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Synthetic Mycomelanin Thin Films as Emergent Bio-Inspired Interfaces Controlling the Fate of Embryonic Stem Cells

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Materials and methods	S2-S3
Figure S1. FT-IR spectroscopy imaging	S4
Figure S2-S8. AFM study of 1,8-DHN allomelanin thin film morphology	S5-S8
Figure S9. Correlation between the mycomelanin film thickness and the concentration of the 1,8-DHN methanol solution spin coated.	S9

Materials and methods

1,8-Dihydroxynaphtalene was purchased from Sigma and used without further purification.

UV-Vis spectra were recorded with a Jasco V-560 instrument.

MALDI spectra were recorded on a Sciex 4800 MALDI ToF/ToF instrument using 2,5-dihydroxybenzoic acid as the matrix. The laser was operated at 3700 Hz in the positive reflectron mode. The mass spectrometer parameters were set as recommended by the manufacturer and adjusted for optimal acquisition performance. The laser spot size was set at medium focus (B50 mm laser spot diameter). The mass spectra data were acquired over a mass range of m/z 100− 4000 Da, and each mass spectrum was collected from the accumulation of 1000 laser shots. Raw data were analyzed using the computer software provided by the manufacturers and reported as monoisotopic masses.

Micro ATR spectra of the powders in transmission mode have been recorded with a Cary 660 FTIR Spectrometer with a Germanium crystal and a liquid nitrogen cooled Mercurium Cadmium Telluride (MCT) detector in the range from 4000 to 900 cm^{-1}, with 32 scans both for background and samples, with a resolution of 4 cm^{-1}.

FTIR spectroscopic imaging was conducted using an Agilent Cary 620 microscope coupled to a Cary 660 Spectrometer. The microscope was equipped with a Germanium crystal and a 64X64 Focal Plane Array (FPA) detector, cooled with liquid nitrogen. At each image pixel corresponds a unique infrared spectrum, which allows to determine chemical composition in an area of 1.1 μm^2. Spectral range was comprised from 3200 to 800 cm^{-1}, with 256 scans both for background and for sample, with a spectral resolution of 4 cm^{-1}.

Thin layer depositions were performed by spin coating using a Laurell WS-650MZ-23NPP/LITE spin coater.

The AFM investigation was carried out using the Dimension ICON-PT Scanning Probe Microscope from Bruker, that enables imaging at sub-nanometer resolution. The machine was operated as a self-optimizing AFM for high-resolution imaging, by using ScanAsyst Imaging and NanoScope software for automating the Peak Force Tapping mode of operation.

The water contact angle was measured by using a OCA20 Dataphysics instrument and the reported value of WCA is the average of six separate measurements.

Bleaching test

The solution for the bleaching test was prepared by adding hydrogen peroxide (100 mM) in aqueous sodium hydroxide (10 mM). Each substrate, 1,8-DHN allomelanin coated or DHI eumelanin coated) was partially soaked into the bleaching solution and the integrity of the thin film monitored by UV-vis spectroscopy.

Table S1

GENE NAME	FORWARD PRIMER 5'-3'	REVERSE PRIMER 5'-3'
Cxcr4	gtaaccaccacggctgtaga	agtagatggtgggcaggaag
Gapdh	cggagtcaacggatttggtcgtat	gaagatggtgatgggcttcc
Gata2	agctcatgactatggcagca	ccggttctgtccattcatct
Nanog	aaccagtggttgaagactagcaatggtc	ttccagatgcgttcaccagatagc
NeuroD	gctccagggttatgagatcg	ctctgcattcatggcttcaa
Oct3/4	ccgtgtgaggtggagtctggagac	cgccggttacagaaccatactcg
Rex1	cagaagaaagcaggatcgcctc	gccacttgtctttgccgttttc
FoxA2	ctgggagccgtgaagatggaag	tccagcgcccacataggatg
Bry	catcggaacagctctccaacctat	gtgggctggcgttatgactca
Gsg	accatcttcaccgatgagcagc	cttggctcggcggttcttaaac

Statistical analysis

Graphpad Prism 7 software was used to perform statistical analysis. Values are presented as mean ± SD. P-values were determined using a two-tail unpaired t-test. $P < 0.05$ was used as a threshold for statistical discernibility.

FT-IR spectroscopic imaging

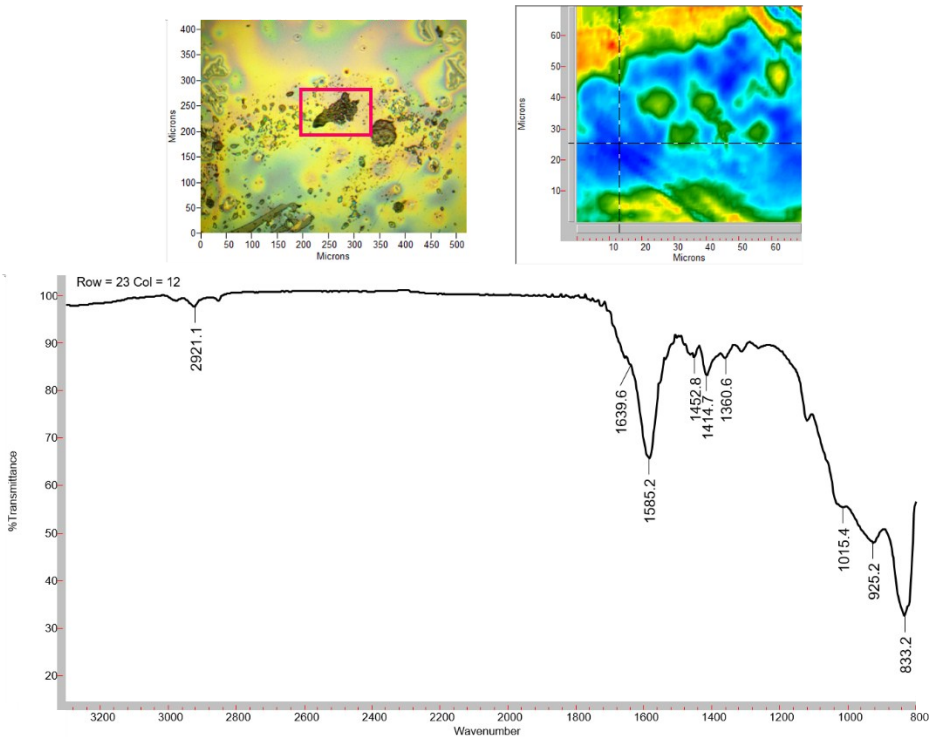


Figure S1. Visible and FPA images associated with infrared spectra (Peak reference at 1585 cm^{-1}: blue-more concentrated, red-less concentrated)

AFM analysis

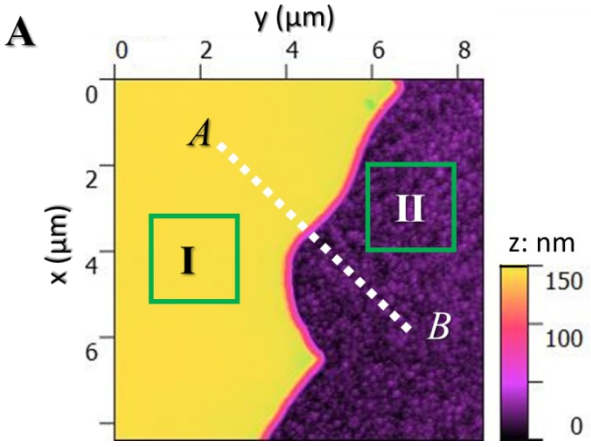


Figure S2.AFM analysis of the mycomelanin thin film from 1,8-DHN. A) Colorplot of one of the AFM maps measured across sharp edges of the film.

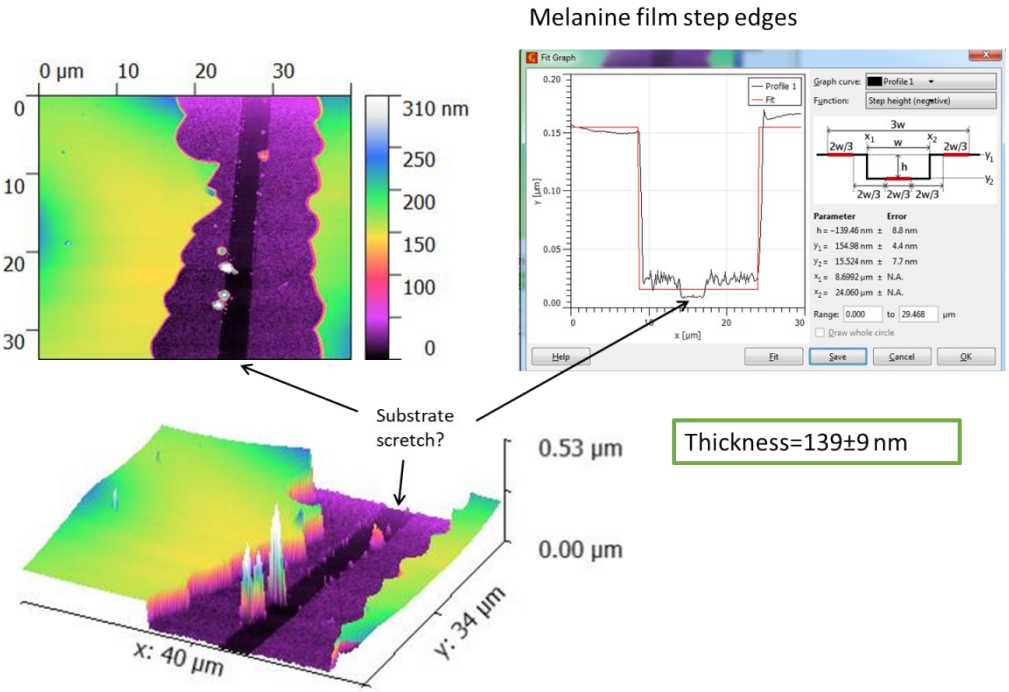
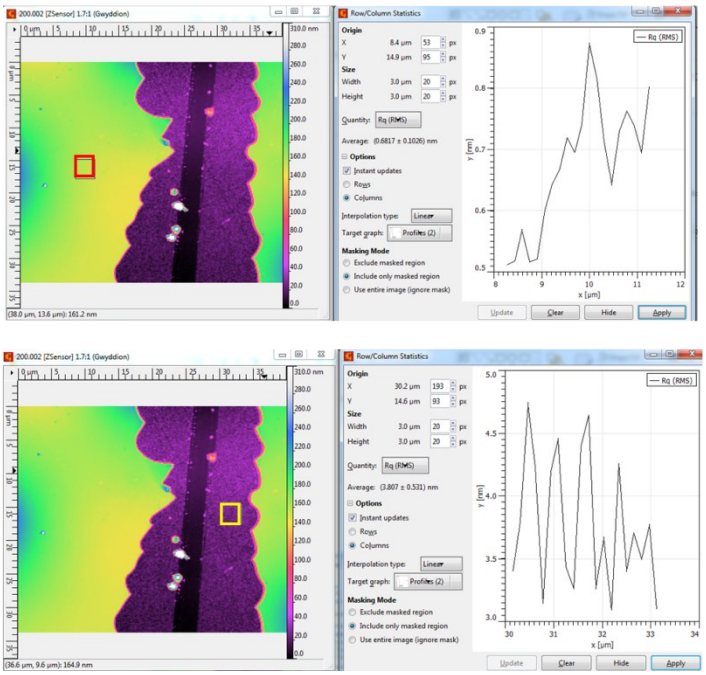


Figure S3. Extensive AFM study

Roughness



film roughness

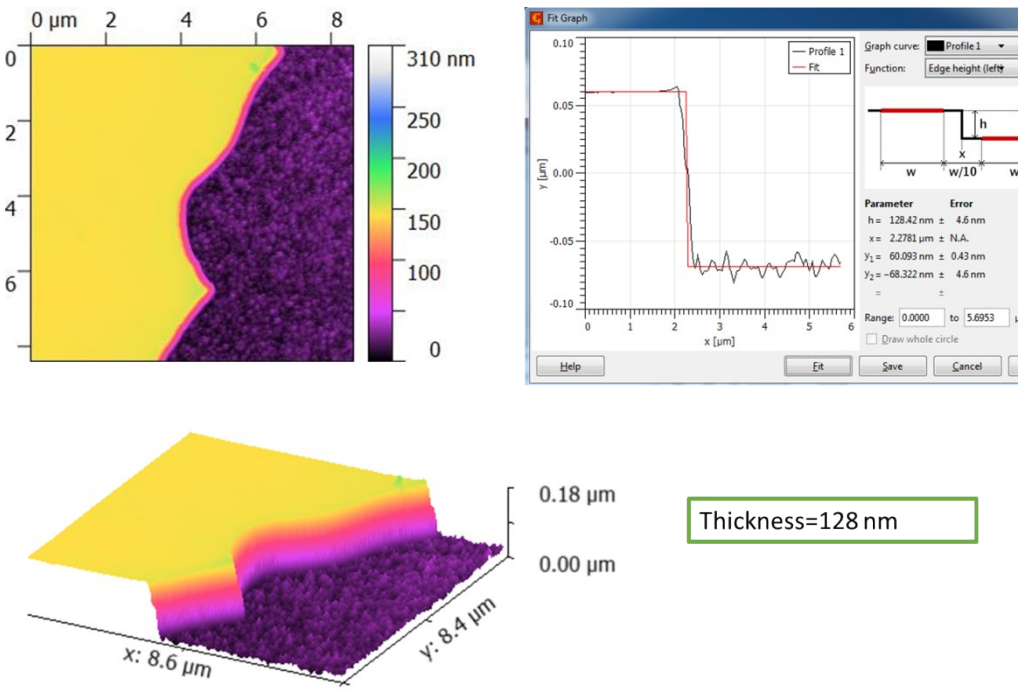
Rms= 0,7±0,3 nm

Substrate roughness

Rms= 3,8±0,5 nm

Figure S4. Extensive AFM study

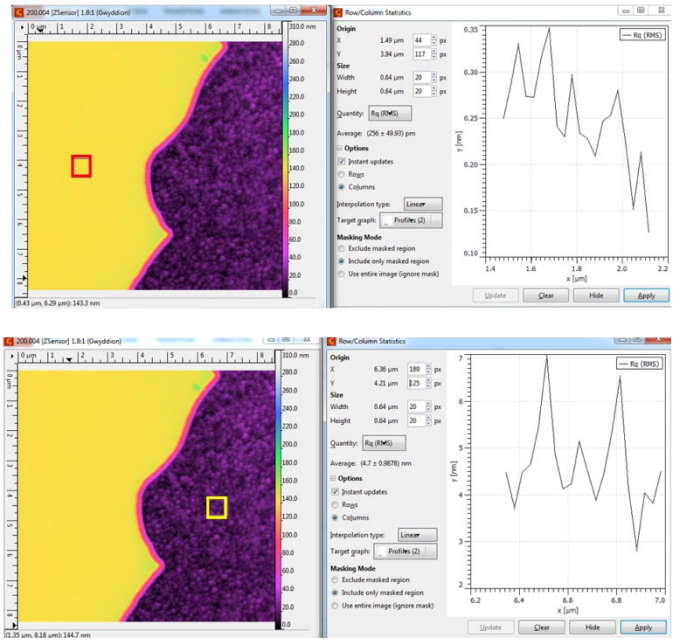
Melanine film step edge



Thickness=128 nm

Figure S5. Extensive AFM study

Roughness



film roughness

Rms= 0,25±0,5 nm

Substrate roughness

Rms= 5±1 nm

Figure S6. Extensive AFM study

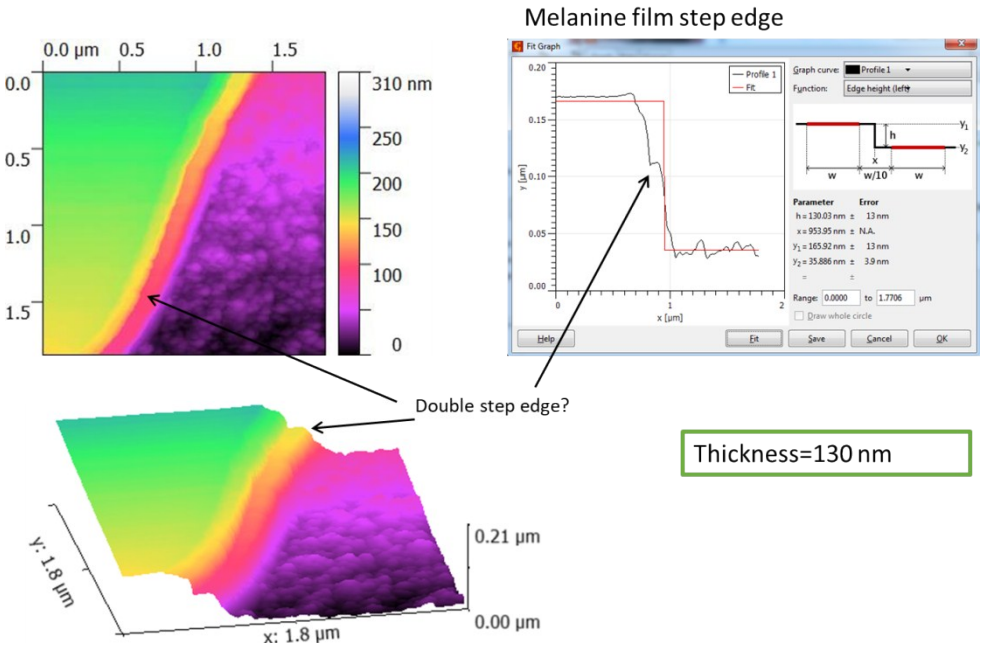
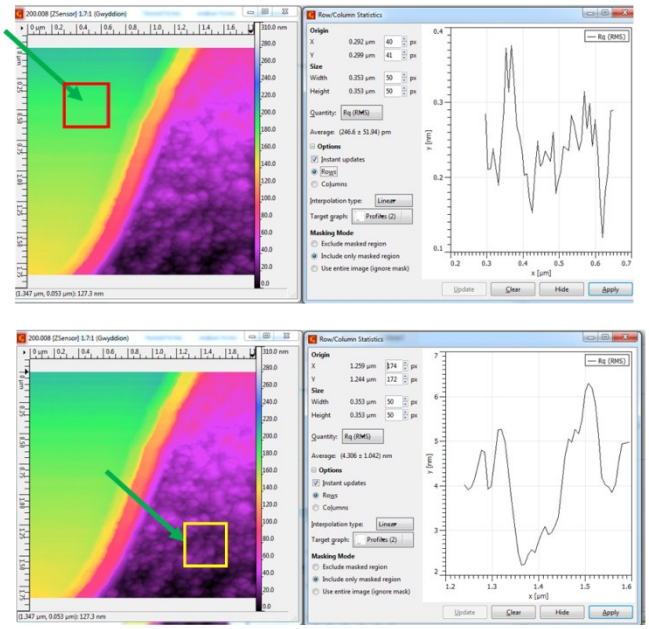


Figure S7. Extensive AFM study

Roughness



film roughness

Rms= 0,25±0,5 nm

Substrate roughness

Rms= 4±1 nm

Figure S8. Extensive AFM study

1,8-DHN methanol solutions spin coated

CONCENTRATION

20 mM 50 mM 100 mM 200 mM

Mycomelanin thin films

THICKNESS

25 nm 50 nm 130 nm 250 nm

Figure S9. Mycomelanin thin films on quartz prepared via AISSP: correlation between the film thickness and the concentration of the 1,8-DHN methanol solution spin coated.