Supporting Information

Cucurbit[8]uril-derived graphene hydrogels

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S.1 Materials and Methods

Chemicals and Reagents

All the starting chemicals used herein were purchased from Sigma Aldrich and used as
received unless stated otherwise. All aqueous solutions were made in a milli-Q water with
a resistivity of 18.2 MΩ/cm at 25 °C.
Cucurbit[8]uril (CB[8]), † MV2+-silane, ‡ MV2+C10-COOH, § MV2+C12 § and HEC-DBF5 were
prepared according to literature procedures. Both the silanol molecules (MV2+-silanol and
AMP-r-silanols were obtained after boiling their respective derivative in ethanol for 6 h under nitrogen and ethanol was removed by the reduced pressure.

**Instrumentation**

Transmission Electron Microscopy (TEM) were analysed using FEI Philips Tecnai 20 microscope under an accelerating voltage of 200 kV. The samples were placed on a carbon grid and plotted with filter paper after 30 s and allowed to dry in air. EDS acquisition and analysis were performed on an Oxford Instruments Aztec Energy X-maxN 80 EDS system attached to a TESCAN MIRA3 FEG-SEM. The samples were placed on a black carbon tape on a sample holder and images were taken at an accelerating voltage of 5 kV and a working distance of 5.0 mm. Atomic Force Microscope (Agilent technologies 5500) in tapping mode was used to image graphene sheets. A dilute suspension of graphene was deposited on a freshly cleaned mica substrate and allowed to completely dry at 37 °C in an open atmosphere. The sample was then scanned with a silicon tip (Bruker, OTESPA-R3). Software Gwyddion was used to remove any artefacts and measure graphene sheet thickness.

$^1$H-NMR spectra were recorded on a Bruker Avance III HD NMR spectrometer (400 MHz) and are reported as follows: chemical shift $\delta$ (ppm) (multiplicity, coupling constant $J$ (Hz), number of protons, assignment). $D_2O$ ($\delta_H = 4.79$ ppm) was used as an internal standard. Chemical shifts are reported in ppm to the nearest 0.01 ppm for $^1$H NMR.

ATR FT-IR spectras were recorded using a Perkin-Elmer Spectrum 100 series FT-IR spectrometer equipped with a universal ATR sampling accessory. Raman spectra were recorded using Horiba LabRAM HR Evolution equipped with several excitation lines. Herein, 532 nm laser was used to excite the samples. A 100x objective and a grating of 2400 grooves/nm was used. The instrument was calibrated by using an in-built standard sample. It is important to note here that, the raman spectra of GR-MV$^{2+}$ was measured after drop-casting solution on the silicone wafer.
X-ray photoelectron spectroscopy (XPS) experiments were carried out on the Thermo ESCA-CAlab 250Xi with Al K-alpha x-ray source. The Shirley background has been subtracted for clarity.

Thermogravimetric analysis (TGA) was carried out with a TA instruments Q-500 thermogravimetry analyzer at a scan rate of 1 °C/min under argon atmosphere.

All rheological sweeps were conducted on an AR-G2 Rheometer (TA Instruments, New Castle, DE, USA) with a 20 mm parallel plate geometry between 20.0 – 20.5 °C. Environmental temperature was recorded using the built in platinum resistance thermocouple in standard AR-Series Peltier lower plates. Zero gap, rotational mapping (precision bearing mapping; 2 iterations), geometrical inertia and friction calibrations were done prior to each use of the rheometer. Hydrogel samples were loaded onto the rheometer with a 600-1000 μm loading gap. Water was placed in the 20 mm parallel plate trap to minimize dehydration.

**General procedure to exfoliate graphite into graphene in water**

The stable and non-flocculated solution of graphene was obtained by using MV$^{2+}$-silane. To this end, 100 mg of graphite with 10 mg of MV$^{2+}$-silane were dispersed in 50 mL water and sonicated for 9 h at 15 °C. The solution was left to settled down for 15 d, decanted and centrifuged at 5k rpm for 1 h and further decanted. Finally, the solution was dialysed for 9 d against water to remove unbound MV$^{2+}$-silane from the solution and afforded an exfoliated-graphene solution called GR-MV$^{2+}$. The concentration of the solution was found to be 0.035 mg/mL. Apparently, the defects mediated carboxylic and hydroxyl groups formed after the ultrasonication would help MV$^{2+}$-silane to covalently bound on the surface of GR. Based on our reagent amounts for graphite and MV$^{2+}$-silane on estimates of the graphene surface area, a reasonable estimate of the tethered density is 0.1 MV$^{2+}$-silane/nm$^2$. The tethered-chain spacing on the surface is then approximately 2-3 nm, which is smaller than or comparable to the size of the tethered MV$^{2+}$-silane. With such a spacing, decreasing the
tethered-chain spacing (increasing the surface density) has a significant entropic cost.\textsuperscript{6}Thus, tethering MV\textsuperscript{2+}-silane to only one side of the graphene is entropically unfavorable. Because we sonicate for 9 h, we believe the MV\textsuperscript{2+}-silane has sufficient time to access the favorable configuration of tethering to both sides.

\textbf{General procedure to obtain CB-mediated supramolecular hydrogel}

Firstly, 1mg of CB [8] was added to 1 mL of GR-MV\textsuperscript{2+} (0.125 mg/mL) solution and sonicated for 10 s. Secondly, 12.5 mg of HEC-DBF was added to this solution. Finally, the mixture was heated (@50 °C) and vortexed to produce a supramolecular hydrogel.
**Impedance Spectroscopy**

Hydrogels were cast between two copper plate electrodes separated by a Teflon spacer (0.5 mm), where the active area was fixed at 0.85 cm$^2$. Potentiostatic electrochemical impedance spectroscopy (PEIS) spectra were recorded using a BioLogic VSP Potentiostat from 1 MHz to 1 Hz with an applied amplitude of 20 mV$_{rms}$ relative to the open circuit potential. Raw Nyquist plots were fit using the EC-Lab ZSim Software (BioLogic) to a modified Randle’s circuit model, $R_s + Q/R_{ct}$, where $R_s$ is the solution resistance, $Q$ is a constant phase element acting as a non-ideal capacitor, and $R_{ct}$ is the charge transfer resistance. Conductivity was calculated from the modeled $R_s$ values using the cell geometry according to Eq. 1, where $K$ is the cell constant, $l$ is the gel thickness and $A$ is the active area.

$$\sigma = \frac{l}{R_s A} = \frac{K}{R_s} = \frac{0.06 \text{cm}^{-1}}{R_s}$$ (1)

**Biological Assays**

Cytotoxicity of gels was screened against normal adult mouse neural stem cells (ANS) derived from the subventricular zone of adult black 6 mice. Cells were cultured in DMEM/HAMS-F12 (500 mL; Sigma D8347) with glucose (7.25 mL; Sigma G8644), MEM NEAA 100x (5 mL; LifeTech/Gibco 11140-035), penicillin/streptomycin (5 mL; LifeTech/Gibco 15140-122), bovine serum albumin solution (800 µL; LifeTech/Gibco 15260-037), bMercETOH 50 mM solution (1 mL; LifeTech/Gibco 31350-010), B27 Supplement 50x (5 mL; LifeTech/Gibco 17504-044), N2 Supplement 100x (2.5 mL; LifeTech/Gibco 17502-048), with mouseEGF (peprotech) at 10 ng/ml, humanFGF (peprotech) at 10 ng/ml, and Trevigen Cultrex 3D Laminin1 1 µg/ml. Cells were cultured and passaged on laminin coated T25 flasks. Cells were plated onto laminin coated 96-well plates to generate confluence curves and for immunohistochemistry staining. To incubate cells with the GR based supramolecular gel, the
samples were irradiated in a UV chamber for 1 h before infusing into the media. As a control, the supramolecular gel was dissolved in cell culture media at the noted concentrations and screened with these cells to account for potential heterogeneity. Confluence curves were generated using an IncuCyte® S3 Live Cell Analysis System. Percent confluence was calculated using Standard Analysis (n=4/well).
**Supporting Figures**

**Figure S2**: (a) Demonstrating that a hydrogel with CB[8] is achieved with dialysed graphene solution whereas, (b) hydrogel is not formed without dialysis of graphene solution. (c) Oscillatory strain sweep, plotting $G'$ and $G''$ against strain at 1 rad/s, and (d) frequency sweeps of a solution shown in vial b, plotting $G'$ and $G''$ against frequency at 20 °C in the linear viscoelastic region.
**Figure S3:** TEM images of GR-MV$^{2+}$. Different sizes and thicknesses of GR nanosheets can be readily seen.

**Figure S4:** Atomic force microscopy (AFM) images of GR-MV$^{2+}$. (a) Graphene sheets with small graphene/carbon particles. (b-d) Graphene sheet at the different locations on mica, (e) Corresponding height profile of exfoliated graphene sheets shown in B-D. This clearly confirms that after the exfoliation there are upto 5 sheets together.
Figure S5: XPS survey spectra of graphite and GR-MV$^{2+}$. Spectra clearly shows that graphite predominantly has carbon species, C1s at 284.5 eV (~90%) and little oxygenated species, O1s at 532.9 eV (~1%). GR-MV$^{2+}$ also has C1s at 284 eV (~92%) and O1s at 532 eV (~4%). Additionally, we found that GR-MV$^{2+}$ also has nitrogen, N1s at 399.5 eV (~2%), silicon, Si2p at 101.7 eV (~1%) and halides elements. Presence of nitrogen and silicone species is only possible if MV$^{2+}$-silane is present on the surface of GR. Higher intensity of O1s peak in GR-MV$^{2+}$ than graphite one, confirms that during the exfoliation process oxygenated functional groups were introduced on the surface of exfoliated GR.
Figure S6: Narrow XPS spectra of graphite and GR-MV$^{2+}$. (a) and (b) deconvulated C1s spectra of graphite and GR-MV$^{2+}$, respectively. The binding energy (BE) peak at 284.4 eV for C-C species is the same in both materials. The peak at 285.7 eV of -C-O species and at 290.0 eV for ester species and $\pi-\pi^*$ shake-ups of GR-MV$^{2+}$ is broader and more intense than that is in graphite. This clearly shows that after the sonication, exfoliated graphene has been oxidized and have higher density of oxygenated functional groups than graphite (consistent with the survey spectra). However, considering C1s spectra of GR-MV$^{2+}$ and comparing it with graphite one, we can say that an exfoliated GR still have very good graphene structures (high density of $sp^2$ carbon) and the defects on the surface are limited. (c) The narrow N1s spectra of GR-MV$^{2+}$ depicts two different nitrogen species one at BE at 401.5 eV is from viologen moiety and another one at 399.6 eV is from amide moiety of MV$^{2+}$-silane molecule. On the other hand, (d) the narrow Si2p spectra also depicts two silane species. The first one at a BE of 102 eV represents the bond of silicon with oxygen originating from the GR, (-Si-O-GR), the second one at 102.7 eV is apparently attributed to the siloxane (-Si-O-Si-), resulting from the partial hydrolysis of MV$^{2+}$-silane molecules during the silanization reaction on the GR surface.
Figure S7: ATR-FTIR spectra of graphite, MV$^{2+}$ and GR-MV$^{2+}$.

Figure S8: $^1$H-NMR data of MV$^{2+}$-silane and graphite + MV$^{2+}$-silane before and after 9 h of sonication. Upfield shift could be seen in the -RSiCH$_2$CH$_2$CH$_3$R- of graphite + MV$^{2+}$-silane mixture, i.e. GR-MV$^{2+}$. 
Figure S9: EDS mapping and elemental analysis of graphite and GR-MV\textsuperscript{2+}. Silicon was only seen in the GR-MV\textsuperscript{2+} indicating that MV\textsuperscript{2+}-silane was bound to the GR.
**Figure S10**: Oscillatory strain sweeps of controls (no CB and CB[7]) as well as the supramolecular hydrogel system with CB[8]. Plots show $G'$ and $G''$ at 20 °C and 1 rad/s. The storage and loss moduli for the CB[8] sample overlap.
Figure S11: Once HEC-DBF was mixed with GR-MV$^{2+}$ the controls and hydrogel formed with CB[8] was kept for 7 d to observe graphene stability in the matrix. Interestingly, supramolecular composite hydrogel formed after addition of CB[8] did not show any changes and the dispersion remained the same. However, controls with no CB and with CB[7] have shown phase separation and GR sheets readily precipitated from solution.

Figure S12: SEM image of the CB[8]-mediated GR-hydrogel, Scale bar: 100 micron
Figure S13: GR-MV\textsuperscript{2+} stability in the presence of Na\textsubscript{2}S\textsubscript{2}O\textsubscript{4}, which is a reducing agent for MV\textsuperscript{2+}. Once reduced, MV\textsuperscript{2+} forms 2:1 homoternary complexes with CB[8]. After complex formation, GR-MV\textsuperscript{2+} starts to flocculate and precipitation can be seen in the second image from the left. Whereas, solubility of GR-MV\textsuperscript{2+} remains the same without any CB and with CB[7] within the same time frame.
Figure S14: The water solution in each vial is the supernatant of the respective solution after 9 h sonication at 15 °C and spin down at 5k rpm for 1 h. Several controls have been carried out to see the importance of MV$^{2+}$-silane as a surfactant. Flat molecules with the same chain length and silane moieties AzoB-silane and Np-Silane could not exfoliate graphite. This could be due to the $\pi-\pi$ stacking between the flat organic molecules and graphene that could not form a stable dispersion of GR. Similarly, graphite, and MV$^{2+}$ and other small-neutral aliphatic silane molecules like AMpr-Silane could not exfoliate the graphite into GR.
The water solution in each vial is the supernatant of the respective solution after the 9 h sonication at 15 °C and spin down at 5k rpm for 1 h. Here, the viologen moiety was kept, however, the chain length and silane group were removed. Clearly, MV$_2^+$-silanol could not exfoliate graphite. This indicates that silane (triethoxysilane) plays a crucial role and forms a covalent bond at the surface of GR after hydrolysis. Surprisingly, other molecules like MV$_2^+$C$_{12}$ and MV$_2^+$C$_{10}$-COOH with long aliphatic chains could not exfoliate graphite into graphene. Considering our observations while doing $^1$H-NMR (Figure S2) and keeping previous reports in mind, we found that hydrolysis and condensation of triethoxysilane produces ethanol as a byproduct. Thus, keeping conditions the same, we used 1 mL of ethanol with MV$_2^+$C$_{12}$ and MV$_2^+$C$_{10}$-COOH to exfoliate graphite and found that the results were the same, no GR could be seen in the solution. The color in the second vial from the left is the color of the MV$_2^+$-silanol itself.
Figure S16: Representative Nyquist plots from PEIS measurements of the GR-MV$^{2+}$/HEC-DBF systems with no CB, CB[7] and CB[8], experimental points (symbols) and fits (dotted lines): (a) across the full fitted frequency range; (b) in the high-frequency range (indicated above) relevant to the $Z_{re}$ axis intercept and fitting of the solution resistance, $R_s$.

Figure S17: Electrical and cytocompatibility characterization of GR-MV$^{2+}$/HEC-DBF systems. (A) Conductivity of each system measured via potentiostatic electrochemical impedance spectroscopy (PEIS; see Fig. S11 for Nyquist plots). (B) Kinetic growth curves over time of the same systems. (C) Terminal plate confluence of adult mouse neural stem cells after 5 d co-incubated with systems without CB, with CB[7], and with CB[8], **p<0.01.
**Figure S18**: CB[8]-mediated GR-hydrogels without rhodamine B (left) and with rhodamine B (right). This illustrates that while there are hydrophobic components, water-soluble small molecules do not crash out.
References


