Supporting Information

Thin-Film Composite Polyamide Membranes Functionalized with Biocidal Graphene Oxide Nanosheets

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MATERIALS AND METHODS

Materials and Chemicals. *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 98%), ethylenediamine (ED, BioXtra), N-hydroxysuccinimide (NHS, 98%), polysulfone beads (PSf, 22 kDa), HEPES (>99.5%), MES monohydrate (>99.0%, BioXtra), 1-methyl-2-pyrrolidinone (NMP, anhydrous, 99.5%), 1,3-phenylenediamine (MPD, >99%), and 1,3,5-benzenetricarbonyl trichloride (TMC, 98%) were obtained from Sigma-Aldrich (St. Louis, MO). Graphite powder (99%, -300 mesh, Alfa Aesar) was obtained from VWR (Radnor, PA). Isopar-G, a proprietary non-polar organic solvent, was obtained from Univar (Redmond, WA). Polyester non-woven fabric (PET, Grade 3249) was obtained from Ahlstrom (Helsinki, Finland). Sodium chloride (NaCl, crystals, ACS reagent) was obtained from J.T. Baker (Phillipsburg, NJ). The LIVE/DEAD BacLight bacterial viability kit, containing propidium iodide (PI) and Syto 9, was obtained from Invitrogen (Molecular Probes, Carlsbad, CA). Unless specified, all chemicals were dissolved in deionized (DI) water obtained from a Milli-Q ultrapure water purification system (Millipore, Billerica, MA).

Graphene oxide synthesis. Graphene oxide was produced by chemical exfoliation from graphite powder according to a method adapted from.[1] Briefly, 1.5 g of graphite was added to 200 mL of a 9:1 mixture of H_2SO_4:H_3PO_4 and bath sonicated (26 W L^{-1}, FS60 Ultrasonic Cleaner, Fisher Scientific Co., Pittsburgh, PA) for 5 min. The reaction vessel was then placed in an ice bath and 9 g of $KMnO_4$ was added to the mixture under constant stirring. The solution was then slowly heated to 50^{o}C and stirred for 12 h. Special care was taken in this step to keep the temperature at 50^{o}C, since Mn_2O_7, formed when $KMnO_4$ is added to concentrated sulfuric acid, can detonate at temperatures higher than 55^{o}C. Next, the reaction was allowed to cool to room temperature overnight and poured on DI ice (~400 mL) with 3 mL of H_2O_2. The reaction was diluted with DI water to 2 L, passed through a 44 μm standard metal testing sieve (W.S. Tyler) and the solid fraction of the filtrate collected on a 5 μm PTFE filter by vacuum filtration. The solid material was then washed in succession with 100 mL of DI water (2x), 100 mL of 1:10 HCl (2x), 100 mL of DI water (2x) and 100 mL of ethanol (2x). For each washing step, the mixture was centrifuged (4000 g for 4 h) and the supernatant decanted away. Finally, the material was purified by dialysis for 72 h. The material was then filtered on a 0.45 μm PTFE filter and vacuum dried. Before use, graphene oxide was suspended in DI, probe-sonicated for 10 min (6.5

kW L^{-1}, Misonix 3000, Misonix Inc., Farmingdale, NY), and centrifuged for 30 min at 2000 g to remove non-exfoliated material.

Graphene Oxide Characterization. Raman spectra of graphene oxide were acquired using a Horiba Jobin Yvon HR-800 Raman spectrometer with 532 nm laser excitation. X-ray photoelectron spectroscopy (XPS) analysis was performed on a ThermoScientific ESCALAB 250 with a monochromatized AI X-ray source (150 eV for survey scans, 20 eV for composition scans, 500 μm spot size) at the University of Oregon CAMCOR facility. Atomic Force Microscopy images were taken in tapping mode with a Bruker Multimode (Digital Instruments, Plainview, NY) AFM equipped with a Tap300Al-G cantilever (BudgetSensors). For AFM measurements, 3 μL of a graphene oxide suspension (50 μg mL^{-1}) was drop casted on a 1 cm x 1 cm silicon wafer cleaned under a 20 min UV-ozone treatment (UV/Ozone ProCleaner, BioForce Nanosciences, Ames, IA).

Thin-Film Composite Membrane fabrication. TFC membranes were prepared by interfacial polymerization of polyamide onto a hand-cast PSf support layer, as described in a previous publication.[2] PSf (12% wt) was stirred in NMP at room temperature until completely dissolved and then stored in a desiccator overnight. PSf was first formed by phase inversion on the PET fabric. The PET fabric was attached to a clean glass plate using water-proof adhesive tape (Fisher) and then wet with NMP. The excess solvent was then removed with a Kimwipe (Kimberly-Clark, Roswell, GA). The PSf solution (~10 mL) was poured on one side of the wet PET fabric and then drawn over the fabric using a casting knife (Gardco, Pompano Beach, FL) with the gate height fixed at 250 μm (~10 mils). The plate was immediately immersed in a DI precipitation bath at room temperature to initiate the phase separation. The support membrane was allowed to sit in the precipitation bath for 10 min and then stored in DI until polyamide formation.

The polyamide active layer was formed on top of the PSf support via interfacial polymerization, as described in a previous publication.[2] Briefly, the PSf support was first immersed for 2 min in an aqueous MPD solution (3.4% wt). The excess solution on the membrane surface was removed with an air knife. The MPD-saturated membrane was then immersed in a 0.15% wt TMC solution in Isopar-G for 1 min to form the polyamide active layer. The membrane was cured in DI at 95 °C for 2 min, dipped in a 200 ppm NaOCl aqueous solution

for 2 min and then dipped in a 1000 ppm $NaHSO_3$ aqueous solution for 30 s. The membrane was finally cured at 95 °C for 120 s. The final TFC membrane was rinsed thoroughly and stored in DI at 4 °C.

Membrane Functionalization. TFC membranes were functionalized with graphene oxide by a method adapted from Tiraferri and co-workers.[3] Membranes were placed on a glass plate and covered with a frame leaving only the active (top) surface exposed. The membranes and frames were sealed with clips to prevent any leakage and placed on an orbital shaker at 70 rpm at room temperature for the whole functionalization process.

A solution of 4 mM EDC, 10 mM NHS, 0.5 M NaCl in 10 mM MES buffer was prepared and the pH was adjusted to 5 with HCl/NaOH before contacting it with the membrane surface. This step converted the native carboxyl groups of the active layer into amine-reactive esters. After 1 h the solution was removed, the membrane surface was washed twice with DI and then contacted 30 min with a solution of 10 mM ED, 0.15 mM NaCl in 10 mM HEPES buffer at pH 7.5. ED was attached to the active layer by the formation of amide bounds with the activated esters. The membrane surface was then washed twice with DI to remove unlinked ED.

Graphene oxide powder (10 mg) was dispersed in 10 mL of 10 mM MES buffer at pH 6. Graphene oxide was probe sonicated for 10 min (6.5 kW L^{-1}, Misonix 3000) to exfoliate the graphene oxide sheets and form a stable suspension. Graphene oxide suspensions were centrifuged for 30 min at 2000 g to remove non-exfoliated graphene oxide. The supernatant was diluted in 40 mL of 10 mM MES buffer (pH 6) 15 min before contacting the graphene oxide suspension with the ED-functionalized TFC membrane, 2 mM EDC and 5 mM NHS were added to the graphene oxide suspension to convert the carboxyl groups present on graphene oxide to amine-reactive esters. The addition of EDC and NHS lowered the pH of the solution to 5.5. After 15 min, the pH was raised to 7.2 and the graphene oxide suspension was contacted with the membrane at room temperature under constant agitation (70 rpm). The graphene oxide functionalized membranes were rinsed with DI and bath-sonicated (26 W L^{-1}, FS60 Ultrasonic Cleaner) for 2 min to remove unbound graphene oxide from the membrane surface. Membranes were stored in DI at 4 ^{o}C until use.

Membrane Characterization. The presence of graphene oxide on the membrane was verified by SEM observation and Raman spectroscopy. For SEM, a membrane coupon was dried

in a desiccator for at least 24 h and then coated with a 15 nm chromium layer. SEM micrographs were taken on a Hitachi SU-70 SEM. For Raman spectroscopy, dried membrane coupons were taped to a clean glass slide and Raman spectra were collected with a Horiba Jobin Yvon HR-800 Raman Microscope using a 532 nm laser excitation. Raman mapping was done using 25 measurements on a 200 x 250 μm grid. For individual Raman spectrum, care was taken to set the focal point of the laser on the top of the membrane surface. For Raman mapping measurements, an area where the focal plane was homogeneous on the whole membrane surface was selected to avoid artifacts due to change in the laser focus.

Intrinsic transport properties of Ctrl-TFC and GO-TFC were evaluated in a laboratory-scale 6-cell cross flow reverse osmosis unit. The effective membrane area was 20.02 cm^2, the crossflow velocity was fixed at 21.4 cm s^{-1}, and the temperature was constant at 25 ± 0.5 °C. The membranes were compacted with DI at 450 psi (31 bar) until the permeate flux reached a steady-state (compacted overnight). The pressure was then reduced to 400 psi (27.6 bar) and run until the permeate flux stabilized. Pure water flux, J_W, was calculated by dividing the volumetric permeate rate by the membrane area. From a 5 M stock solution, NaCl was added to reach a final concentration of 50 mM and salt rejection was measured using a calibrated conductivity meter (Oakton Instruments, Vernon Hills, IL).

Intrinsic water permeability, A, was determined by dividing the water flux by the applied pressure, $A=J_W/\Delta P$. Observed NaCl rejection, R, was determined from the difference in bulk feed (C_b) and permeate (C_p) salt concentrations, $R = 1 - C_p/C_b$. The rejection values for each sample are the average of three different measurements. The solute permeability coefficient, B, was determined from:

$$B = J_w^{RO}\frac{1-R}{R}\exp\left(-\frac{J_w^{RO}}{k}\right)$$

where k is the mass transfer coefficient of the crossflow cell.[4,5]

The hydrophilicity of the membrane surface was evaluated by measuring the contact angle for DI water using the sessile drop method (VCA Video Contact Angle System, AST Products, Billerica, MA). The left and right contact angles were obtained using the VCA Optima XE software. Membrane coupons were desiccator dried for at least 24 h before measurements. To

account for the variability due to hand-cast membranes, contact angles from a total of 50 random locations from 5 different membrane samples were collected.

Antimicrobial Activity of Graphene Oxide Functionalized Membranes. The antimicrobial activity of graphene oxide functionalized TFC membranes was evaluated by a plate counting method. Before evaluating a GO-TFC membrane for antimicrobial activity, the presence of graphene oxide on the membrane was confirmed by performing water contact angle measurements for each membrane. Membranes showing a contact angle higher than 65° were not used for antimicrobial activity assessment. Overnight cultures of *Escherichia coli* K12 (Coli Genetic Stock Center #7740) were grown in LB medium at 37 °C. The cultures were then diluted in fresh LB and grown until log phase (~2 h), verified by measuring the optical density at 600 nm. The bacterial cells were washed three times with fresh sterile 0.9% saline solution before being diluted to 10^7 CFU mL^{-1} in sterile 0.9% saline solution. For membrane exposure, coupons of 1.5 cm^2 were punched from Crl-TFC and GO-TFC membranes and placed in a plastic holder to expose only the active layer to the bacteria. Bacterial suspensions (0.5 mL per cm^2) were contacted with Ctrl-TFC and GO-TFC membranes for 1 h at room temperature. The excess bacterial suspension was then discarded and the membrane coupons were washed thoroughly with sterile 0.9% saline solution. The membrane coupons were placed in a 50 mL Falcon tube containing 10 mL of 0.9% saline solution and bath-sonicated (26 W L^{-1}, FS60 Ultrasonic Cleaner) for 7 min to remove the bacteria from the membrane surface. Bacteria were spread on LB agar plates and incubated overnight at 37 ^oC.

For SEM imaging of bacterial cells on the membrane surface, *E. coli* suspensions were exposed as described above. After contacting the cells with the membrane, the membrane coupons were washed with 0.9% sterile saline solution and fixed with Karnovsky's fixative (2% paraformaldehyde, 2.5% glutaraldehyde in 0.2 M Sorenson's buffer, pH 7.2) for 3 h. Samples were then dehydrated by a sequential immersion (10 min) in water:ethanol (50, 70, 80, 90, 100%) and ethanol:freon (50, 75, 100%), and left to dry overnight in a desiccator at room temperature. Samples were then sputter-coated with chromium and imaged by SEM.

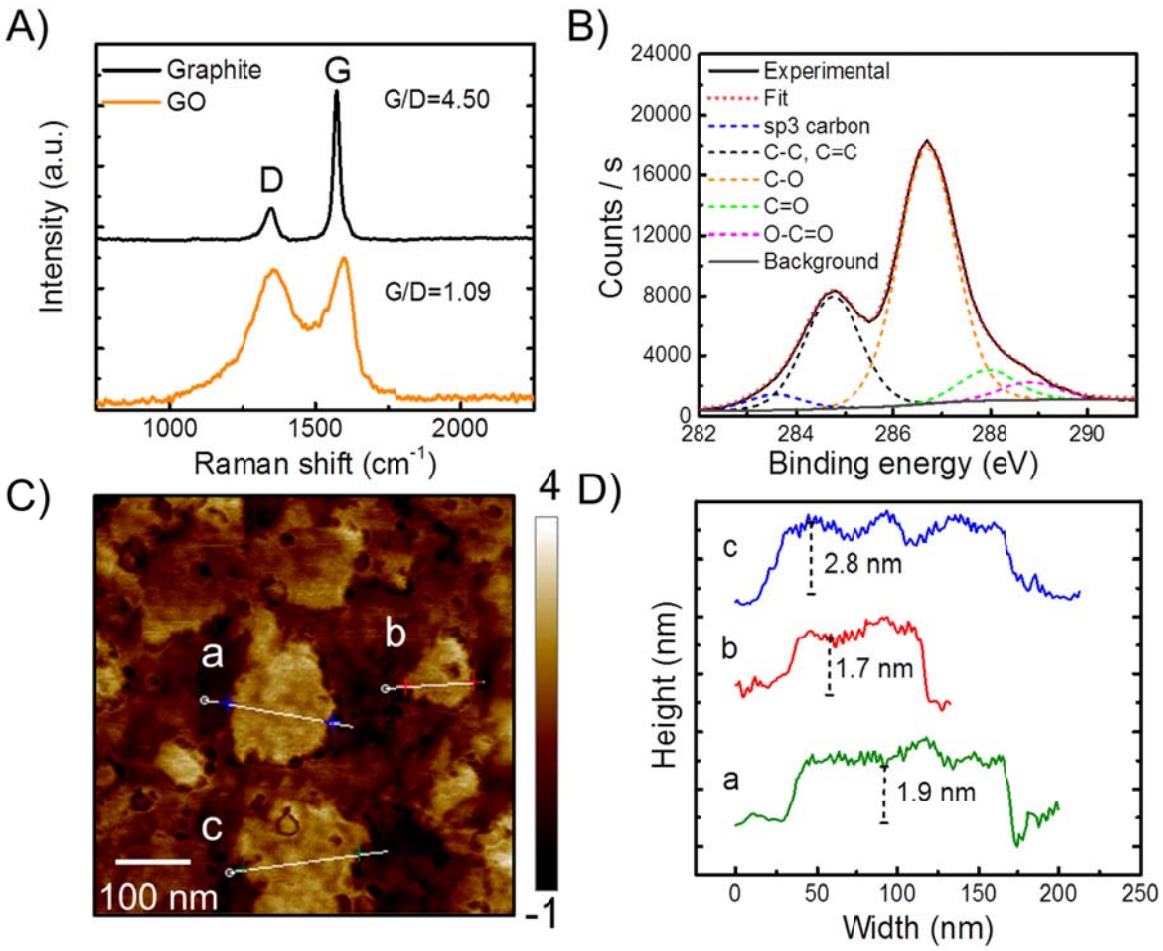

FIGURE S1. Graphene oxide characterization. (A) Raman spectra of graphite and graphene oxide showing the G and D bands. The G/D ratio indicates the level of defects introduced in the graphitic structure by the oxidation process. (B) XPS spectroscopy data of graphene oxide. (C) Representative AFM image of graphene oxide nanosheets. (D) Height profile of the graphene oxide nanosheets determined from the AFM image analysis.

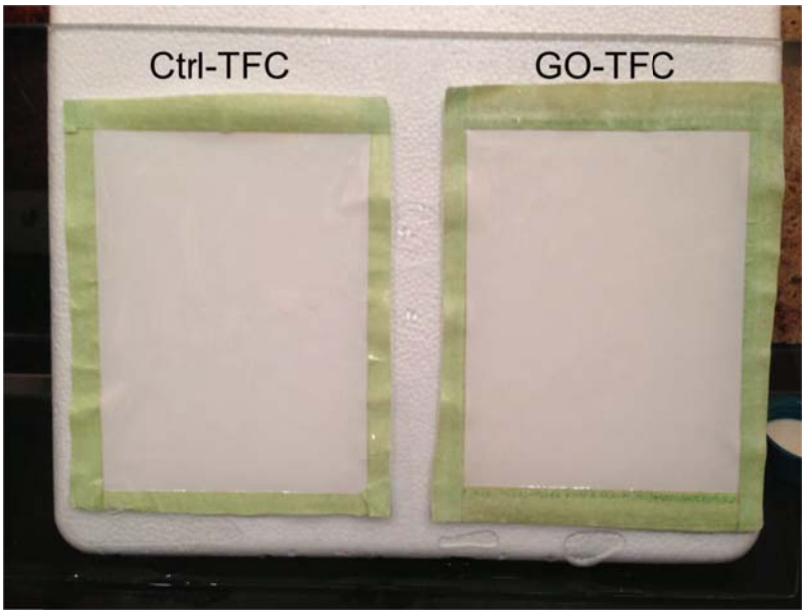

FIGURE S2. Pictures of the Ctrl (Ctrl-TFC) and graphene oxide functionalized TFC (GO-TFC) membranes. The graphene oxide layer is not visible on the membrane surface.

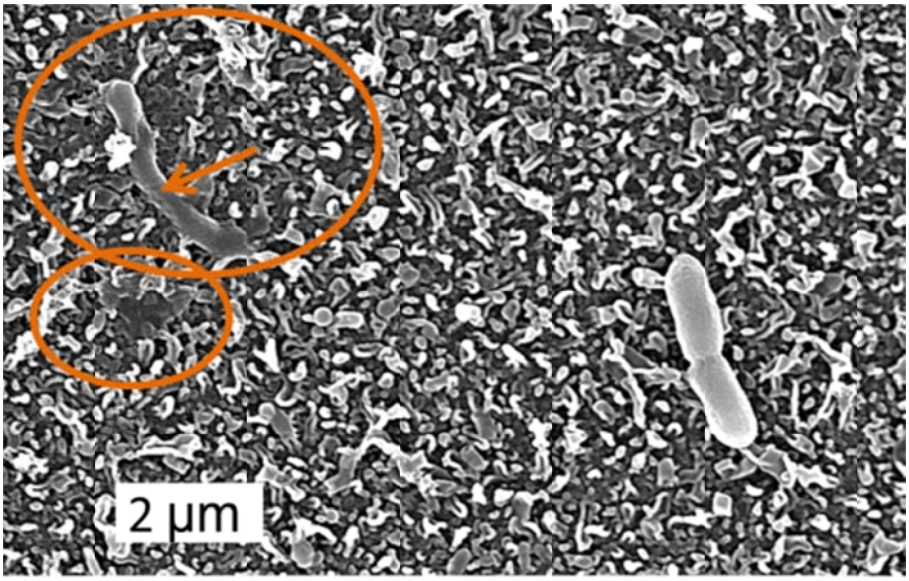

FIGURE S3. SEM micrograph showing *E. coli* cells at the surface of the GO-TFC membrane active layer. The darker regions indicating graphene oxide bound to the polyamide active layer are circled on the image. Compromised cell is indicated with an arrow.

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