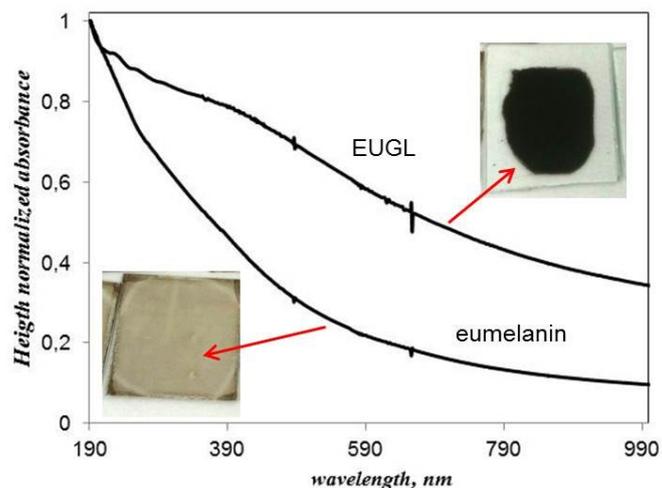


## Supporting Information

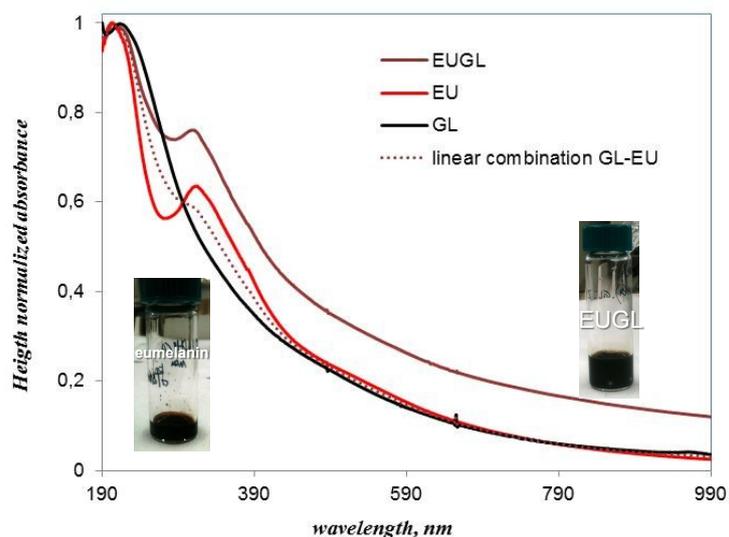
### **Supplementing $\pi$ -Systems: Eumelanin and Graphene-Like Integration Towards High Conductive Material for Mammalian Cell Culture Bio-interface.**

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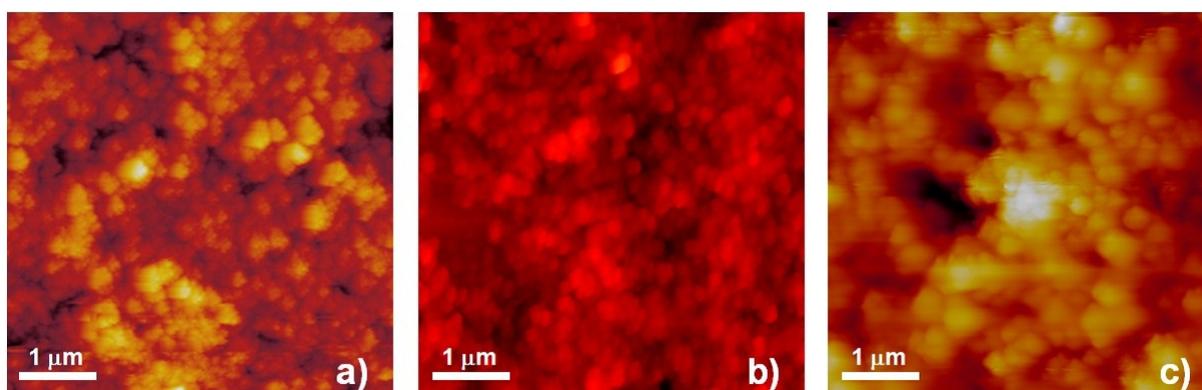
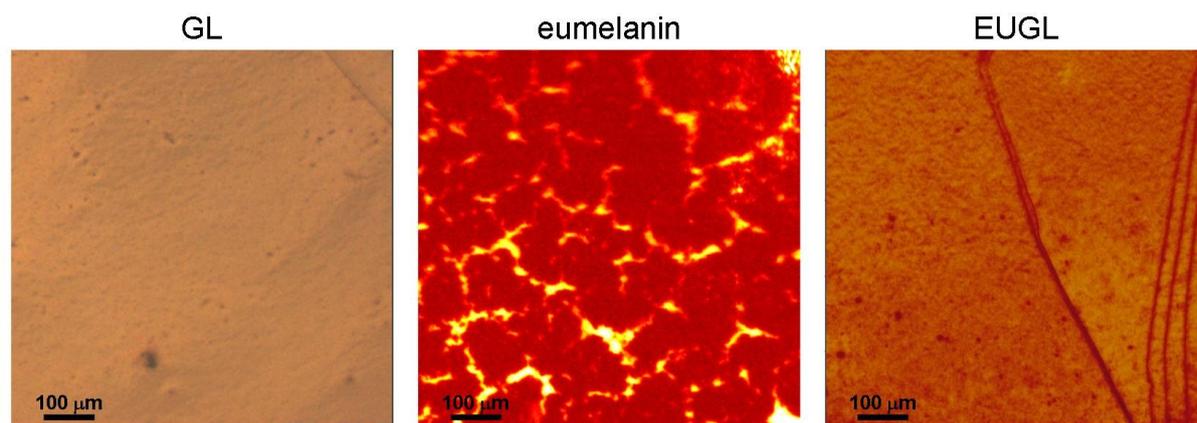
**Figure S1a.** UV –Vis plots of eumelanin and EUGL hybrid films on quartz substrates.



**Figure S1b.** UV–Vis plots of eumelanin and EUGL in water suspension. A 1:1 linear combination of the UV –Vis spectra of GL layers and eumelanin is also reported. Spectra absorption are normalized at 200 nm

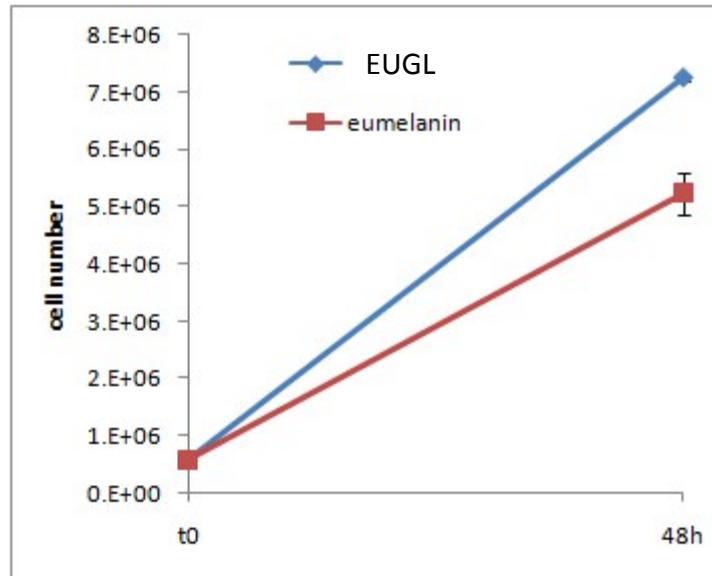
The absorbance ratios (300/600, listed below), evidenced the higher absorption in the visible region of EGUL with respect to neat eumelanin and to a 1:1 linear combination of the GL layers and EU.

Absorbance ratios: EUGL:2,96; EU:4,21; GL: 4,33; linear combination of GL-EU: 4,27.

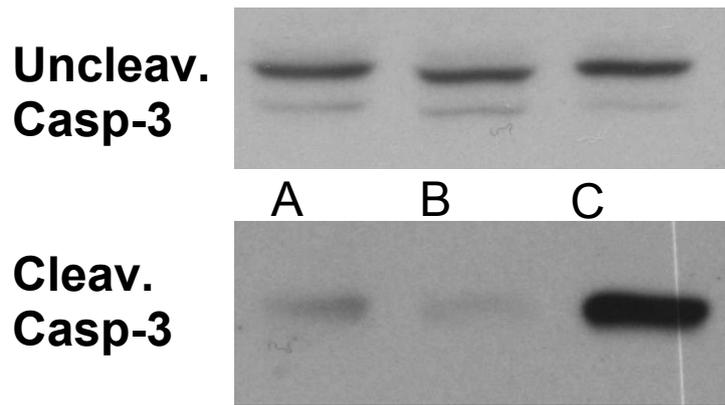


**Figure S2.** Non-contact large scale AFM images of GL layers, eumelanin and EUGL films. The effect of GL/Eumelanin ratio on EUGL film morphology was also inspected for GL/Eumelanin ratio of 1/3; 1/1; 3/1 (images a)-c) respectively)

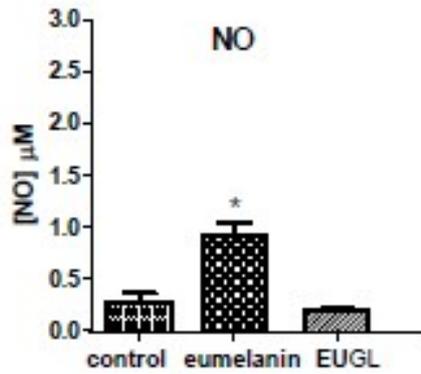
Below, pictures of corresponding mica substrates.



**Figure S3.** Growth curves of ESCs counted at 2 days from plating over EUGL and gelatin.



**Figure S4.** Western blot analysis of uncleaved and cleaved caspase-3. ESCs were grown on (A) gelatin or on (B) EUGL-coated dishes and after 48 hours were collected. The positive control (C) are ESCs exposed to 15Gy of X rays to induce apoptosis.



**Figure S5.** Effects of melanin and EUGL hybrid on rat microglial production of nitric oxide (NO). Primary rat microglial cells were grown on melanin alone and EUGL hybrid for 24h. NO production was evaluated in microglial culture supernatants by measuring nitrite, a stable end product of NO. Values are expressed as mean  $\pm$  S.D (n=4).

## Materials and Methods:

**Eumelanin (EU):**DHI-DHICA mixture (3:1 in weight) was dissolved in methanol by ultrasonic agitation (20 mg/mL).The suspension was kept for 10 min under agitation with a magnetic stirrer afterwards pH was adjusted to 8 by the addition of ammonia solution (28% in water) allowing indole autoxidation and polymerization. After 1 h the reaction was quenched by adding acetic acid (1 M) until pH 4 was established. A EUGL film was obtained by drop casting the suspension onto clean substrates (well plates for biological essays, quartz and mica plates). For bulk analyses the suspension was dried in oven at 80 °C. After drying the EUGL hybrid resulted insoluble in water.

**Graphene Like (GL):**GL layers in water suspension were obtained from carbon black (CB) through a two steps oxidation/reduction strategy<sup>1, 2</sup>.Briefly, 500 mg of CB powder was oxidized with 10 mL of nitric acid (67 wt. %) at 100 °C under stirring for 90 hours. After cooling at room temperature, the aqueous suspension was centrifuged (3500 rpm, 30 min) obtaining a solid dark brown hydrophilic residue. The oxidized hydrophilic nanoparticles (20 mg) were suspended in 20 mL of water and reduced by using 450 µL of hydrazine hydrate (100 °C, 24 h). At the end of the reaction the excess of hydrazine was neutralized with nitric acid (4 M) and the resulting black solid (graphene-like layers, GL) was recovered by centrifugation (3000 rpm, 30 min) and washed with distilled water three times in order to remove all traces of unreacted reagents. The pH of the GL aqueous suspension was 3.70.

## Methods:

Elemental composition (C, H, N wt.%) was estimated by a Perkin–Elmer 2400 CHNSO elemental analyzer. Fourier Transform Infrared (FTIR) spectra were acquired on the powdered sample in the 600–4000 cm<sup>-1</sup> on a Nicolet iS10 spectrometer using the attenuated total reflectance (ATR) method by using a germanium crystal.

The thermal stability of the samples was evaluated by thermogravimetric analysis (TGA) performed on a Perkin–Elmer Pyris 1 thermogravimetric analyzer. The materials were heated in an oxidative environment (air, 30 mL min<sup>-1</sup>) from 50 °C up to 800 °C at a rate of 10 °C min<sup>-1</sup>.

UV–Vis spectra were acquired on an HP 8453 Diode Array spectrophotometer by using 1 cm path-length quartz cuvette (eumelanin and EUGL water/methanol suspensions) and a quartz substrate (dried eumelanin and EUGL films).

The determination of GL surface acidic functionalities was performed adapting a fluorimetric test<sup>1,3</sup> based on specific interaction between the cationic thionin acetate (THA) and carboxylic functionalities of complex carbonaceous materials.

Morphological AFM images were taken by means of an XE100 Park instrument operating in non-contact mode (amplitude modulation, silicon nitride cantilever from Nanosensor) at room temperature in ambient conditions. Film thickness was estimated by scratching the film down to the substrate with a needle and measuring by AFM the height of the resulting trench. Film roughness was estimated as Root Mean Square (RMS) from several AFM scans on the same sample. Samples for AFM imaging were prepared by drop-casting the hybrid in water-suspension onto freshly cleaved mica substrate (grade V-1, Electron Microscopy Sciences).

A four contacts geometry (van der Pauw configuration<sup>4</sup>) was employed to estimate the dc electrical conductivity, while impedance spectroscopy measurements were carried out by supplying ac voltages and measuring the resulting current (amplitude and phase) by using a Stanford Research SR830 lock-in oscillator and amplifier in a standard ratiometric configuration<sup>5</sup>.

**Primary Rat Microglial Cell Cultures** All the animal related procedures were conducted in accordance with European Communities Council Directive n° 86/609/EEC.

Primary microglial cells were obtained from the cerebral cortex of 1- or 2-day old decapitated rats as previously described<sup>6</sup>. Briefly the cortices were dissected and digested for 20 min at 37° C in 0.125% trypsin and for further 5 min in presence of 50 KU/ml of Dnase I.

Cells were plated at a density of  $4.5 \times 10^4$  cells/cm<sup>2</sup> in T75 flasks in 10ml D-MEM/F12 (Dulbecco's modified Eagle's medium/F12) supplemented with 10% Fetal Calf Serum (FCS) (Invitrogen) and antibiotics (100 IU/ml of penicillin and 100 mg/ml of streptomycin). The medium was changed within 24 h, and then twice a week. After 10 -14 days from dissection, microglia was detached from the astrocyte monolayer by shaking, and the cells re-suspended in D-MEM/F12 (10% FCS and antibiotics). Thereafter the cells were placed in 24-well plastic plates at a density of  $5 \times 10^5$  cells/ml, or  $5 \times 10^4$  in cells viability assay, and incubated at 37° C in a humidified atmosphere containing 5% CO<sub>2</sub>. Purity of microglial cell populations (>98%) was verified by staining with IBA-1 (1:1000) antibody. After 24h and 48h the supernatants were collected for lactate dehydrogenase (LDH), MTT and nitric oxide (NO) assessment.

**Cell Viability.** The effects on viability of microglial cells was assessed with two experimental approach. Firstly, the potential cytotoxicity of the two different cell substrates: EUGLand eumelanin alone (as control), was measured as the release from damaged cells of the cytosolic

enzyme lactate dehydrogenase by using a LDH diagnostic kit (Sigma Chemicals Co. ,St. Louis, MO, USA). LDH leakage due to membrane damage was assessed by measuring the activity of LDH in the cells and media as described elsewhere with some modifications<sup>7</sup> .

Microglial cells ( $5 \times 10^4$ ) were seeded into cell culture plates coated with EUGL hybrid or neat eumelanin to test cell viability

Then, 100  $\mu$ l of supernatant was removed from each well and transferred into the corresponding wells of a new 96-well plate.

LDH activity was determined by adding 100  $\mu$ l of reaction mixture (included with kit) to each well and incubating for 30 min in the dark at room temperature. Formation of reduced nicotinamide adenine dinucleotide (NADH), during the LDH-mediated catalysis of the oxidation of lactate to pyruvate, results in an increased absorbance at 490 nm that is proportional to the LDH activity in the sample. Absorbance measurements were taken at 490 nm on a microplate reader with a reference wavelength at 650 nm.

Subsequently, we performed another cytotoxicity test, the MTT test [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)], in order to evaluate the capacity of both the substrates to affect the cell mitochondrial function. Mitochondrial function was evaluated spectrophotometrically by measuring the degree of mitochondrial reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to (aqueous insoluble product) formazan by succinic dehydrogenase (5). MTT assay has greater applicability in the detection of cells which are not dividing but are still metabolically active. It can, therefore be used to distinguish between metabolically active cells and dead cells. (6). In this assay, mitochondrial dehydrogenase enzyme of living cells converts yellow MTT to purple formazan, which is spectrophotometrically measured. In brief, microglial cells at a density of  $5 \times 10^4$  cells/well were seeded in into 24-well plates coated with EUGL hybrid or neat eumelanin. The cells were incubated with MTT (0.5 mg/mL) for 4 h at 37° C. Formazan crystals in the cells were solubilized with DMSO. The level of formazan in each well was determined by measuring its absorbance at 570 nm.

**NO Production by Microglial Cells.** After 24 h NO production in microglial culture supernatants was evaluated by measuring nitrite, a stable end product of NO. Nitrite was determined by a colorimetric assay with Griess reagent. One-hundred microliter of culture medium reacted with an equal volume of Griess reagent (one part of 1% sulfanilamide dissolved in 5%  $H_3PO_4$  and one part of 0.1% naphthylethylenediamine dissolved in distilled water) in 24-well culture plates for 10 min at room temperature. The absorbance was

measured with a microplate reader at 545 nm using a calibration curve of sodium nitrite standards (0.7–50  $\mu$ M).

### **Embryonic Stem Cell Cultures and treatments:**

E14Tg2a (BayGenomics, San Francisco, CA, USA) mouse embryonic stem cells (ESCs) were maintained on feeder-free, gelatin-coated plates (BD Biosciences) in the following ESC medium: GMEM (Glasgow Minimum Essential Medium, Sigma) supplemented with 2 mM glutamine, 100 U/mL penicillin/streptomycin, 1 mM sodium pyruvate, 1  $\times$  non-essential amino acids (all from Invitrogen), 0.1 mM - mercaptoethanol (Sigma), 10% fetal bovine serum (HyClone Laboratories) and 103 U/mL leukemia inhibitory factor (LIF, Millipore). For the growth curve,  $6 \times 10^5$  ESCs were plated in triplicate on 60-mm dishes coated with gelatin or with neat eumelanin or EUGL. The number of cells was evaluated at 2 days and 4 days after trypsinization. Phase contrast images were captured with an inverted microscope (DMI4000, Leica Microsystems) by using LAS AF software.

Neural differentiation was induced adapting the methods of Ying et al., 2003<sup>8</sup>. Briefly,  $\alpha$ 1-tubulin–EGFP cells<sup>9</sup> were plated onto gelatine- or melanin-coated dishes (both EUGL and neat eumelanin) at low density ( $1.5 \times 10^4$  cells/cm<sup>2</sup>) in ESC medium. After 24h the cells were cultured in the following differentiation medium: 1 vol of DMEM/F12 combined with 1 vol of Neurobasal medium, supplemented with 0.5% N2 supplement, 1% B27 supplement, 2 mM glutamine (Invitrogen). Within four days in these conditions the cells undergo neuronal differentiation and start to express GFP.

For apoptosis induction ESCs were irradiated with 15 Gy of X rays by using RS2000 Biological Irradiator (Rad Source) and incubated for 24h before analysis. Apoptosis was revealed by measuring the levels of cleaved caspase 3.

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