Electronic Supplementary Information

Graphene as Membrane for Encapsulation of Yeast Cells: Protective and Electrically Conducting.

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Contents

Procedure for the synthesis of graphene oxide

Graphene oxide was synthesized via the modified Hummer's method. Graphite flakes (~ 325 mesh size, Alfa Aesar) were oxidized using a combination of powerful reagents i.e., sulphuric acid (H_2SO_4), potassium per sulphate ($K_2S_2O_8$), phosphorous pentoxide (P_2O_5). 3g of graphite flakes were dissolved in 10 ml of H_2SO_4. Oxidizing agents $K_2S_2O_8$ and P_2O_5 were added to the graphite in sulphuric acid and stirred at 90 ºC till the flakes were dissolved. The stirring continued for 4 more hours at 80 ºC and the solution was then diluted with 500 ml of Milli-Q Millipore™ water (Ultrapure water > 16MΩ). After the dilution, the solution was stirred overnight, filtered, washed with de-ionised water and then dried to get the powdered form of graphene oxide.

Pre-oxidized GO powder was then subjected to further oxidation with 125 mL of H_2SO_4 and 15g of Potassium permanganate ($KMnO_4$) in an ice bath where the solution was stirred for 2 more hours. 130 mL of Milli-Q Millipore™ water was added to the mixture and this caused the temperature to rise to 95 ºC. After 15 minutes, 15 mL of Hydrogen Peroxide (H_2O_2) was added to reduce the manganese in the solution to manganese sulphate (Mn→ $MnSO_4$). Finally, the solution was diluted with 400 mL of Milli-Q Millipore™ water and resultant yellow suspension was stirred overnight. GO was filtered and washed till the rinsed water pH was found to be approximately 7.[1, 2]

Surface functionalization

For this purpose, Silicon chips patterned with gold electrodes were washed with 2-propanol and Milli-Q Millipore™ water and subsequently sonicated in 50:50 by volume solution of 2-propanol and Milli-Q Millipore™ water to remove any residual particles on the surface. Their surface was then hydroxylated by placing them in "piranha" solution (7:3 concentrated H_2SO_4: 30% H_2O_2) (**CAUTION**: piranha solution reacts violently with organic compounds!). The chips were then washed thoroughly with Millipore™ water. The chips were then placed in a solution of (3-Aminopropyl)triethoxysilane (1mL in 100 mL of Millipore™ water) for 10 minutes. The chips were then annealed on a hot plate at 120 ºC for 30 minutes. Once cooled, the chips were used for the deposition.[2]

Reduction of graphene oxide

Reduction of graphene oxide to graphene was accomplished using a reducing sugar i.e., glucose. 40mg of glucose was added to a solution of graphene oxide (0.1mg /1mL) in a vial and stirred for 40 min. 20µL of ammonia solution (28% W/W NH_4OH) was then added and the mixture was stirred at 90 ºC for 60 min. The resultant solution was centrifuged (10,000 rpm), washed with Millipore™ water and then re-suspended in Millipore™ water for further use. [3]

Calcium-Gold solution (Ca-Au)

Calcium-Gold solution was found to be a favourable binding agent and it was prepared by mixing calcium chloride solution with the gold colloid in the ratio 1:11 respectively. Calcium chloride solution was prepared by dissolving anhydrous calcium chloride in Millipore™ water (1mg/1mL solution). Thus prepared calcium chloride solution was mixed with gold colloid (10nm, BBI International) and stirred for 24 hours. The change in color from wine red to blue indicated the formation of gold nanoparticle chain. [4]

Graphene oxide-Calcium-Gold solution (GO-Ca-Au)

Graphene oxide is attached to the Calcium-Gold nanoparticles by mixing the solutions of GO and Calcium-Gold (Ca-Au). GO (0.5mg/1mL) solution is centrifuged at 500 rpm for 3 min

to remove micron sized particulates and then added to preformed Calcium-Gold solution in the ratio 1:20 and stirred for 8 hours to get homogeneous solution of GO-Ca-Au.

Harvesting of Saccharomyces Cerevisiae (Yeast cell)

S. cerevisiae strain BY4741 (MATa his3Δ leu2Δ met15Δ ura3Δ) is used in the experiments. 2g of yeast-peptone-dextrose broth (YPD) broth powder was dissolved into 40mL of Millipore™ water and autoclaved to get the broth solution.Yeast cells were grown in YPD broth at 30 ºC for 8 hours, shaking at 200 rpm. After 8 hours, the cells reach a logarithmic growth phase and they are centrifuged and subsequently washed with Millipore™ water to remove any YPD residue. Washed cells were re-suspended in water for further deposition with graphene.

Saccharomyces Cerevisiae (Yeast cell) on CRG-Ca-Au

Yeast cells harvested after 8 hours of growth in the YPD broth were centrifuged and washed. 100µL of such cell suspension is added to 1mL of CRG-Ca-Au solution and allowed to mix for an hour.

Live-Dead test

The viability of the yeast cells after the deposition with Ca-Au-CRG was studied using a Molecular probes® L-7009 LIVE/DEAD® Yeast Viability Kit (Invitrogen™). The kit consists of two-color fluorescent probe FUN® 1 which stains the cell body, with a fluorescent fungal surface labeling reagent Calcofluor™ White M2R.We mixed 260.3mg of HEPES powder in 100mL of Millipore™ water to get 10mM of Na-HEPES buffer; 2g of glucose was added to HEPES buffer and the resultant solution was used as the medium for staining the cells with the dye reagent. 3mL of 10µM and 2mL of 60µm FUN 1 reagent solutions were prepared by mixing the stock 10mmdimethylsulfoxide (DMSO) cell stain into sterile GH solution.Saccharomyces Cerevisiae (yeast cells) were grown in YPD broth for 8 hours, centrifuged and washed 2 times before mixing it with the CRG-Ca-Au solution as described in the previous sections. After incubating for an hour, the solution was centrifuged and re-suspended in GH solution. CRG-Ca-Au-yeast cells in GH solution were finally stained with FUN 1 reagent solutions (10µM and 60µM) by mixing it in the ratio 50:50. 5µL of 25µm Calcofluor solution prepared from its 5µm stock solution.

Apparatus

AFM was conducted with a Veeco DI Dimension 3100 Nanoscope IV scanning probe attached with a tapping tip. Field Emission Scanning electron microscopy (FESEM) was done with a LEO 1530 Gemini field emission gun attached with EDX/OIM. TEM images were obtained with a LEO 912ab energy filtered transmission electron microscope (EFTEM). Optical

spectroscopy was carried out using USB4000 (ocean optics) spectrometer. Electrical characterization was done using a custom-built IV setup consisting of Agilent 6614C DC power supply and Hewlett Packard 3458Amultimeter. Raman spectra were obtained on a J-Y T64000 Raman spectrometer with 514.5 nm wavelength incident laser light. Confocal Microscopy was done on an upright Leica DM 6000B microscope.

Characterization and Results

The AFM images of the synthesized GO sheets deposited on silicon wafers is shown in Fig. S-1. A typical thickness of ~ 1-1.25 nm with size of microns is observed for the sheets.

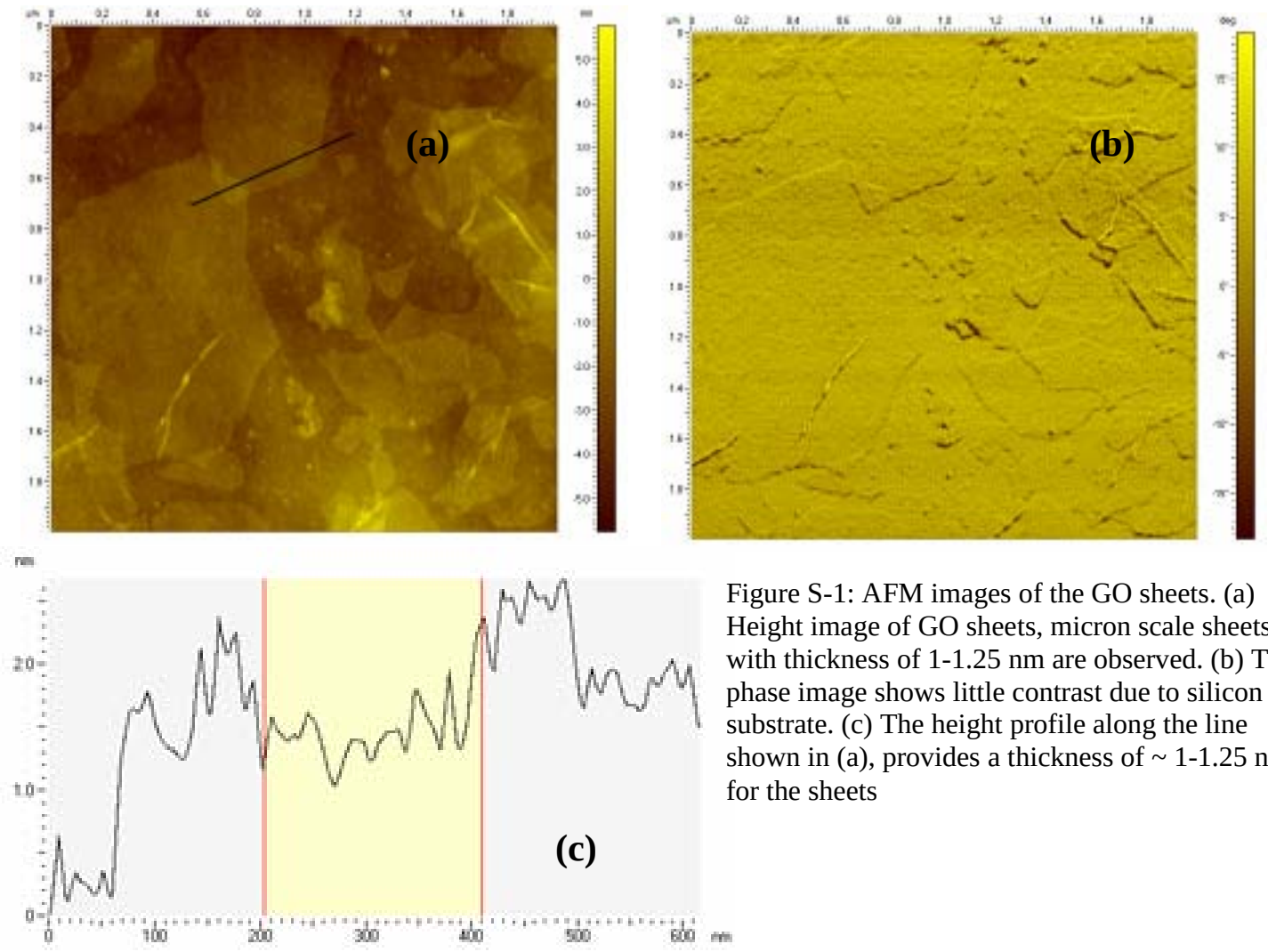
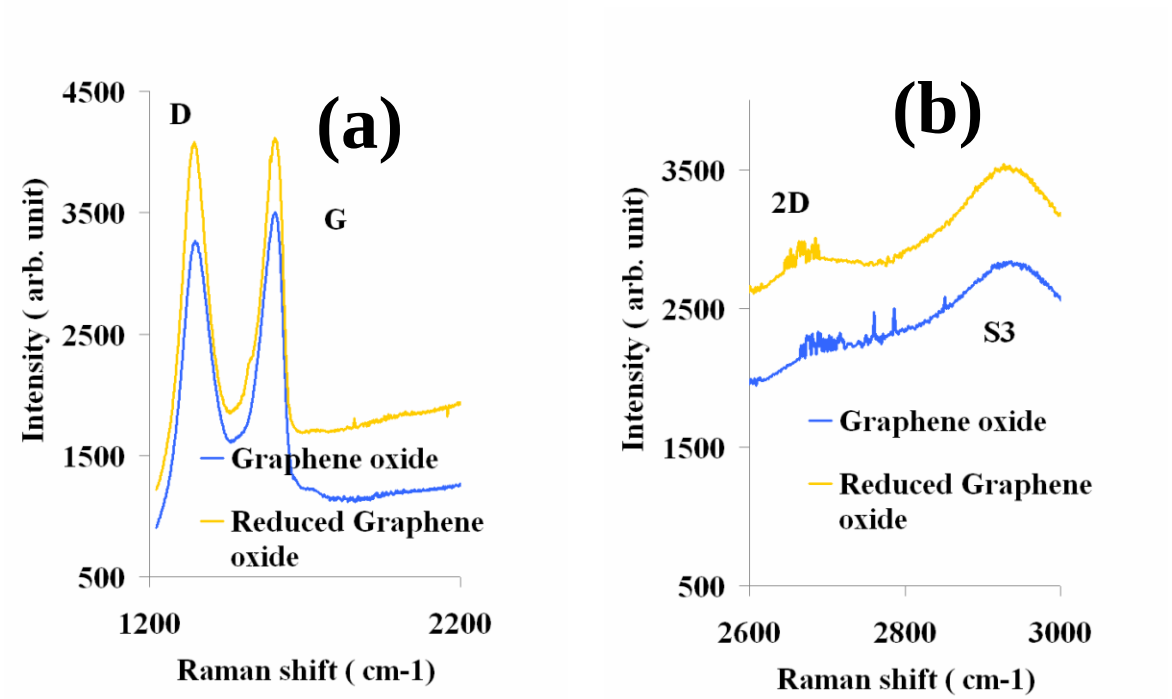
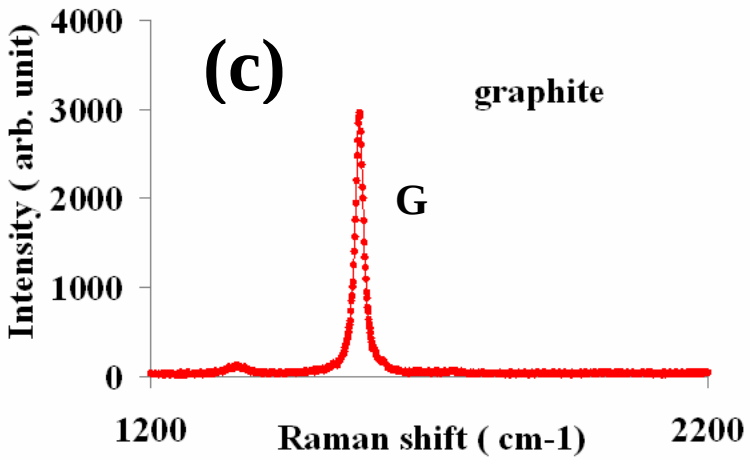


Figure S-1: AFM images of the GO sheets. (a) Height image of GO sheets, micron scale sheets with thickness of 1-1.25 nm are observed. (b) The phase image shows little contrast due to silicon substrate. (c) The height profile along the line shown in (a), provides a thickness of ~ 1-1.25 nm for the sheets

The Raman spectrum for the GO sheets shows the typical G and D bands at ~ 1600 cm^{-1} and 1363 cm^{-1} respectively (Fig. S-2a). The presence of D band signifies the presence of oxidized region (unordered) in the sp2 hybridized matrix of graphene sheets. On reduction to graphene the relative intensity of the D/G bands increases from 0.93 to 0.99 due to a reduction in the average size of the ordered regions. This occurs due to formation of small ordered regions from the previously unordered oxidized regions in the sheets.[5]

The 2D band is observed as a shoulder at ~2720 cm^{-1} in the graphene oxide as it is only partially oxidized form of graphene. On reduction to CRG the intensity of the 2D band increases (Fig. S-2b). For graphite only a very sharp G band at 1574 cm^{-1} is observed due to the sp2 network of carbon atoms.[6] Also observed is a prominent 2D band at 2730 cm^{-1} a characteristic for graphitic material.[6] These observations are consistent with the reported observations on graphene oxide and the effect of reduction. [5, 6, 7]





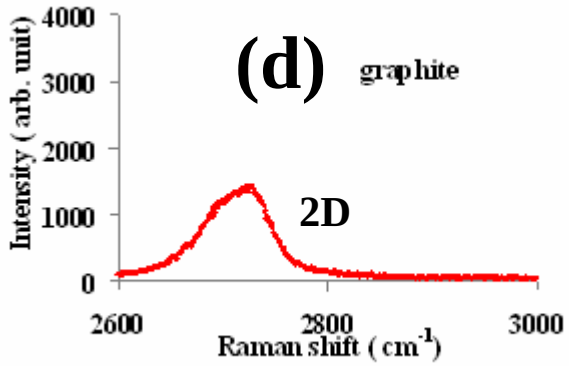


Figure S-2: (a) & (b)The Raman spectra for GO shows the typical G band at 1600 cm^{-1} and the D band at ~ 1363 cm^{-1}. On reduction to CRG both the bands are still observed but the D/G intensity increases. The 2D band is observed at 2720 cm^{-1} as a weak shoulder in the GO and CRG samples. Its intensity increases on reduction of GO to CRG. (c) &(d) For graphite sample a very prominent G band is observed ~ 1574 cm^{-1}. Also observed is a very sharp 2D band at 2730 cm^{-1}.

There is minimal change observed in the fluorescence spectrum of the FUN1 dye after incubation with CRG-Ca-Au sheets (Fig. S-3). The primary effect is only a reduction in the fluorescence intensity. This is a common effect observed with graphene sheets, where the absorbance of dye molecules on the sheets leads quenching.[8]

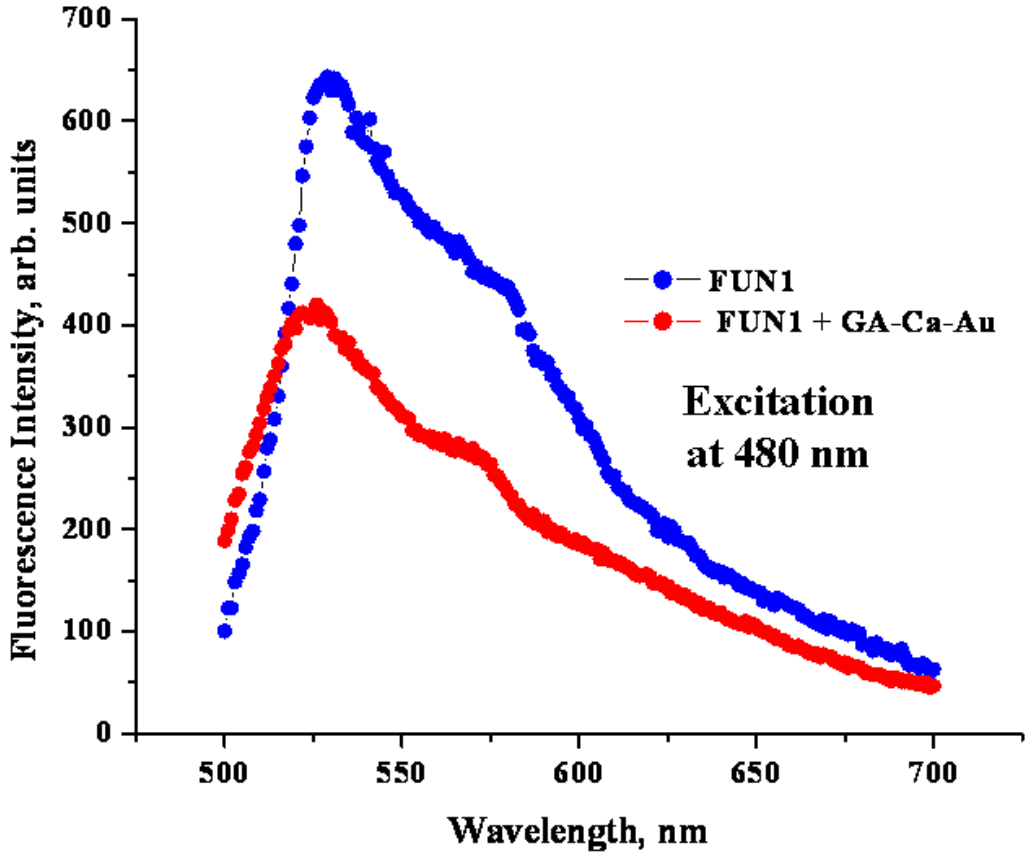


Figure S-3: Both plain FUN1 dye and FUN1 incubated with CRG-Ca-Au solution shows solution shows the characteristic emission at ~520 nm (green) and a shoulder at ~570 nm. No shift in fluorescence is observed for the dye on incubation with CRG-Ca-Au solution.

Due to the reduction in cell volume in FESEM, the graphene sheets not only wrinkle but also the spacing between them decreases.

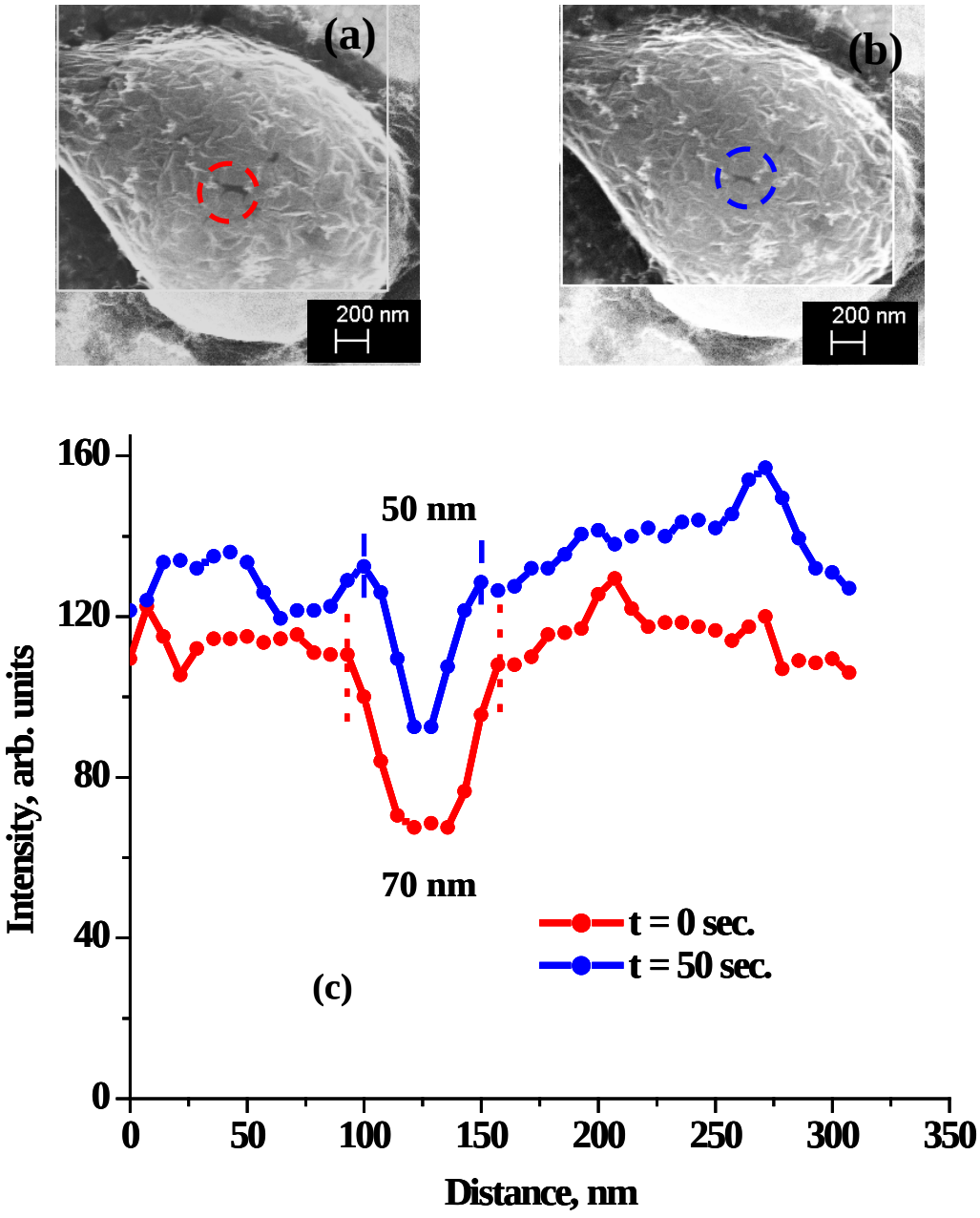


Figure S-4: FESEM images of CRG-cell. (a) FESEM image of the GRC-cell after 4 minutes in FESEM. Encircled is an opening between the GRC sheets on the cell. (b) After further 3 minutes in the high vacuum of FESEM the opening shrinks. (c) From the lines scan it is calculated that the opening shrinks from ~ 70 nm to 50 nm in 3 minutes.

Plain SaC cells and CRG sheets fail to interface in absence of Ca-Au nanoparticles, as seen in Fig. S-5.

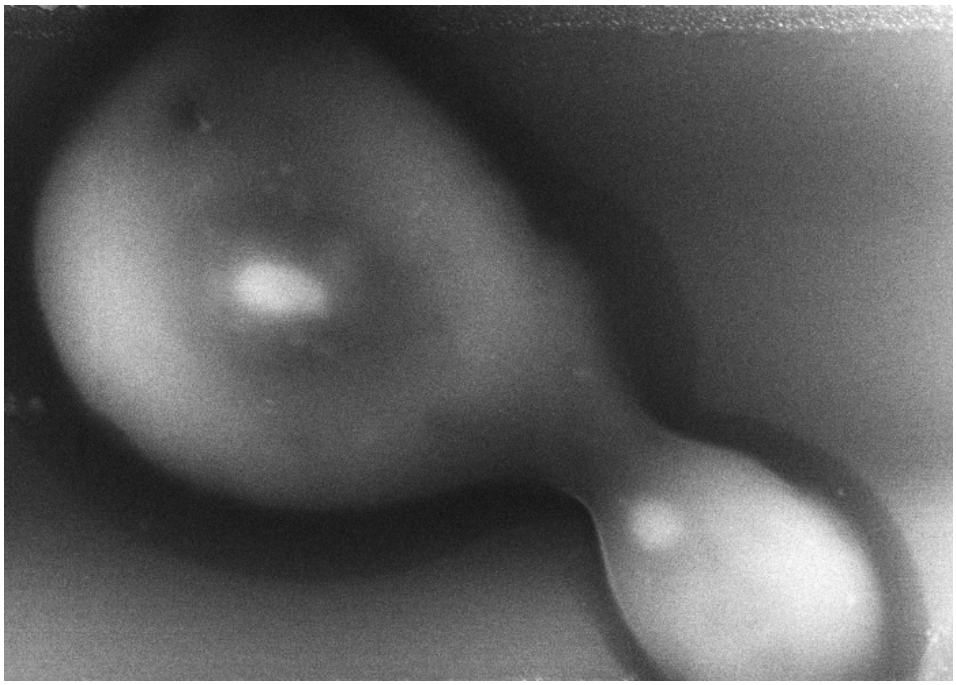


Figure S-5: FESEM image of SaC cells showing that they do not interface with GRC in absence of Ca-Au nanoparticles.

The binding between SaC cells and GO-Ca-Au is qualitatively similar to that of CRG-Ca-Au (Fig. S-6). However when GO is reduced to CRG its conductivity increases by more than three orders of magnitude.[9] This is important for application of these cells as electrical devices and sensors. The reported Young's modulus for CRG is ~ 0.3 TPa and that of GO is ~ 0.6 TPa, hence their mechanical properties are similar.[10]

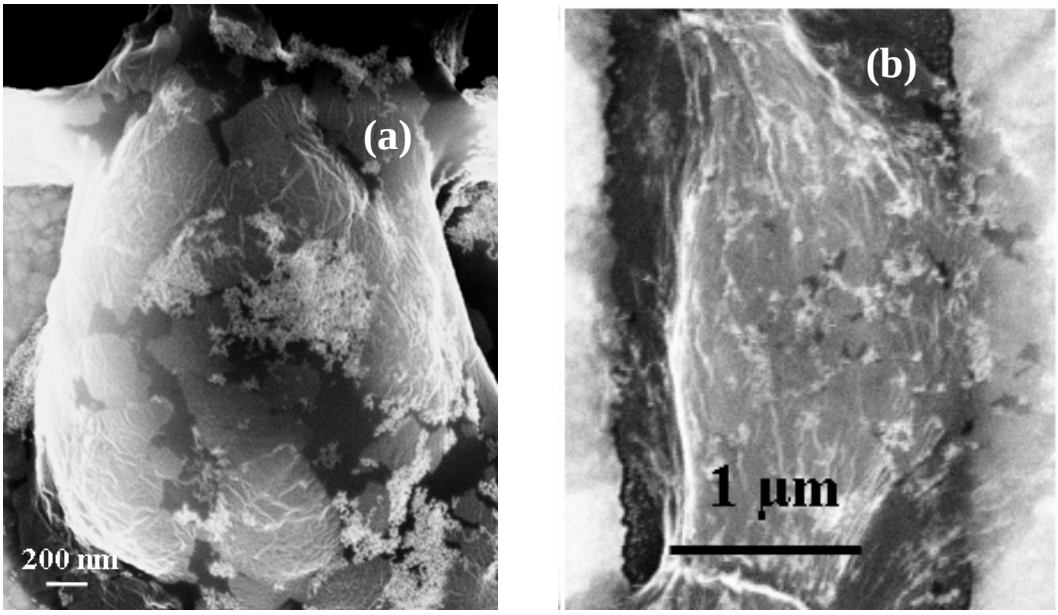


Figure S-6: FESEM image show that SaC bind with GO-Ca-Au sheets with qualitatively identical results as observed with CRG-Ca-Au

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