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Electronic Supplementary Information

Encapsulation of Yeast Displaying Glucose Oxidase on their Surface in Graphene Oxide Hydrogel Scaffolding and its Bioactivation

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GO Synthesis

GO was synthesized from exfoliated graphite by a modified Hummers method[3] and a detailed protocol is described in our previous article.[4] First, exfoliated graphite powder (1 g) was added to a solution of $K_2S_2O_8$ (1.67 g) and P_2O_5 (1.67 g) in 8 mL concentrated H_2SO_4. The mixture was kept at 80 °C for 4.5 h on a hot plate. After the mixture was cooled to room temperature, it was diluted with 0.35 L of deionized water (DIW) and filtered. Then the preoxidized material was washed with DIW and dried at 60-70 °C overnight. Next, preoxidized carbon was redispersed in 40 mL of concentrated H_2SO_4 with the mixture kept in an ice bath. Subsequently, 5 g of potassium permanganate were added gradually under constant stirring to avoid overheating. The mixture was stirred at 35 °C for 2 h and then slowly diluted with 80 mL of DIW upon cooling in the ice bath.

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The mixture was stirred for an additional 2 h and then 250 mL more DIW were added, followed by the addition of 6 mL of 30% H_2O_2 to react with the excess of permanganate. The color of the solution changed to yellow after addition of the peroxide. The oxidized product was filtered and washed with 100 mL HCl (1:10) to remove metal ion impurities, followed by washing with 300 mL of DIW and by dialysis to remove the acid. A dispersion of GO in water was prepared by dispersing the oxidized material in DIW in an ultrasound bath for 2 h. Aqueous GO dispersions were stable for at least a few months. The concentration of GO in this aqueous solution was 0.99 g per 100 ml of the solution.

Strains and cultivation media

Shewanella oneidensis MR-1 (ATCC number 700550) culture was grown in soy broth. *Saccharomyces cereviciae* EBY100 strain (wild type; WT) culture was grown in YPD media (yeast extract 20 g/L, peptone 20 g/L, and D-glucose 20 g/L), or on SC-ura plates. Yeast expressing glucose oxidase (GOx) on the cell surface (*S. cereviciae* EBY100 strain harboring the pC-GOx plasmid)[1] were grown in SDCAA media (D-glucose 20 g/L, yeast nitrogen base without amino acids 6.7 g/L, Bacto casamino acids 5 g/L, $Na_2HPO_4 \cdot H_2O$ 6.8 g/L, and $NaH_2PO_4 \cdot 2H_2O$ 9.6 g/L) or SC-ura-trp in the case of non-induced culture. Induced culture was grown in 80% SGCAA (similar to SDCAA with galactose instead of glucose), and 20% SDCAA.

GO-Hydrogel preparation

Induced yeast expressing GOx, WT EBY100 yeast and S. *oneidensis* cultures were grown over night at 30 °C with continuous shaking (250 rpm) to an OD_{600nm} of 1 (equivalent to 10^7 yeast cells/mL and to 10^{10} *S. oneidensis* cells/mL).[2] Following centrifugation of either 1 minute at 13000 rpm for volumes less than 1.5 mL or 10 minutes at 4000 rpm for larger volumes, the pellets from each culture were resuspended in either phosphate buffer (pH 7; PB) or 100 mM glucose in PB; except for S. *oneidensis* that was resuspended in soy broth for the capacitance measurements. The resuspension volume was half of the initial volume of the culture. Additionally, free commercial GOx was dissolved in either PB or 100 mM glucose in PB to achieve final enzyme concentration of 10-50 mU/mL. The resulting cultures and enzyme solution then were mixed carefully with similar volume of

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GO (*i.e.* a ratio of 1:1 between GO and microorganisms culture or enzyme solution volumes). Likewise, similar amounts of either PB or 100 mM glucose in PB were mixed with GO as controls (1:1). Hence, when glucose was present in the sample, its final concentration in the GO hydrogel was 50 mM, and when microorganisms were present in the sample, the final number of cells within the hydrogel was 10^7 yeast cells/mL or 10^{10} *S. oneidensis* cells/mL.

Characterization

Sample preparation was similar to the described procedure in the hydrogel preparation section however; the final free GOx concentration was 10 mU/mL. This enzyme concentration was similar to the calculated enzyme produced by GOx-expressing yeast at OD_{600nm}=1.[1] All hydrogels were incubated at room temperature for 5 days. Duplicates of 0.5 mL from each hydrogel were extracted at the starting point and every 24 hours of the experiment. Immediately after the extraction, all samples were incubated at 80 °C for 60 minutes in order to inactivate enzymes and microorganisms.

Scanning electron microscope (SEM) imaging was performed at 3-5 kV using the FEI Magellan^{TM} 400L (Eindhoven, Holland). The specimen was prepared by deposition of a drop of the ethanol suspension of the sample onto 400 mesh copper grid with lacey carbon support film or on silicon substrate.

X-ray photoelectron spectroscope (XPS) measurements were performed on a Kratos Axis Ultra X-ray photoelectron spectrometer (Manchester, UK). High resolution spectra were acquired with a monochromated Al Kα (1486.6 eV) X-ray source with 0° takeoff angle. The pressure in the test chamber was maintained at $1.7 \cdot 10^{-9}$ Torr during the acquisition process. Data analysis was performed with Vision processing data reduction software (Kratos Analytical Ltd.) and CasaXPS (Casa Software Ltd.).

Raman spectroscopy was conducted using the laser wavelength of 488 nm on a CRM200 confocal Raman spectrometer, WITec.

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Bioelectrocatalysis

Samples preparation was as previously described in the hydrogel preparation section. The final concentration of free GOx was 50 U/mL. All samples (200 µL each) were incubated at room temperature for 24 h. The hydrogels in the presence of glucose were covered gently with 100 mM glucose in PB up to the top of the tube to assure similar electrode coverage. Likewise, hydrogels in the absence of glucose were covered with PB. A conventional three-electrode set up was assembled with graphite tip (area 0.022 cm^2) as working electrode, coiled Pt wire as counter electrode and Ag/AgCl (NaCl 3M) electrode as reference electrode (ALS, Tokyo, Japan). Cyclic voltmmetry (CV) was performed with VSP potentiostat (BioLogic science instruments, Claix, France) between 0.3 V to 0.9 V at scan rate of 0.5 mV/sec. Prior to cell conduction, the Pt wire was polished with alumina slurry, washed with ethanol and distilled water, and dried out with air flow. The working electrode was submerged in the hydrogel while the counter and reference electrodes were submerged in the PB top solution.

Capacitance measurements

Following the described sample preparation in the hydrogel preparation section (Free GOx concentration was 50 U/mL), cyclic voltammetry was performed using a PalmSense potentiostat (Palm Instruments, Houten, The Netherlands). A three electrode home-made cell was constructed with a graphite disc as the working electrode (0.6 cm diameter) on the cell bottom, Pt wire as counter electrode and Ag/AgCl as reference electrode (ALS, Tokyo, Japan). A sample of 100 µL of each GO hydrogel was loaded on top of the

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working electrode and was covered gently with 5 ml PB. The working and reference electrodes were submerged in the top PB solution. Prior to cell conduction, the graphite electrode was polished with sandpaper and Pt wire was polished with alumina slurry. The two electrodes then were washed with ethanol and distilled water, and dried out with air flow. All CVs measurements (-1 V to 1 V, 100 mV/sec) were performed at room temperature for the duration of 5-14 days.

Live cell assay

Hydrogels were prepared as previously described in the hydrogel preparation section with induced GOx-expressing yeast and WT EBY100 yeast cultures in either PB or 100 mM glucose in PB. The hydrogels then were incubated at room temperature. Triplicates of 100 μL were taken from each hydrogel at the starting point and after 7 days. Ten-fold serial dilutions were made with sterile PB up to 10^{-6} fold. A sample of 100 μL from each dilution was plated on SC-ura plates for WT yeast and SC-ura-trp plates for GOx-expressing yeast. Plates were incubated at 30 °C for 36-48 h and individual colonies were counted. Moreover, the absorbency of ten-fold diluted hydrogels was checked at 600 nm wavelength.

Additional Results

1. Minimal gelation concentration

The minimal gelation concentration was determined by preparing a set of mixtures of different concentrations of GO and 10^7 cells/mL yeast or 10^{10} cells/mL *S. Oneidensis* cultures.

Gelation of different solutions was tested by inverting an eppendorf and testing whether the gel stays atop. Fig. S1 shows a set of different concentrations of graphene oxides mixed with 10^7 cells/mL of *S. cereviciae* EBY100.

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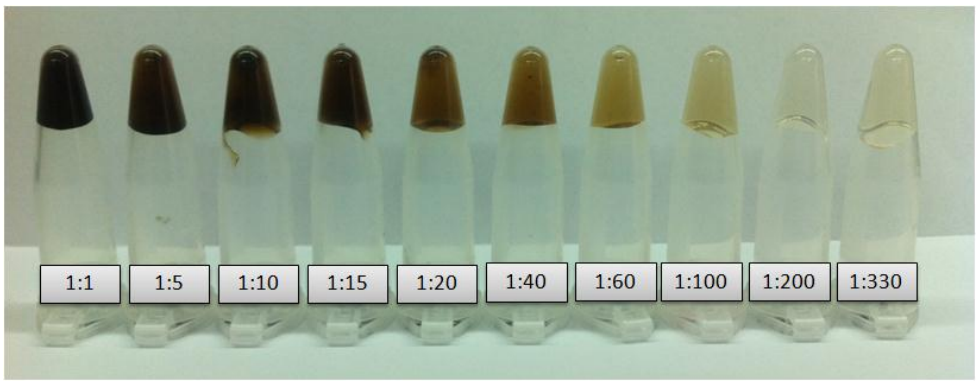

Fig. S1 The inverted beaker test of hydrogels made with different concentrations of graphene oxide and 10^7 cells/mL *S. cereviciae* EBY100. GO: yeast culture weight ratios are indicated.

2. Raman Spectroscopy of graphene oxide after exposure to different biohydrogels

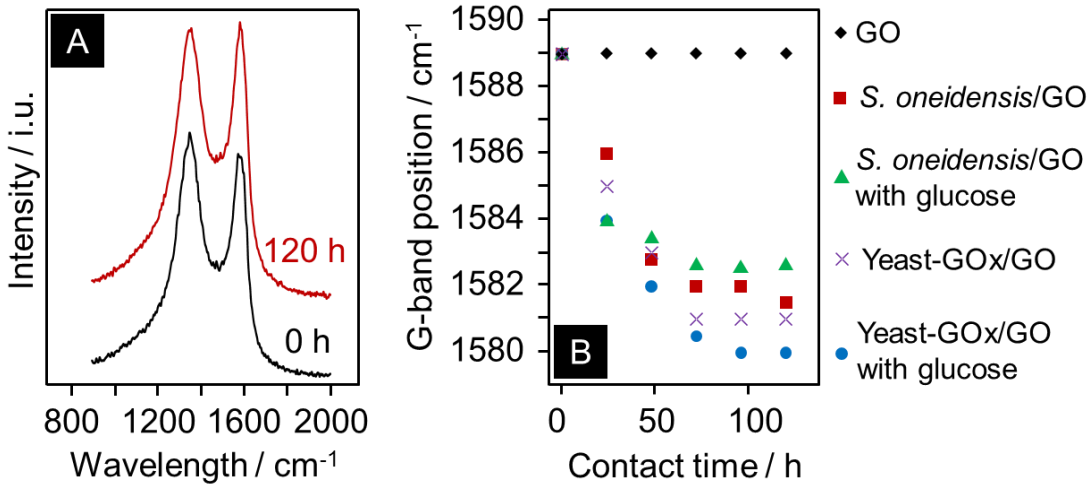

Fig. S2 Characteristic features of the Raman D and G bands as a function of contact time of the GO and the different microorganisms. (A) Raman spectra collected from the sampled Yeast-GOx/GO supplemented with glucose immediately after preparation and after 120 h of GO exposure to Yeast-GOx and glucose. (B) Peak intensity ratios I_D/I_G (obtained from Raman spectra) *vs.* the contact time between GO and the solution.

3. X-ray photoelectron spectroscopy

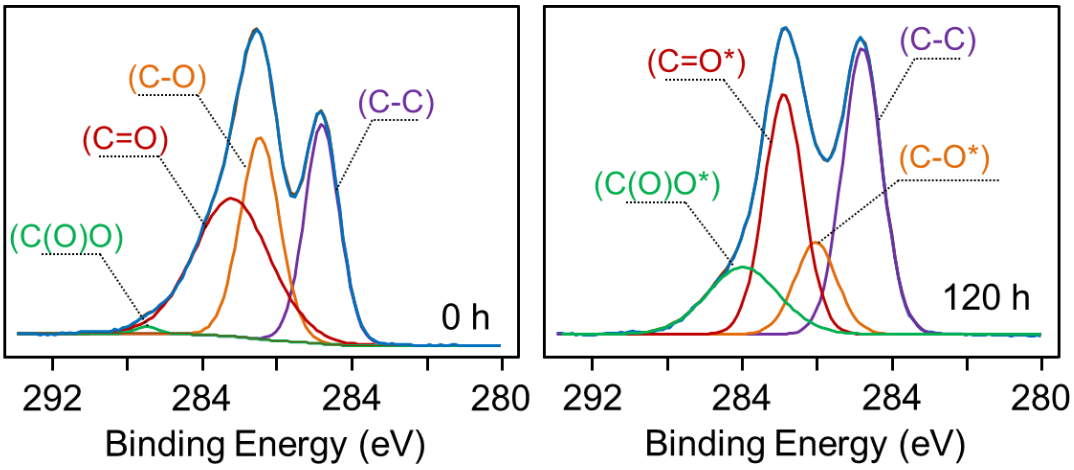

Fig. S3 XPS spectra of C1s collected from the sample of Yeast-GOx/GO supplemented with glucose immediately after preparation and after 120 h of GO exposure to Yeast-GOx and glucose. The right peak on each spectrum is attributed to C-C bonded carbon and the left one to oxygenated carbon. The fraction of oxygenated carbon is considerably reduced after the exposure to the genetically modified yeast in the presence of glucose. However, this figure is of limited quantitative value since we could not separate the yeast from the GO prior to the measurements.

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