleaned thoroughly with chloroform and ethanol. Afterwhich, millipore water (resistivity >18 MΩ·cm, 20 °C) was poured on the trough. A total of 2 mL GO solution (1:5 water:methanol mixture to a final concentration of 1mg/mL) was slowly spread onto the air-water interface using a 50 μL glass syringe. The material was allowed to equilibrate for 15 minutes and was then compressed at 3 mm/min barrier speed. LB transfer of the film was performed upstroke at a pulling speed of 1 mm/min. Repeated deposition was performed to obtain 1, 2 and 3 layers of GO film. After every deposition, the films were dried for 12 hours in the vacuum oven operating at room-temperature.

Antibacterial Assay: GO-LB films were individually placed in a well-plate (Falcon). To each well was added 1.0 mL of bacterial culture and then incubated at 37 °C for 2 h. The samples were then removed and, immediately prior to viewing, were stained with 3mL of L 7007 Live-dead stain solution for 10 minutes from Molecular Probes (Leiden, The Netherlands) containing a green fluorescent dye (Syto 9) marking viable bacterial cells and red fluorescence dye (PI) for detection of dead cells. The surfaces were placed in microscope slides, covered with a cover slip and imaged using BX 51 Olympus Fluorescent Microscope equipped with a DP72 digital camera under 100x objective. FITC filter for green fluorescence from SYTO 9 in all bacteria and a TRITC filter for red fluorescence from PI in membrane-compromised bacteria were used. All images were acquired and analyzed using cell Sens Dimension software (Olympus). Percent inactivation was expressed as the percent of the ratio of the total number of dead cells to the total number of bacteria attached.

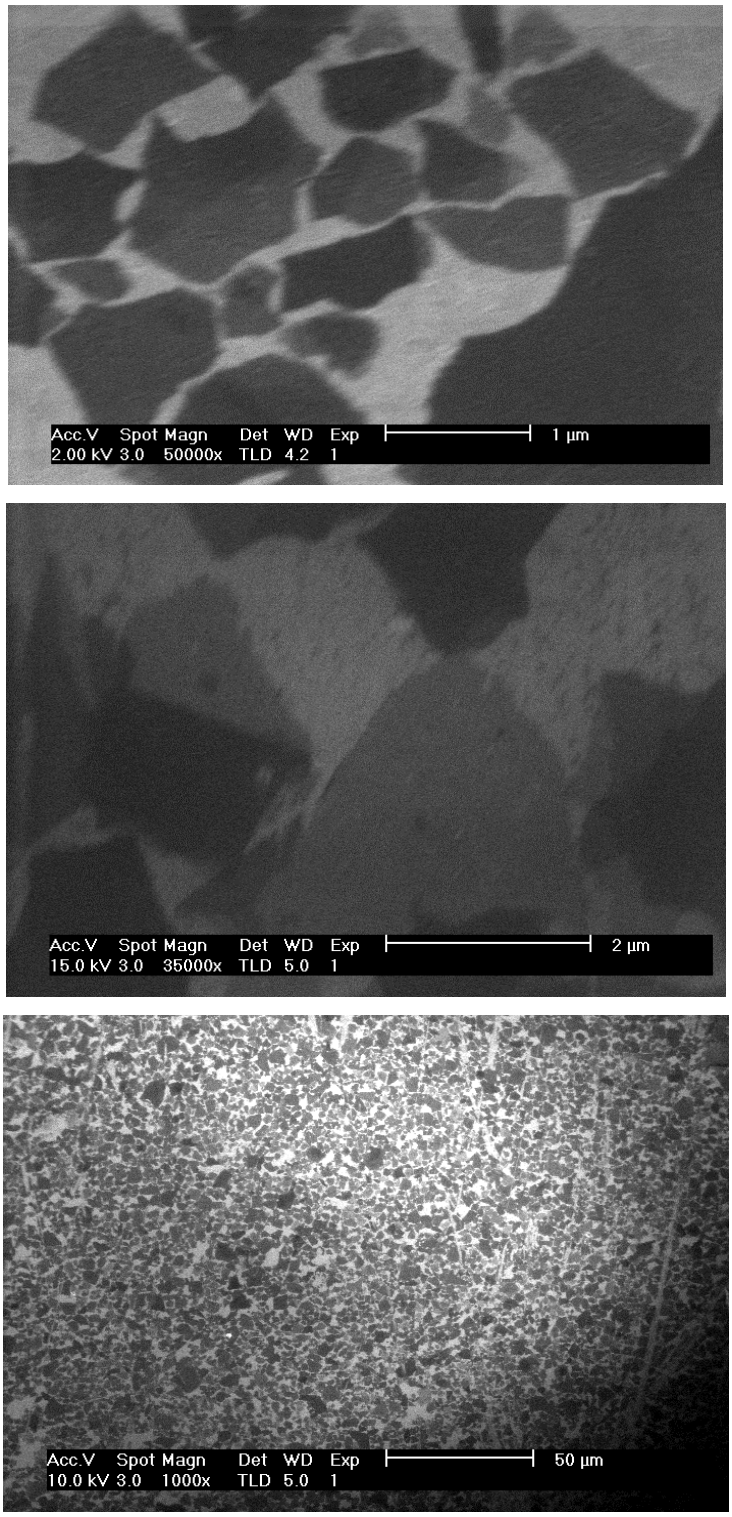

Fig. S1. SEM images of GO film deposited by LB. Note that the GO sheets are oriented flat on the surface of the substrate.

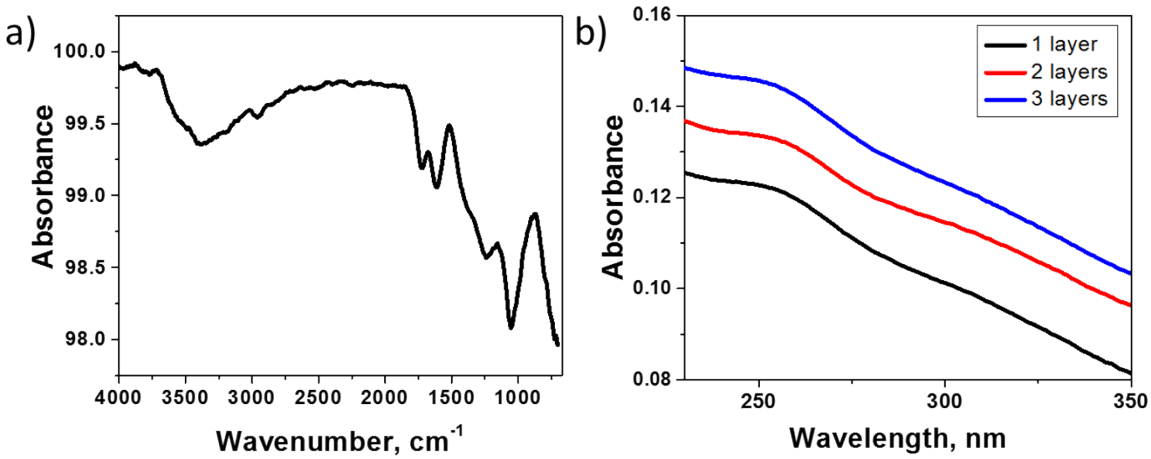

Fig. S2. (a) ATR-FTIR spectrum of GO. The identity of the as-synthesized GO was further confirmed by ATR-FTIR spectroscopy, revealing characteristic bands at 1051 cm^{-1} (C-O stretching vibrations), 1239 cm^{-1} (C-OH stretching vibrations), 1608 cm^{-1} (skeletal vibrations of the unoxidized graphitic domains), 1723 cm^{-1} (C=O stretching from carbonyl groups) and a broad band centered at 3368 cm^{-1} (O-H stretching vibrations).[4] **(b)** Absorption spectra of 1, 2 and 3 layers of GO deposited by LB. The increase in the absorbance suggests more deposited materials as the number of LB layers is increased.

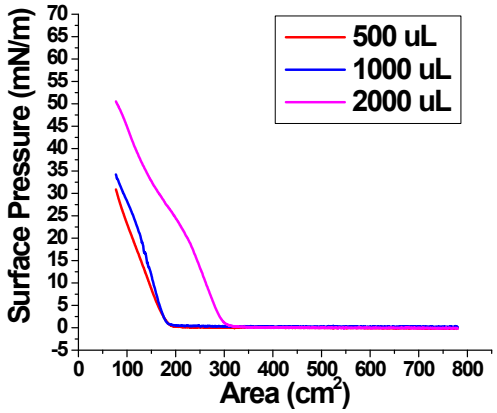

Fig S3. π-area isotherm of 500 µL, 1000 µL and 2000 µL GO dispersed in 1:5 water: methanol mixture to a final concentration of 1 mg/mL. 2 mL of sample loading shows a well-define classic π-area isotherm (smooth gas to liquid to solid phase transitions) upon lateral compression.

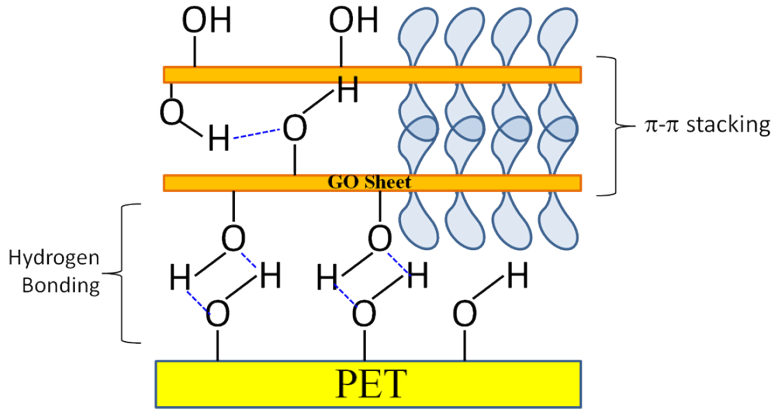

Fig. S5. Proposed explanation for the observed stability of GO sheets on PET substrates. The stability may be due to the strong hydrogen bonding of GO to the hydroxy groups of PET film. Secondly, as the number of layers was increased by repeated deposition, the π-π stacking of the GO sheets may have contributed to the observed stability.

References:

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