Supporting Information

Stem Cell-Compatible Eumelanin Biointerface by Chemically-Controlled Solid State Polymerization

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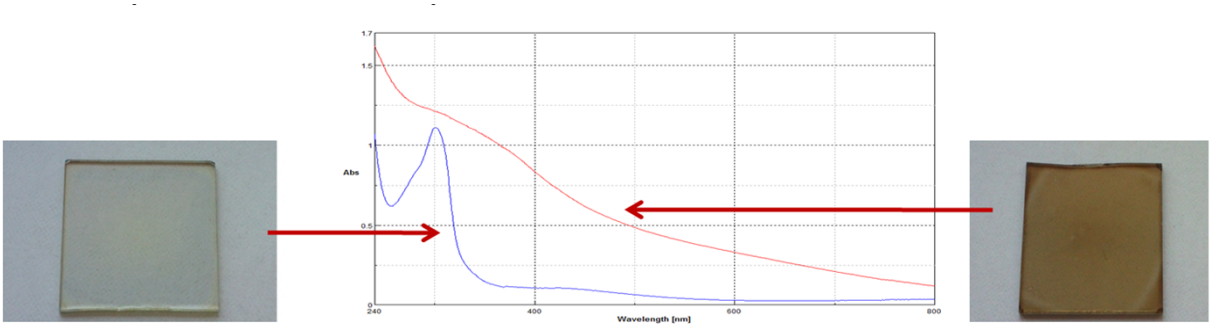
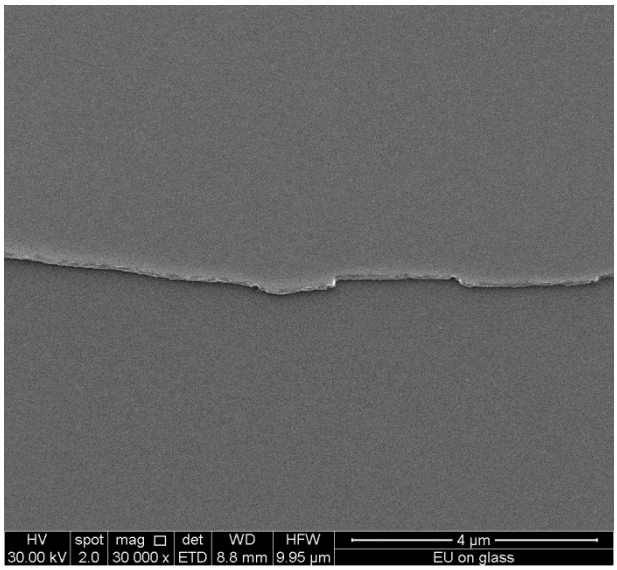
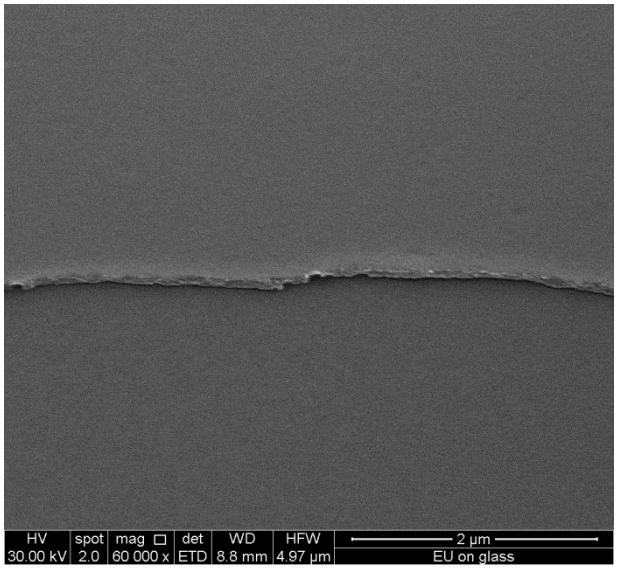


Figure S1. UV –vis plots of DHI and DHI melanin (120 min AISSP time) films on quartz substrates are showed with the corresponding substrate picture: DHI, left and eumelanin: right.





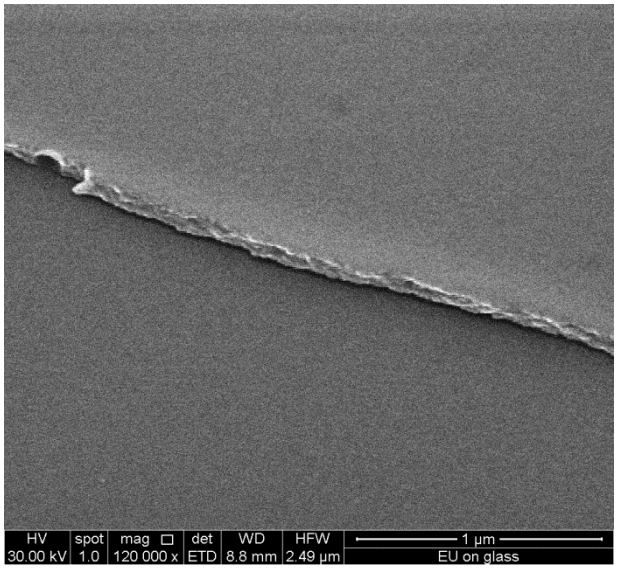


Figure S2. (A) SEM picture of DHI melanin thin film grown on glass. The film has been scratched to inspect the quality of the adhesion.

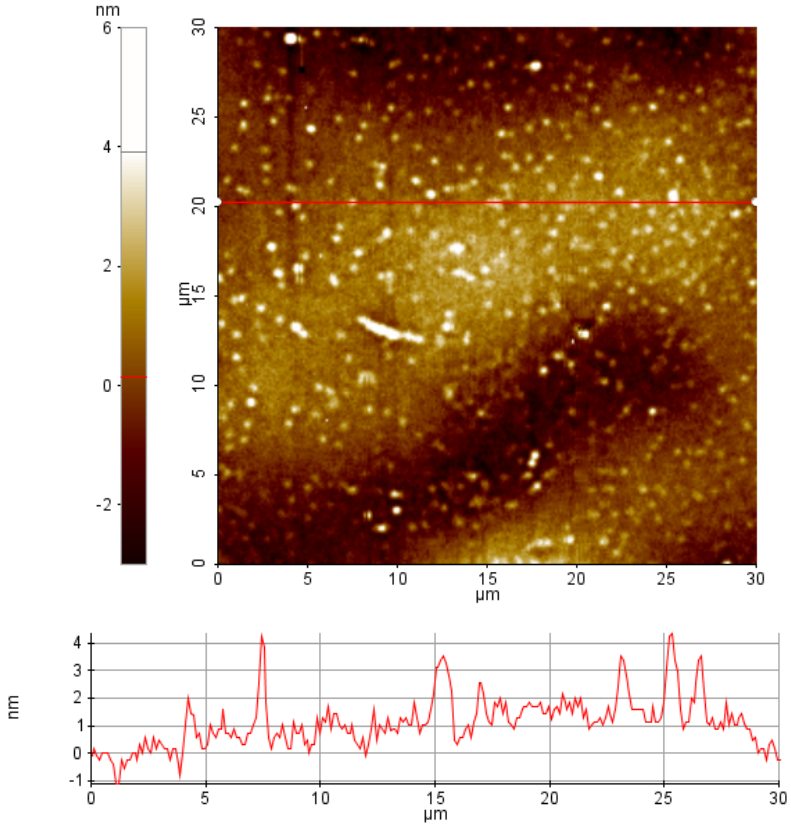


Figure S3. AFM image taken on a 30µm *30 µm scale for DHI melanin thin film grown on glass. Below, the height profile caught from the red line on the image is reported.

DHI units	Pseudomolecular ion peak (m/z)
2	296.4
3	441.2
4	591.3
5	738.5
6	881.4

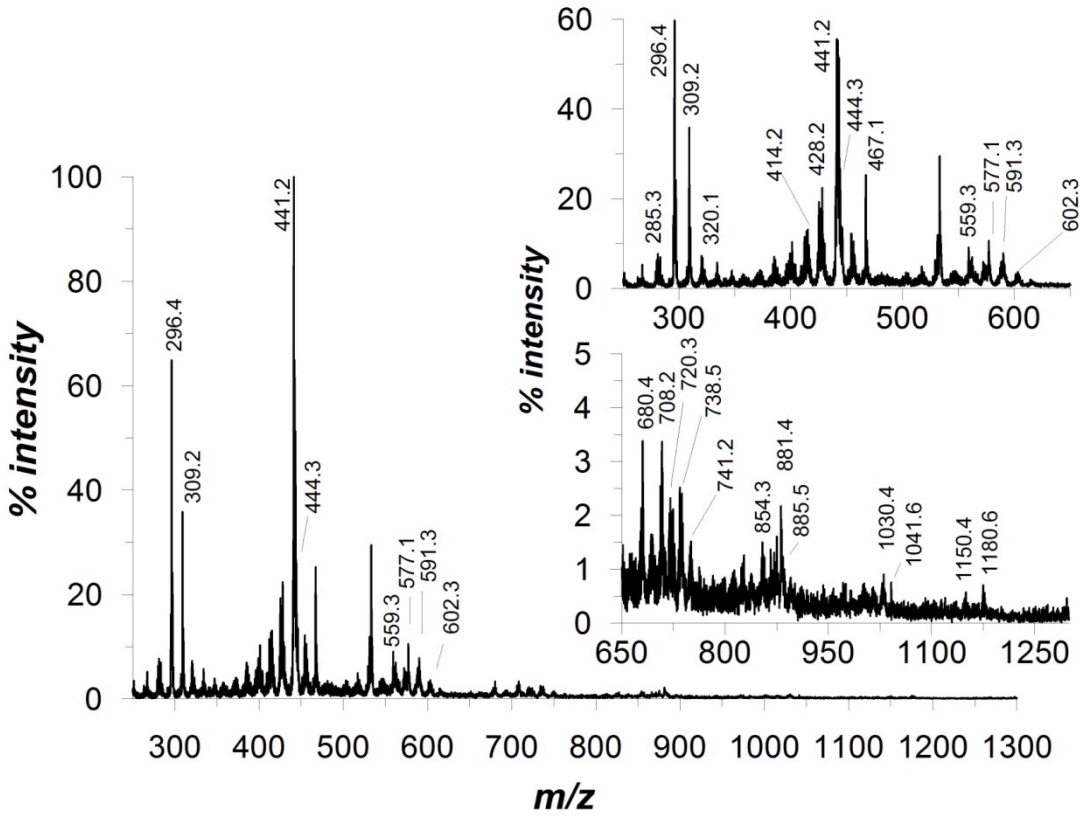


Figure S4. Identification the main components of DHI melanin film detected by comparison of MALDI-MS of .

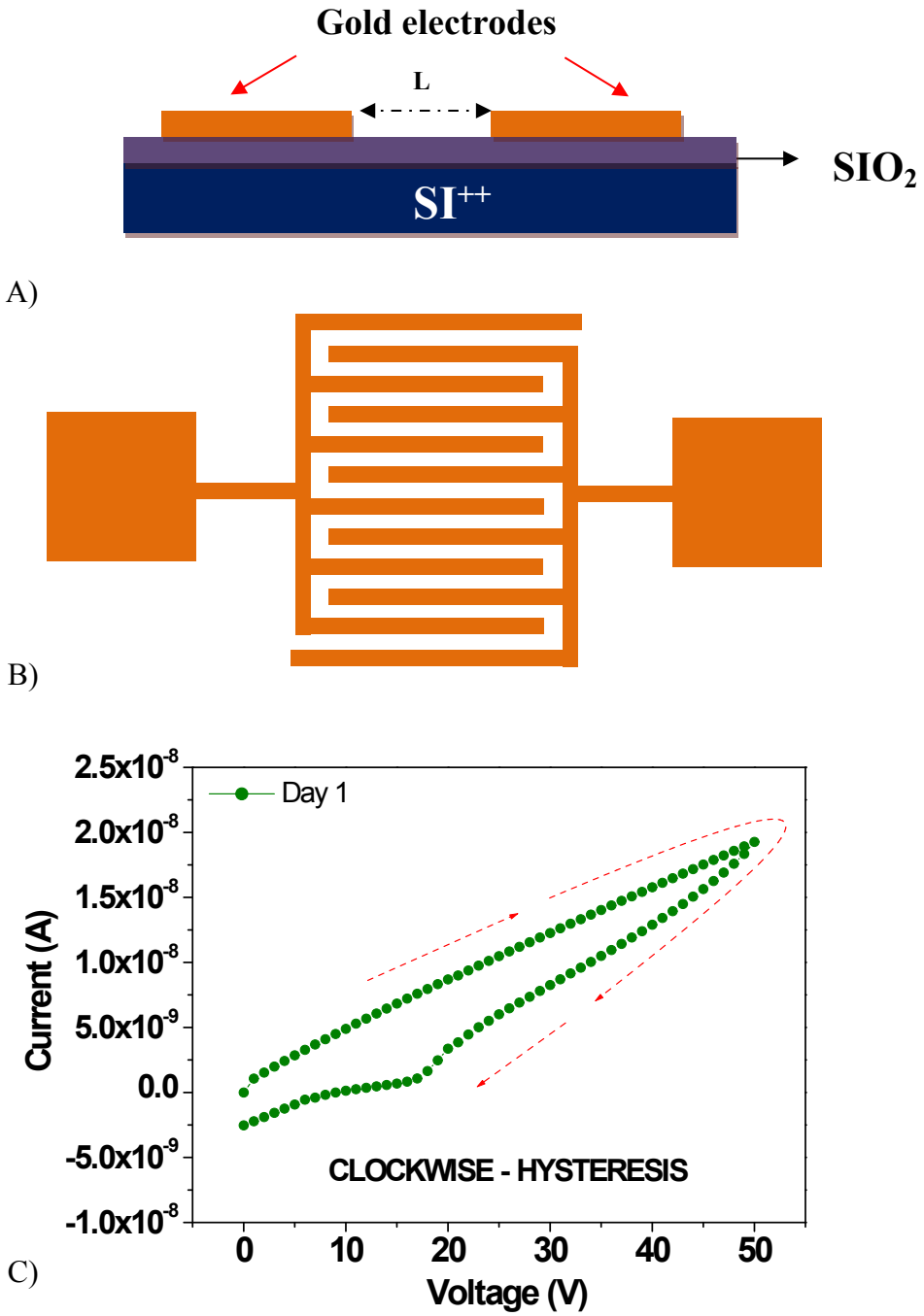


Figure S5. (A) Side view of the highly doped (Si^{++})/SiO_2/Au multilayer substrates used for the electrical tests on DHI-eumelanin films. (B) Layout of the interdigitated gold electrodes. (C) Current-Voltage (IV) curve with hysteresis loop measured for a DHI melanin thin film during the first day of storage in air.

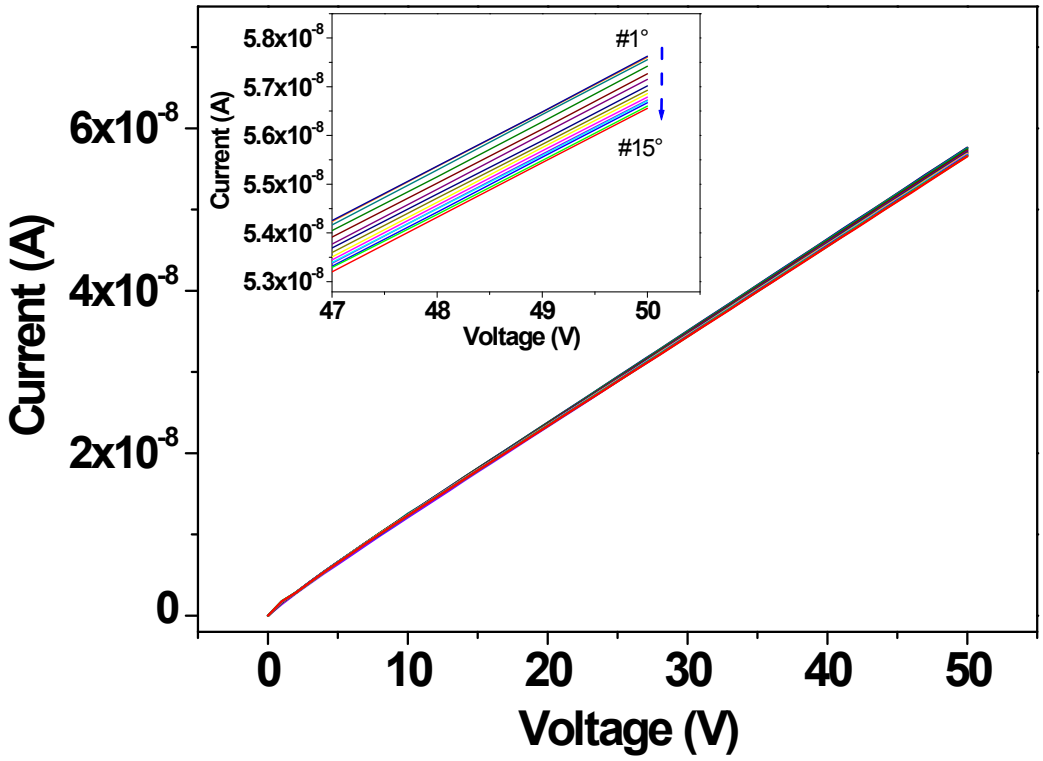
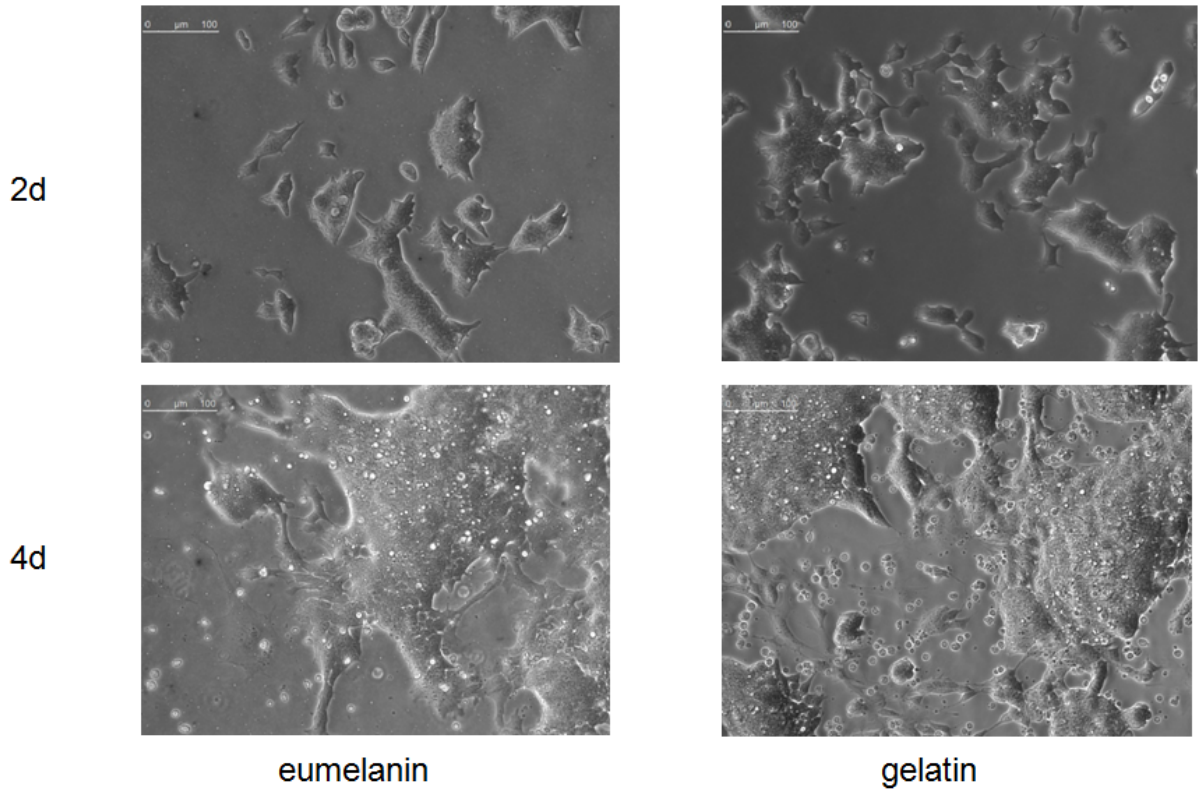


Figure S6. A set of repeated IV curves measured for a fresh AISSP eumelanin channel



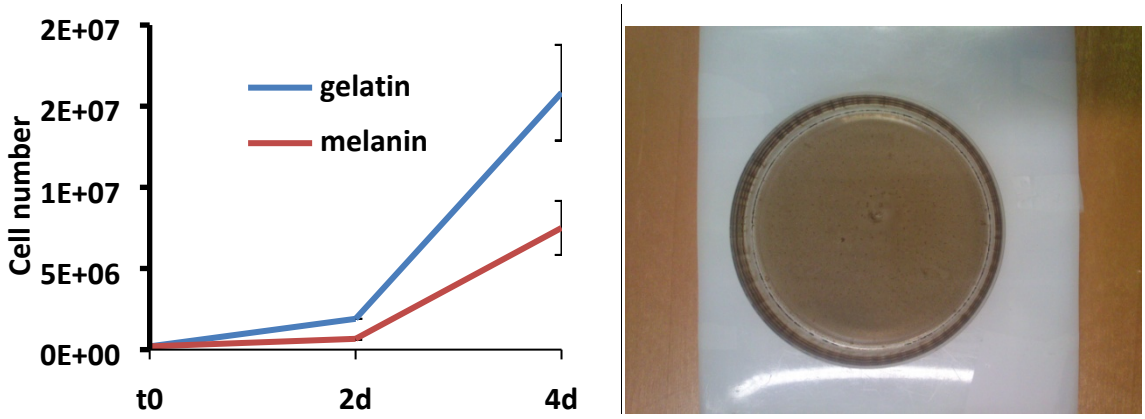
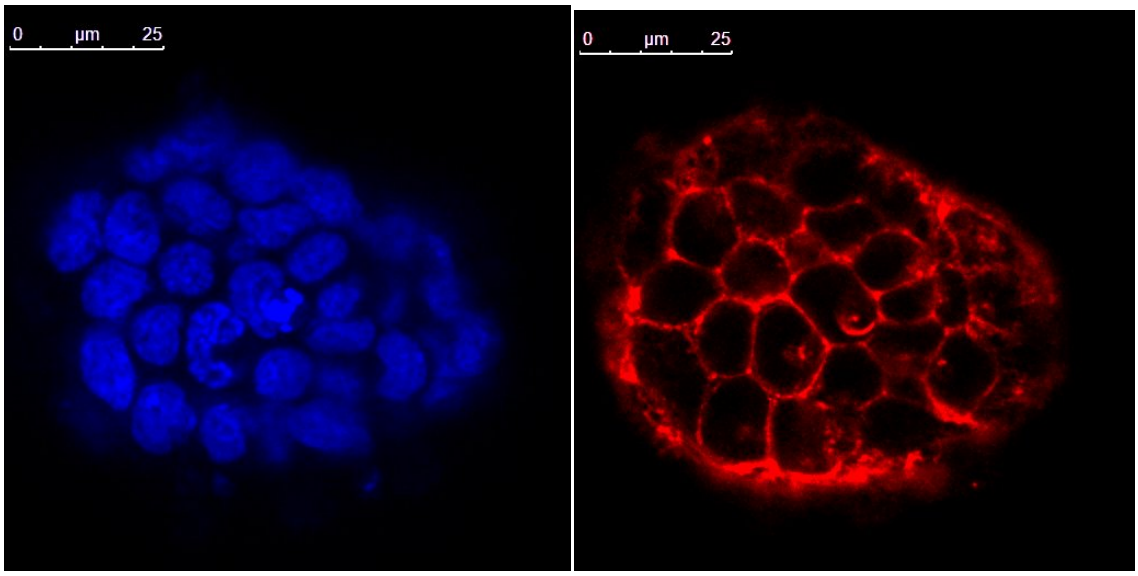


Figure S7. Phase contrast images of ESC colonies seeded on melanin and gelatin for 2 and 4 days (Scale bars: 100µm) and the corresponding growth curves of ESCs counted at 2 and 4 days from plating. The data are reported as mean±standard error.
A picture of an eumelanin coated plate use for seeding is also reported.

EUMELANIN



GELATIN

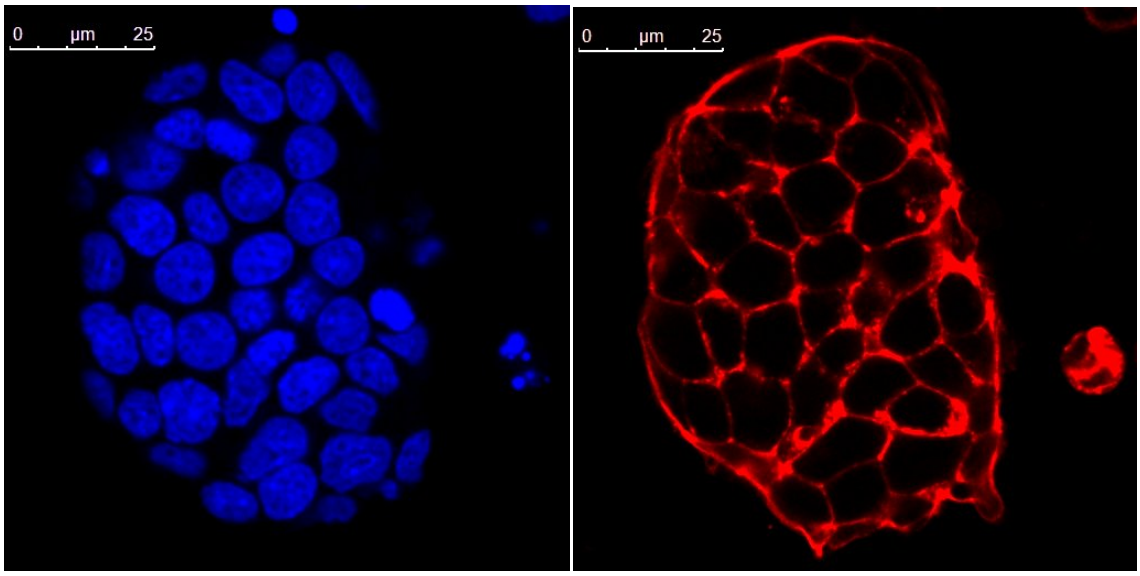


Figure S8. Confocal analysis of undifferentiated ESCs grown for 2 days on eumelanin or gelatin-coated plates and stained with TRITClabeledphalloidin that binds to F-actin.Nuclei were counterstained with DRQ5. Scale bars: 25µm.

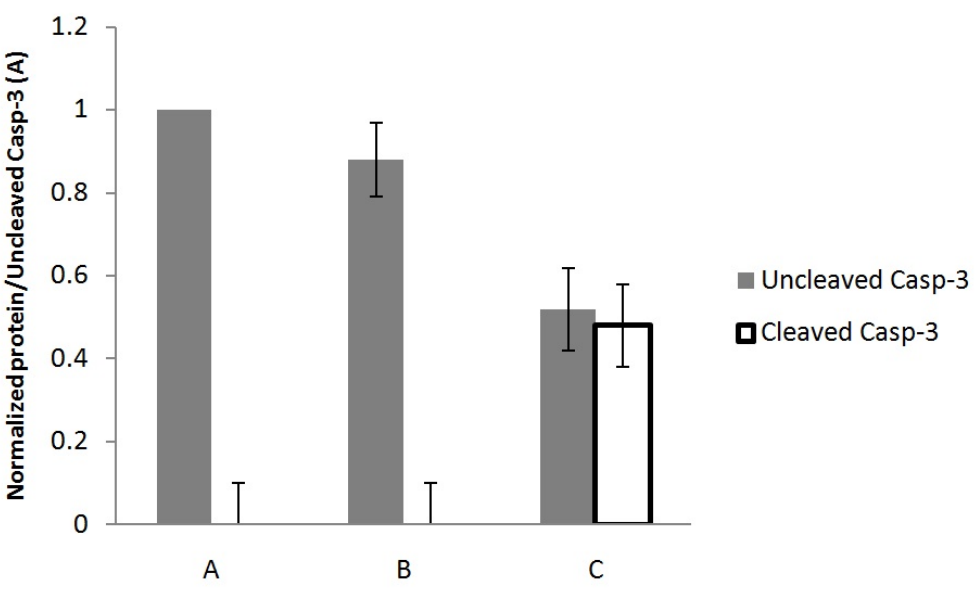
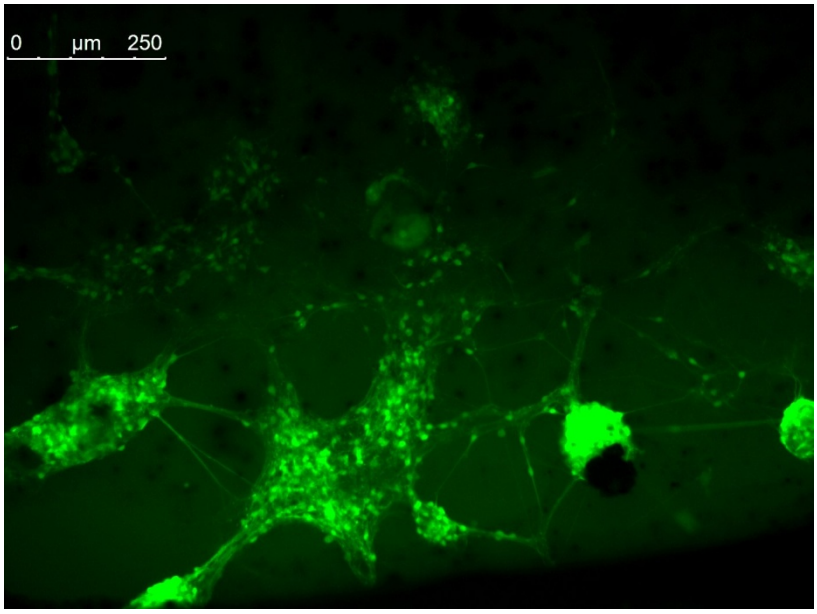


Figure S9. Bar graphs depicting the western blot analysis of uncleaved and cleaved caspase-3.

EUMELANIN



GELATIN

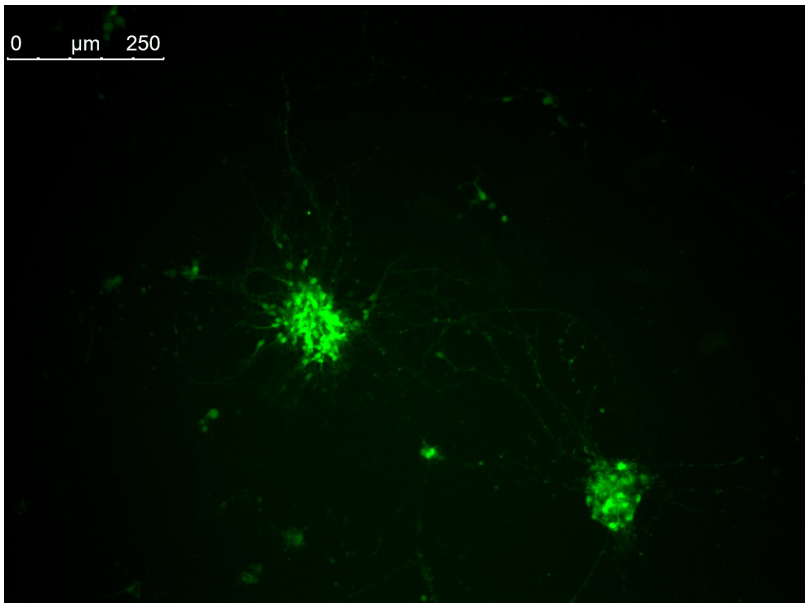


Figure S10. Florescent analysis of neurons after 11 days of differentiation through SFEB formation on eumelanin or gelatin-coated plates. α1-tubulin-GFPESCs were induced to differentiate though SFEBs in differentiation medium for 4 days when the neuronal precursors are fully developed. Then, SFEBs were dissociated and the cells plated on gelatin or melanin and grown for more 7 days in differentiation medium to allow the further differentiation into mature neurons.Scale bars: 250µm.

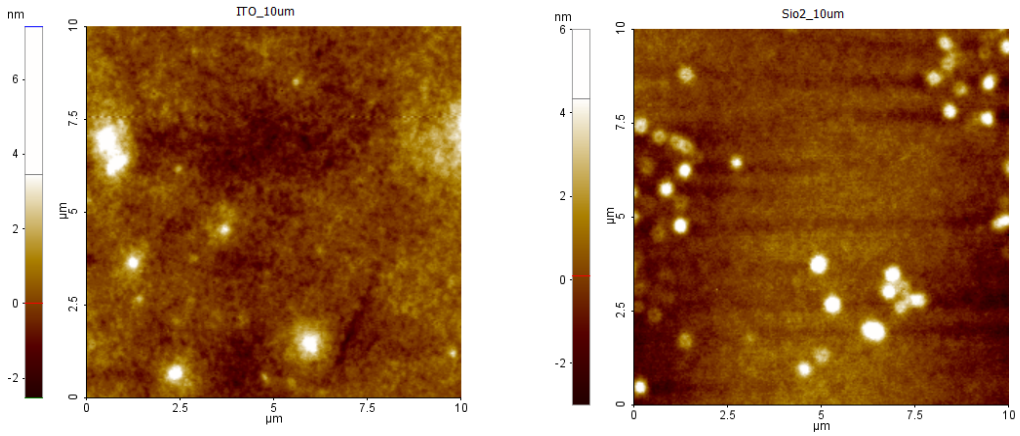


Figure S11. AFM image taken on a 10µm *10 µm scale for DHI melanin thin films grown on (left) ITO and (right) SiO_2 substrates.

Materials and Methods:

All commercially available reagents were used as received and all the solvents were analytical grade quality. Anhydrous solvents were purchased from commercial sources and withdrawn from the container by syringe, under a slight positive pressure of argon. 5,6-Dihydroxindole (DHI) was prepared according to a reported procedure. [1]

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.

Electrical measurements were performed both in air and vacuum (10^{-2} Pa) by using a Janis Probe Station connected to a Keithley 2612A Dual-Channel system source-meter instrument. During all electrical measurements, eumelanin films were kept in darkness. Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionization (AP-MALDI) mass spectra were performed on an Agilent 1100 Series instrument equipped with a MSD Ion Trap (Agilent Technologies, Palo Alto, CA, USA). A nitrogen laser (337 nm, mounted inside a laser and stage control box) produces a 10 Hz pulsed beam of UV light. The ion trap scanned from 50 to 4000 m/z. Each AP–MALDI mass spectrum consists of 100–150 co-added spectra collected on the whole spot surface (1–2 mm in diameter) in order to overcome the lack of homogeneity of the sample on the target plate. Additional details are given in a previous paper. [2]

DHI thin films were prepared by spin coating with Laurell WS-650MZ-23NPP/LITE coater on quartz or on transistor substrates from concentrated methanol solutions (30 mg/mL) after filtering through a 0.2 μm Whatman membrane. Appropriate volume deposition (10 - 50 μL) and speed gradients were used. In some cases the films were annealed at 70 °C for 30' under nitrogen atmosphere. AISSP: Melanin thin films were obtained by exposing the DHI films (100-200 nm thickness) for 18 h to air-equilibrated gaseous ammonia from an ammonia solution (28% in water) in a sealed camera at 1 atm pressure at controlled temperature (25 - 40 °C). Other DHI melanin thin films were produced by previously reported procedure.[3]

Synthesis of dopa-melanin[3]

A solution of DL-dihydroxyphenylalanine (DL-dopa) (10 g, 0.051 mol) in 2 L of deionized water was adjusted to pH 8 by the addition of concentrated ammonia solution. Air was bubbled through the stirred solution for 3 days. Concentrated hydrochloric acid was then used to bring the pH to 2, and the resulting black precipitate was isolated by centrifugation and washed several times with 0.01 M hydrochloric acid and then with deionized water.

DHI Thin Film Deposition:

DHI thin films were prepared by spin coating with a Laurell WS-650MZ-23NPP/LITE coater; thin films were deposited on quartz or on Si^{++}/SiO_2/Au r substrates. Thin films were obtained from a 30 mg/mL solutions of DHI in methanol after filtering through a 0.2 μm nylon membrane, using the following speed gradients: 2000 rpm for 90"; 800 rpm for 10" and 3000 rpm for 60"; 2000 rpm for 60"; and 3000 rpm for 90". In some cases thin films were annealed at 70 °C for 30' under a nitrogen atmosphere.

Ammonia-Induced Solid State Polymerization:

The oxidation of DHI thin films (100-200 nm thickness) to give the melanin polymer has been achieved by exposure to an oxidizing atmosphere (e.g. oxygen atmosphere and ammonia vapors). In the general procedure, the appropriate film was incubated in the oxygen/ammonia atmosphere at controlled temperature (25 - 40 °C). The ammonia vapors were produced by equilibration of the atmosphere with ammonia solution (28% to 7% NH_3 in H_2O) in a sealed camera at 1 ATM pressure. Exposure times varied in the range 2 to 18 h. When appropriate the whole spin coating procedure was conducted under oxidation promoting atmosphere. Oxidative polymerization was followed by UV-vis spectroscopy. Spectra were reordered in the range 240-800 nm at several reaction times form 1 min to 1 day after AISSP was started.

In detail, the film on quartz reported in Figure S1 was obtained with the following parameters:

DHI solution in methanol: 30 mg/mL; speed gradient: 800 rpm for 10" and 3000 rpm for 60"; oxidizing atmosphere: 28% NH_3 in H_2O equilibrated air; oxidation temperature 25 °C; oxidation time: 2 h;

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 × 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, $6x10^5$ ESCs were plated in triplicate on 60-mm dishes coated with gelatin or with melanin. 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. 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.

Embryonic Stem Cell Differentiation:

Neural differentiation was induced adapting the methods of Ying et al., 2003[4]. Briefly, α1-tubulin–EGFP cells[5] were plated onto gelatine- or melanin-coated dishes at low density (1.5x104 cells/cm^2) 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.

ESCs differentiation into serum-free embryoid body (SFEB) formation[6] was induced by placing $1x10^6$ESCs in 100-mm Petri dishes in the following differentiation medium: GMEMsupplemented with 2 mM glutamine, 1 mM sodium pyruvate, 1× nonessential amino acids, 0.1 mMβ-mercaptoethanol and 10% Knock-out Serum Replacement (KSR). After 7 days SFEBs are mainly composed of neuronal precursors and they are plated on gelatin or melanin-coated dishes. After further 3 days the presence of neuronal precursors and mature

neurons (GFP-positive cells) was analysedwith an invertedflorescentmicroscope (DMI4000, Leica Microsystems) byusing LAS AF software.

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.

Antibodies, Western blot analysis and Immunostaining:

For western blot analysis ESCs were lysed in a buffer containing 1 mM EDTA, 50 mMTris-HCl (pH 7.5), 70 mMNaCl, 1% Triton, and protease inhibitor cocktail (Sigma), andanalyzed by Western blot. The following primary antibodies were used: rabbit Cleaved Caspase-3 (1:1000 Cell Signaling), rabbit Caspase-3 (1:1000 Millipore). Antibody protein complexes were detected by HRP-conjugated antibodies and ECL (both from Amersham Pharmacia).

For immunostaining, ESCs were plated on gelatin or melanin-coatedchamber slides (Thermo Scientific) to allow the observation with the confocal microscope. After 48 hours from plating the cells were fixed in 4%paraformaldehyde.The nonspecificblock and permeabilizationwas performed by treatment in 10% FBS, 1% BSA, 0.1% triton and 1X PBS for 10' at room temperature,followed by incubation with The Phalloidin–Tetramethylrhodamine B isothiocyanate(1:500 Sigma). After three washes in PBS 1X for 10' at room temperature the nuclei were counterstained with with DRQ5 (Cell Signaling, 1:1000)

Confocal microscopy was performed with a Leica TCSSMD FLIM microscope (Leica Microsystems) using LAS AF software (Leica Microsystems). When required, the brightness, contrast and color balance of the images were adjusted in Photoshop CS2 (Adobe Systems).

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2. F. Bloisi, A. Pezzella, M. Barra, M. Alfe, F. Chiarella, A. Cassinese and L. Vicari, *Appl Phys a-Mater*, 2011, **105**, 619-627.
3. J. P. Bothma, J. de Boor, U. Divakar, P. E. Schwenn and P. Meredith, *Adv Mater*, 2008, **20**, 3539-+.
4. Q. L. Ying, M. Stavridis, D. Griffiths, M. Li and A. Smith, *Nature biotechnology*, 2003, **21**, 183-186.
5. S. Parisi, F. Passaro, L. Aloia, I. Manabe, R. Nagai, L. Pastore and T. Russo, *Journal of cell science*, 2008, **121**, 2629-2634.
6. S. Parisi, M. Battista, A. Musto, A. Navarra, C. Tarantino and T. Russo, *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 2012, **26**, 3957-3968.