Do Graphene Oxide Nanostructured Coatings Mitigate Bacterial Adhesion?

Electronic Supplementary Information

Karl Wuolo-Journey[1♠], Sara BinAhmed[1♠], Elise Linna[1], Santiago Romero-Vargas Castrillón[1, 2, 3 *]

Environmental Science: Nano

[1] Department of Civil, Environmental, and Geo- Engineering, University of Minnesota, 500 Pillsbury Dr. SE, Minneapolis, MN 55455, USA
[2] Institute for Infrastructure and Environment, School of Engineering, The University of Edinburgh, William Rankine Building, Thomas Bayes Road, Edinburgh EH9 3FG United Kingdom
[3] Institute for Materials and Processes, School of Engineering, The University of Edinburgh, Sanderson Building, Robert Stevenson Road, Edinburgh EH9 3FB United Kingdom

*Corresponding author:
Santiago Romero-Vargas Castrillón, Santiago@ed.ac.uk, sromerov@umn.edu
Tel: +44(0)131 651 3567
♠These authors contributed equally to this work

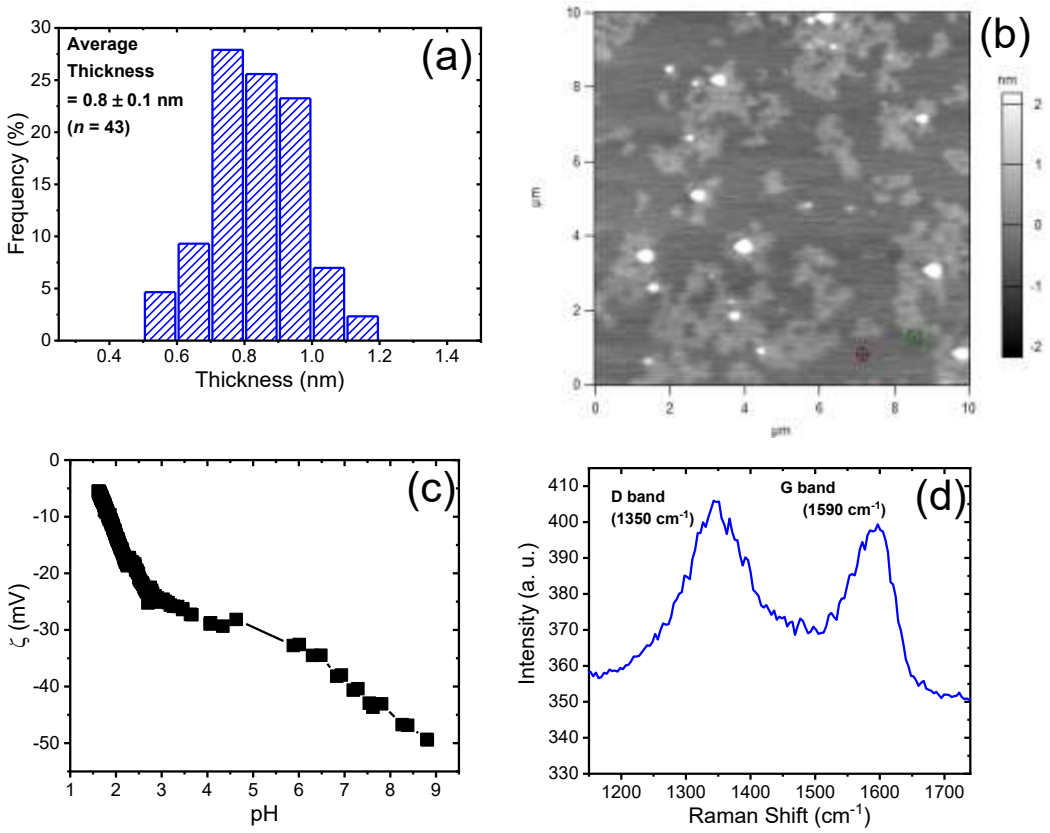

Figure S1. Characterization of graphene oxide (GO) nanosheets: (a) distribution of nanosheet thickness determined by AC mode AFM in air using an AC160TS-R3 Si cantilever (Olympus) with nominal spring constant 26 N m^{-1} and resonance frequency 300 Hz; (b) representative AFM image of GO nanosheets deposited on a Si substrate; (c) ζ-Potential of GO in aqueous dispersion at a concentration of 250 μg mL^{-1}, determined with a Stabino zeta potential analyzer; (d) Raman spectrum of GO nanosheets deposited on a silicon wafer.

Bacterial Deposition Assay. To complement our AFM results, we carried out a bacterial deposition assay to evaluate the bioadhesion propensity of the substrates. The assay entails exposure of the membrane surfaces to a *P. fluorescens* suspension under agitation, followed by colony counting from irreversibly adhered cells.[1] *P. fluorescens* ATCC 13525 was cultured overnight in 50 mL of autoclaved LB broth at 30 °C in an incubator (ThermoScientific MAXQ4450) under stirring (125 rpm). Bacterial suspensions were diluted 1:25 in autoclaved LB broth, and incubated for a further three hours at 175 rpm and 30 °C. Cells were harvested in mid-exponential phase ($OD_{600\ nm} \approx 0.6$) and centrifuged thrice at 5000g (for 1 min), re-suspending the pellet after each centrifugation in 1 mL PBS (pH 7.4). After the final re-suspension, 1-cm^2 substrate coupons were placed at the bottom of scintillation vials and each was immersed in 1 mL of the bacterial suspension, such that the entire coupon was fully covered by the liquid. The scintillation vials were then placed in the incubator (ThermoScientific MAXQ4450) at 30 °C under 175 rpm agitation. After 1 hour, substrates were removed from the suspension, gently rinsed with PBS, and placed in 10 mL of fresh PBS in 50-mL falcon tubes. Following bath sonication for 10 minutes, the resulting suspension was diluted 1:100, and a 50-µL aliquot of the dilution was smeared over an agar plate with a sterilized glass rod. After incubation overnight at 30 °C, the colonies were counted. This experiment was repeated two additional times for each substrate type for a total of three replicates.

The results of the bacterial deposition assay are presented in Fig. S2, showing the number of colony forming units (CFU) normalized by the PES control. Adhesion is significantly mitigated on PES-GO substrates, with the number of colonies on the PES-GO surface being 8.1% of the control PES following a 1-h exposure ($p < 0.05$, one-sided unpaired *t*-test).

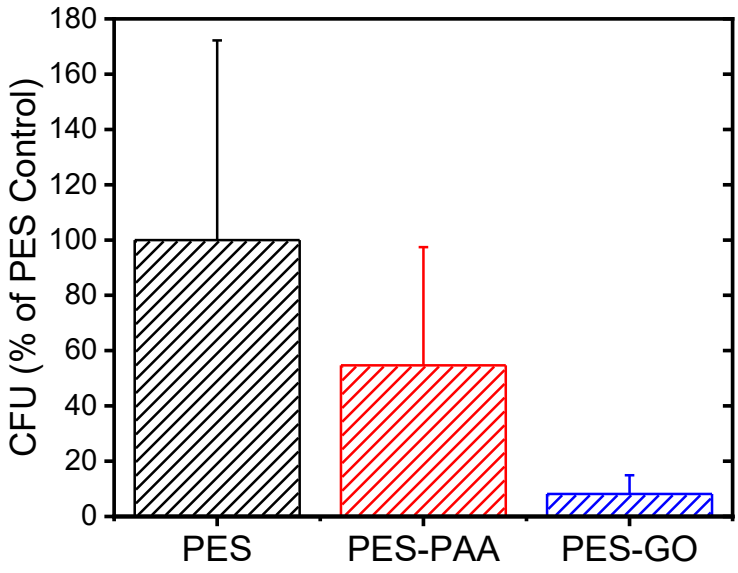

Figure S2. Bacterial deposition assay of pristine PES, poly(acrylic acid) (PAA)-modified PES (PES-PAA), and GO-modified PES (PES-GO) substrates. Colony-forming units (CFU) are shown as % of the PES control. Error bars denote the standard deviation of three experiments.

It is important to note that the CFU data in Fig. S2 are possibly influenced by the cytotoxicity of GO.[1–4] Thus, the precipitous drop in CFU count on PES-GO compared to PES and PES-PAA may be due to a combination of lower adhesion and GO's biocidal activity. However, the relative contributions to the CFU count of adhesion mitigation (due to the interfacial properties of PES-GO) and biocidal activity cannot be disentangled with this simple colony counting assay, and thus would require further investigation.

Characterization of Membrane Transport Properties. The water permeability coefficient (A) of the membranes was determined in a laboratory-scale filtration apparatus equipped with a crossflow cell (CF042D, Sterlitech, with active membrane area, A_m, of 42.1 cm^2), pump (HydraCell M-03S, Wanner Engineering), and temperature-controlled stainless steel feed reservoir. Membranes were compacted with a distilled water feed for 24 hours at a transmembrane

pressure difference (Δp) of 50 psi and crossflow velocity of 0.08 m s^{-1}. Following compaction, measurements of the steady-state permeate flow rate were recorded every second for 1 hour at Δp = 50 psi and 20 °C with a digital flow meter (SLI, Sensirion). The average permeate flow rate, Q_p, was used to compute the water permeability coefficient from $A = Q_p/(A_m \Delta p)$. For control polyethersulfone (PES) membranes, the flux through the membranes was determined by weighing the permeate, since the permeate flow rate exceeded the maximum flow rate measurable with the digital flow meter. Four poly(acrylic acid)-modified (PES-PAA), four GO-modified (PES-GO) and two control PES membranes were characterized.

Effect of Surface Functionalization on Water Permeability and Ion Rejection. Surface modification of the PES membranes resulted in additional hydraulic resistance that decreased the water permeability coefficient (A). For pristine PES we find $A = 102.1 \pm 3.5$ L m^{-2} h^{-1} bar^{-1}. On the other hand, for PES-PAA membranes (prepared by acrylic acid polymerization with 10-s UV exposure), we find $A = 9.0 \pm 1.8$ L m^{-2} h^{-1} bar^{-1}, while for PES-GO, $A = 7.0 \pm 0.7$ L m^{-2} h^{-1} bar^{-1}, i.e., the covalently bonded GO layer further decreases water permeability. We also determined the Na_2SO_4 rejection coefficient (R) at Δp = 50 psi (feed concentration = 10 mM) for the functionalized membranes using a conductivity probe, finding R = 21.4% and 42.7% for PES-GO and PES-PAA, respectively. The A coefficient and ion rejection of PES-PAA and PES-GO materials are similar to those of nanofiltration membranes.[5,6] Additional experiments with PES-PAA membranes prepared with 20-60 sec UV irradiation resulted in steep loss in water permeability (results not shown), due to the formation of a dense PAA layer (observe the prominent carboxyl band at 1700 cm^{-1} when the irradiation time was ≥ 20 s, Fig. 2).

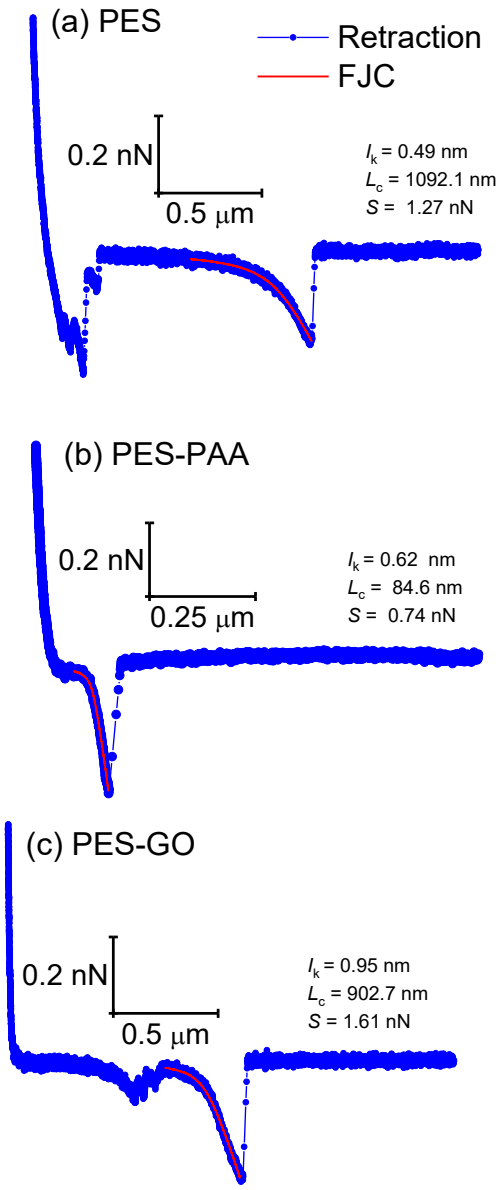

Figure S3. Representative retraction force (F)-elongation (z) curves for different membrane substrates (see caption) recorded with *P. fluorescens* bacterial probes. The data show fits of the extended freely-jointed chain (FJC) model, given by $z(F) = L_c\left[\coth\left(\frac{Fl_k}{k_BT}\right) - \frac{k_BT}{Fl_k}\right]\left(1 + \frac{F}{S}\right)$, where L_c is the contour length, l_k is the Kuhn length, and S is the stretch modulus of the polymer; k_B and T = 298.15 K are Boltzmann's constant and absolute temperature, respectively. Best-fit values of L_c, l_k and S are given in the caption. Due to the thermal noise underlying the measurements (≈ 30 pN), the fitted region of the force-extension curves was smoothed using a locally weighted least-squares smoothing algorithm (loess) implemented in Origin 2018 (Northampton, MA). FJC parameters were obtained by non-linear regression of the smoothed data using the function nlinfit in Matlab R2018a (MathWorks, Natick, MA).

Literature Cited

1. Perreault, F., Tousley, M. E. & Elimelech, M. Thin-Film Composite Polyamide Membranes Functionalized with Biocidal Graphene Oxide Nanosheets. *Environ. Sci. Technol. Lett.* **1**, 71–76 (2013).
2. Perreault, F., De Faria, A. F., Nejati, S. & Elimelech, M. Antimicrobial Properties of Graphene Oxide Nanosheets: Why Size Matters. *ACS Nano* **9**, 7226–7236 (2015).
3. Akhavan, O. & Ghaderi, E. Toxicity of graphene and graphene oxide nanowalls against bacteria. *ACS Nano* **4**, 5731–5736 (2010).
4. Gurunathan, S., Han, J. W., Dayem, A. A., Eppakayala, V. & Kim, J. H. Oxidative stress-mediated antibacterial activity of graphene oxide and reduced graphene oxide in Pseudomonas aeruginosa. *Int. J. Nanomedicine* **7**, 5901–5914 (2012).
5. Bouchoux, A., Roux-de Balmann, H. & Lutin, F. Investigation of nanofiltration as a purification step for lactic acid production processes based on conventional and bipolar electrodialysis operations. *Sep. Purif. Technol.* **52**, 266–273 (2006).
6. Do, V. T., Tang, C. Y., Reinhard, M. & Leckie, J. O. Effects of hypochlorous acid exposure on the rejection of salt, polyethylene glycols, boron and arsenic(V) by nanofiltration and reverse osmosis membranes. *Water Res.* **46**, 5217–5223 (2012).