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Observation of Electron Transfer Between Bacteria and High Conductivity Graphene-PEDOT composites

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Supporting Information

Experimental Section:

Graphene Dispersion

Natural flake graphite (Sigma Aldrich) with a bulk particle size of less than 2µm was used as received and all suspensions were prepared in MilliQ filtered water. Graphene was produced using a liquid phase exfoliation technique as described previously1, 2. The surfactant employed was the triblock copolymer L64 (Sigma Aldrich) and used as received. The bulk layered material (10g equivalent to 1% w/w) was added to 1L of MilliQ. Sonication at a power of approximately 100W was commenced with the 10% w/w surfactant solution in MilliQ water added drop wise over a period of 1 hour. .. Following exfoliation, the suspensions were centrifuged at 2500 rpm for 20 minutes to remove the larger, unexfoliated particles. Exfoliation down to single and few layer graphene was confirmed using Raman spectroscopy and transmission electron microscopy as described in previous publications2, 3.

Vapor Phase Polymerization of composites

Fe(III) tosylate (Fe(Tos)3) was received from HC Stark as a 40 wt % CB40 solution in n-butanol. 3,4-Ethylenedioxythiophene (EDOT) monomer and triblock copolymer P123 were obtained from Aldrich.

A series of oxidant solutions were prepared using the pristine oxidant solution and diluting to 16 wt % Fe(Tos)3 using a solution of graphene diluted in ethanol. This diluent solution contained 70% graphene dispersion and 30% ethanol by weight. After addition of the graphene dispersion into the oxidant solution, oxidant thin films were deposited on glass microscope slides, and then exposed to the vapour phase polymerisation process. The glass substrates (prior to coating) were washed using a mild detergent, then ethanol, and finally rinsed using high purity water. Prior to spin-coating, the substrates were air plasma treated (Diener, Plasma etcher NANO, Germany) for 2 minutes. The oxidant solution was spin-coated (400B-6NPP, Laurell Technologies Inc.) at a speed of 1500 RPM for 25 seconds and then placed on a 70 °C hot plate for 30 seconds. Immediately after heating, samples were placed into a 115 L vacuum chamber oven (Binder, Germany) set to 35 °C. The chamber was pumped down to 45 mbar, at which time the vessel containing the EDOT monomer (heated to 45 °C) was opened, releasing monomer vapour into the volume of the chamber. Samples were removed after 25 minutes and placed on a 70 °C hotplate for 2 minutes to anneal the polymer. Samples were removed from the hotplate and further annealed through cooling to room temperature. After 20 minutes in general lab conditions (25 °C and 35%RH), samples were carefully rinsed in an ethanol bath to remove consumed and unconsumed oxidant, unbound surfactant, and residual un-reacted monomer. At the end samples were dried with an air gun, followed by a final ethanol spray rinse and air-drying step.

Bacterial Strains and Culture Conditions

The bacterial strain used in this work was Pseudomonas aeruginosa ATCC 10145T. Prior to electron transfer experiments the bacteria were cultured in the absence of oxygen to stimulate anaerobic respiratory pathways. Single colonies were selected from nutrient agar plates (Difco, BD) and inoculated into 15 mL of nutrient broth (Difco). The inoculated tubes were incubated at 37 °C with minimal shaking (50 rpm) for 7 days. The cells were harvested by centrifugation, washed and resuspended in phosphate-buffered saline (PBS, pH 7.4). Immediately prior to electron transfer experiments, the cell suspensions were supplemented with glucose (to a final concentration of 2% w/v) as the electron donor for metabolism, and (NH4)2SO4 (0.1 % w/v) to supply essential elements. Cultures were

bubbled with N2 for 30 minutes and the headspace filled before partial submersion of graphene-PEDOT composite and reference non-conductive glass samples, at which point the tubes were sealed. The samples were then incubated at 37 °C with shaking at 50 rpm for up to 2 weeks to allow electron transfer to take place. In an additional set of experiments to probe the mechanism of electron transfer, filter paper was attached to the graphene-PEDOT films to prevent bacterial attachment. The sheet resistance of the film was subsequently measured after culturing under the same conditions.

Cell Viability

P. aeruginosa cells grown overnight in nutrient broth at 37 °C were harvested by centrifugation and resuspended in PBS to OD600 = 0.1. Samples of graphene-PEDOT and non-conductive glass were covered with 250 µL of the resulting suspension and incubated at 37 °C for one hour. Subsequently, the samples were gently rinsed with sterile water and stained using a BACLIGHT bacterial viability kit (Life Technologies, Invitrogen) according to the manufacturer's protocol. Briefly, cells were simultaneously labelled with SYTO9® green fluorescent dye and propidium iodide (PI), which fluoresces red. SYTO9® is able to permeate all cells, thus labelling them with green fluorescence, while PI is only able to enter non-viable cells, but displaces SYTO9®. This resulted in dead cells being labelled red, while living cells remain green. After staining, samples were imaged using a Fluoview FV10i inverted confocal scanning laser microscopy system (Olympus, Japan).

$AgNO_3$ redox reaction

$AgNO_3$ was received from Sigma Aldrich and used as supplied. Bacteria-reduced graphene-PEDOT was exposed to a solution (1% w/w) of $AgNO_3$ in MilliQ filtered water by immersion for 2 minutes.

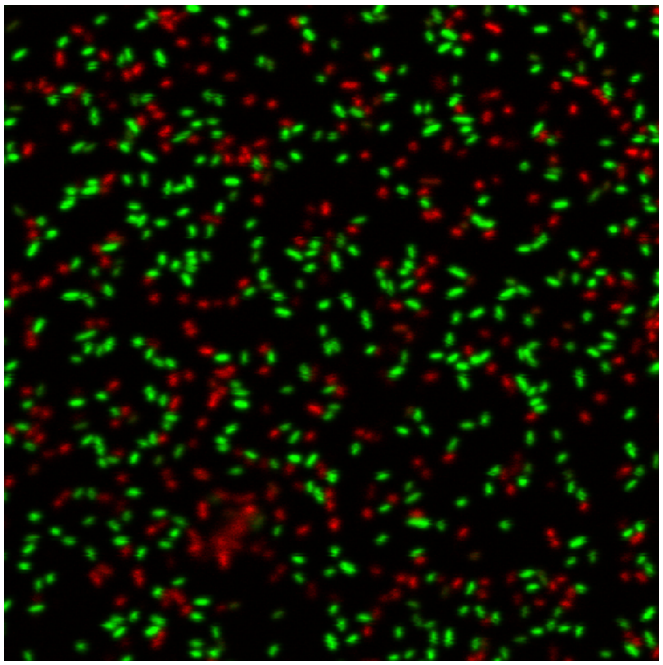
Glucose control

Glucose was used as received from Sigma Aldrich. A sample of gPEDOT was immersed in a 20% solution of glucose for four days, at which point it was rinsed thoroughly, dried, and sheet resistance was measured to be 58.78 Ω/□ demonstrating that little to no direct reduction occurred due to the feedstock.

Electrical Measurement

Electrical analysis of the thin films was conducted using the 4-point probe technique (Multi height probe, RM3, Jandel Engineering). The sheet resistance of the samples in Ω/□ was measured with a tip radius of 100 µm, tip spacing of 1 mm and 60 g preset load. On each sample, up to 6 measurements were taken and the average value reported. The conductivity (σ) in S/cm was calculated from the inverse of the sheet resistance multiplied by the film thickness (as measured using a Dektak XT, Bruker, 0.3g preset load and 25 µm radius tip).

Supplementary Figures



SUPPLEMENTARY FIGURE S1. Viability of *P. aeruginosa* cells adhered to the surface of graphene-PEDOT nanocomposites. Prior to fluorescent imaging, cells were labelled with two fluorescent dyes: SYTO®9, which labels the DNA of all cells with green fluorescence, and propidium iodide (PI), which labels non-viable cells with red fluorescence. PI also binds to DNA, but with higher affinity than SYTO®9, and it is only able to enter cells with compromised outer membranes. The overall proportion of viable cells is approximately 0.7.

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