Electronic Supplementary Information for the manuscript:

Vat Dyes: Promising Biocompatible Organic Semiconductors for Wearable Electronics Applications

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Contents

Biological assay

Sample preparation and cells culturing

Standard Petri dishes (Nunclon Delta, InterMed, Denmark) were coated at the bottom by the films of organic semiconductor materials (100 nm) thermally evaporated in a high vacuum (10^{-5} mbar). The prepared samples were sterilized with ethanol (70% aq.) for 30 min before performing cell seeding.

Human Embryo Lung Fibroblasts (HELF) were obtained from the Research Centre for Medical Genetics (RCMG) collection (fourth passage). Cells were seeded at 1.7×10^4 per mL in DMEM (PanEco, Moscow, Russia), 10% fetal calf serum (PAA, Vienna, Austria), 50 U/ml penicillin, 50 g/mL streptomycin, 10 g/mL gentamycin, and then incubated at 37 Cº for different periods - 30 h, 96 h, 14 d.

Antibodies staining and fow cytometry analysis

HELFs were washed consistently in Versene solution, 0.25% trypsin, and suspended in PBS. Staining of HELFs with various antibodies was also performed. The cells fixation was accomplished by their treatment with paraformaldehyde (Sigma-Aldrich, 2%, 37 Cº, 10 min) with later washing with 0.5% BSA-PBS, and with 0.1% Triton X-100 (PBS, 15 min, 20 Cº). After washing in solution with 0.5% BSA-PBS, the cells were stained with 1 μg/mL primary and secondary antibodies for 2 h (4 Cº).

When a double break occurred in DNA, the H2AX histone is phosphorylated at serine 139 (γH2AX), which was demonstrated by Lobrich with colleagues [1]. It means that antibodies to γH2AX histone are a reliable marker for the evaluation of double breaks DNA damages [2]. The formation of 8-oxoguanine (8-oxo-dG, or 8OxG) is one of the common signatures of DNA damages upon the action of the reactive oxygen species (ROS) [3]. γH2AX and 8-oxodG were analyzed using γH2AX-specific and 8-oxodG specific antibodies labeled with fluorescein isothiocyanate (FITC, US Biological, USA). The analysis of cells was performed by using of CyFlow Space (Partec, Germany). Each experiment was repeated at least three times; subpopulations of the cells were gated as recommended by the CyFlow software.

Optical microscopy

The cells fixation was accomplished by their treatment with paraformaldehyde (Sigma-Aldrich, 2%, 37 Cº, 10 min) with later washing with 0.5% BSA-PBS. The images of the cells were obtained using the AxioScope A1 microscope (Carl Zeiss) immediately after the fixation procedure.

Quantification of mRNA levels

Total mRNA isolation from HELF was performed by a standard method using the YellowSolve kits (Clonogen, Russia). RNA samples were reverse transcribed by Reverse Transcriptase kit (Sileks, Moscow, Russia). The expression profiles were obtained using qRT-PCR with SYBR green PCR MasterMix (Applied Biosystems, Foster City, USA). The mRNA levels were analyzed using the StepOne Plus (Applied Biosystems); the technical error was approximately 2%. The following primers were used (Evrogen, Moscow, Russia):

TBP (reference gene) (F: GCCCGAAACGCCGAATAT; R: CCGTGGTTCGTGGCTCTCT);

TNFα(F:CAGCCTCTTCTCCTTCCTGAT; R: GCCAGAGGGCTGATTAGAGA);

INFγ(F:GGCATTTTGAAGAATTGGAAAG; R:TTTGGATGCTCTGGTCATCTT);

BAX(F: GGAGCTGCAGAGGATGATTG; R: AGTTGAAGTTGCCGTCAGAA);

IL-6_hv(F: AAATTCGGTACATCCTCGACGGCA; R: AGTGCCTCTTTGCTGCTTTCACAC);

IL-1b_vh(F:GGTGTTCTCCATGTCCTTTGTA; R: GCTGTAGAGTGGGCTTATCATC);

NRF2 (NFE2L2) (F: TCCAGTCAGAAACCAGTGGAT; R: GAATGTCTGCGCCAAAAGCTG);

NOX4 (F: TTGGGGCTAGGATTGTGTCTA;R: GAGTGTTCGGCACATGGGTA);

CCND1 (F: TTCGTGGCCTCTAAGATGAAGG; R: GAGCAGCTCCATTTGCAGC);

TLR9 (TGAAGACTTCAGGCCCAACTG; TGCACGGTCACCAGGTTGT);

BCL2 (TTTGGAAATCCGACCACTAA; AAAGAAATGCAAGTGAATGA);

NFKB1 *(*CAGATGGCCCATACCTTCAAAT; CGGAAACGAAATCCTCTCTGTT).

All the obtained results were reproduced at least three times as independent biological replicates. Statistical analysis was performed with GraphPad Prism software.

References

[1] P. Taylor *et al.*, "γH2AX foci analysis for monitoring DNA double-strand break repair: Strengths, limitations and optimization," *Cell Cycle*, vol. 9, no. 4, pp. 662–669, 2010.

[2] E. S. Ershova, V. A. Sergeeva, A. I. Chausheva, D. G. Zheglo, and V. A. Nikitina, "Mutation Research / Genetic Toxicology and Environmental Mutagenesis Toxic and DNA damaging effects of a functionalized fullerene in human embryonic lung fibroblasts," *Mutat. Res. Toxicol. Environ. Mutagen.*, vol. 805, pp. 46–57, 2016.

[3] A. Valavanidis and T. Vlachogianni, "(8-OHdG): A Critical Biomarker of

Oxidative Stress and Carcinogenesis," *J. Environ. Sci. Heal. Part C*, vol. 0501, pp. 120–139, 2009.

Table S1. Contact angle and surface energy of dielectrics used for OFETs manufacture.

Materials	Contact angle (deg.) mean±SD		γ^p (mJ m^{-2}) mean±SD	γ^d (mJ m^{-2}) mean±SD	γ^{tot} (mJ m^{-2}) mean±SD
	DI Water	DIM			
AlOx	63.64 (±2.94)	49.32 (±1.10)	12.33 ±1.67	34.65 ±0.61	46.98 ±2.28
Tetracontane	109.28 (±4.80)	60.14 (±2.16)	0.01 ±0.06	28.50 ±1.24	28.50 ±1.31
Shellac	65.31 (±6.20)	45.27 (±1.67)	10.60 ±3.28	36.87 ±0.89	47.47 ±4.17
BCB	90.39 (±0.88)	16.10 (±2.37)	0.24 ±0.08	48.83 ±0.57	49.07 ±0.65
PS	75.81 (±1.85)	31.18 (±2.84)	4.09 ±0.69	43.73 ±1.21	47.82 ±1.90
PE	103.01 (±2.79)	58.18 (±2.17)	0.15 ±0.20	29.62 ±1.25	29.77 ±1.45

Table S2. The effects of the semiconductor materials on the gene expression in HELFs after 96 h incubation. Relative levels of expression are shown as mean values for 3 – 6 replicates and their standard deviation. (*) $p \leq 0,05$ – against control cells, non-parametric Ordinary One-way Anova with multiple comparisons test (Kruskal-Wallis).

		Material								
	GENES	ADT	Pent	C60	DNTT	VO9	VB20	VY4	VG1	VB4
1	CCND1	0.05 ± 0.02	-0.12 ± 0.15	-0.01 ± 0.04	-0.33 ± 0.35	0.04 ± 0.30	0.12 ± 0.15	0.20 ± 0.08	-0.08 ± 0.04	0.00 ± 0.09
2	NRF2	-0.14 ± 0.02	-0.15 ± 0.07	0.44 ± 0.15	0.26 ± 0.16	-0.47 ± 0.09	-0.68 ± 0.03	-0.16 ± 0.02	0.14 ± 0.20	0.24 ± 0.22
3	NOX4	-0.25 ± 0.22	0.14 ± 0.35	-0.70 ± 0.34	-0.27 ± 0.23	0.06 ± 0.28	0.15 ± 0.11	0.08 ± 0.25	-0.54 ± 0.22	-0.22 ± 0.30
4	Nf-kb	0.20 ± 0.25	0.19 ± 0.27	0.49 ± 0.43	-0.35 ± 0.15	0.02 ± 0.04	-0.02 ± 0.03	-0.03 ± 0.07	-0.05 ± 0.09	0.11 ± 0.05
5	BCL2	-0.06 ± 0.27	-0.65 ± 0.51	-1.30 ± 0.48	-0.74 ± 0.53	1.50 ± 0.21	1.04 ± 0.31	1.25 ± 0.30	-1.13 ± 0.27	-0.62 ± 0.29
6	BAX	-0.56 ± 0.15	-0.94 ± 0.26 **	-0.26 ± 0.13	-0.31 ± 0.01	0.05 ± 0.06	-0.14 ± 0.01	-0.04 ± 0.02	0.16 ± 0.03	0.07 ± 0.09
7	BECN	0.18 ± 0.12	0.09 ± 0.14	0.01 ± 0.12	0.08 ± 0.05	0.09 ± 0.04	0.01 ± 0.10	0.03 ± 0.07	-1.18 ± 0.40 *	-0.36 ± 0.33
8	TNFα	-0.03 ± 0.24	-0.45 ± 0.31	-0.26 ± 0.16	0.03 ± 0.12	0.75 ± 0.30	1.50 ± 0.36 *	1.38 ± 0.27 *	0.11 ± 0.19	0.42 ± 0.4
9	IL-1β	-0.04 ± 0.32	-1.00 ± 0.05	-0.64 ± 0.23	-1.51 ± 0.18	-0.19 ± 0.29	-0.24 ± 0.34	-0.22 ± 0.11	-1.07 ± 0.19	0.26 ± 0.09
10	TLR9	-1.26 ± 0.15	-0.01 ± 0.02	0.24 ± 0.16	-0.83 ± 0.37	-0.21 ± 0.13	-0.04 ± 0.07	0.08 ± 0.05	0.35 ± 0.09 *	0.39 ± 0.19 *
11	INFγ	-0.15 ± 0.03	-0.17 ± 0.09	-0.41 ± 0.14	-0.33 ± 0.08	0.30 ± 0.17	-0.13 ± 0.11	0.57 ± 0.23	-0.23 ± 0.04	-0.03 ± 0.02

Table S3. Deposition conditions for organic dielectric coatings.

Dielectric material	Solvent	Concentration	Spin-coating frequency
BCB	mesitylene	1% (by wt.)	1500 rpm for 20 s 4000 rpm for 20 s
PS	toluene	1 mg/mL	1500 rpm for 10 s 4000 rpm for 20 s
Tetracontane	toluene	1 mg/mL	1500 rpm for 10 s 4000 rpm for 20 s
Shellac	ethanol	1 mg/mL	1500 rpm for 10 s 4000 rpm for 20 s

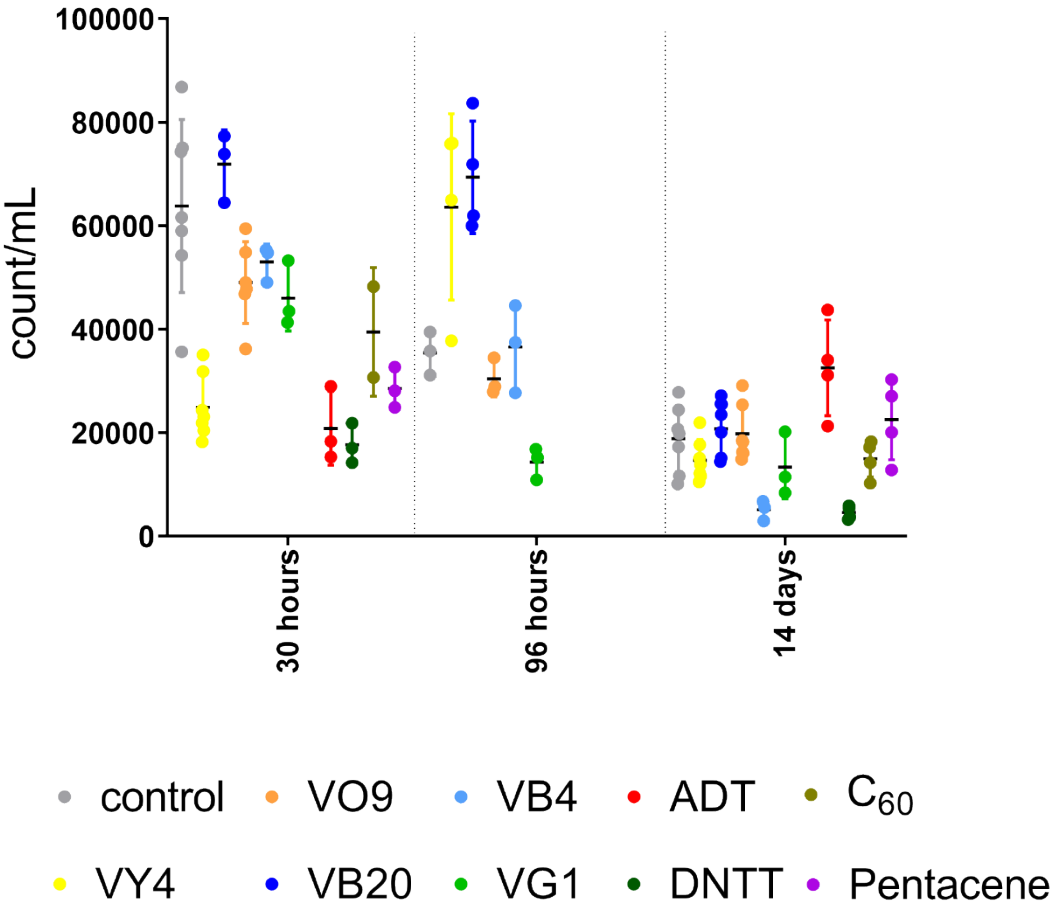

Fig. S1. Cell counts according to the flow cytometry analysis for a series of vat dyes and the reference organic semiconductors.

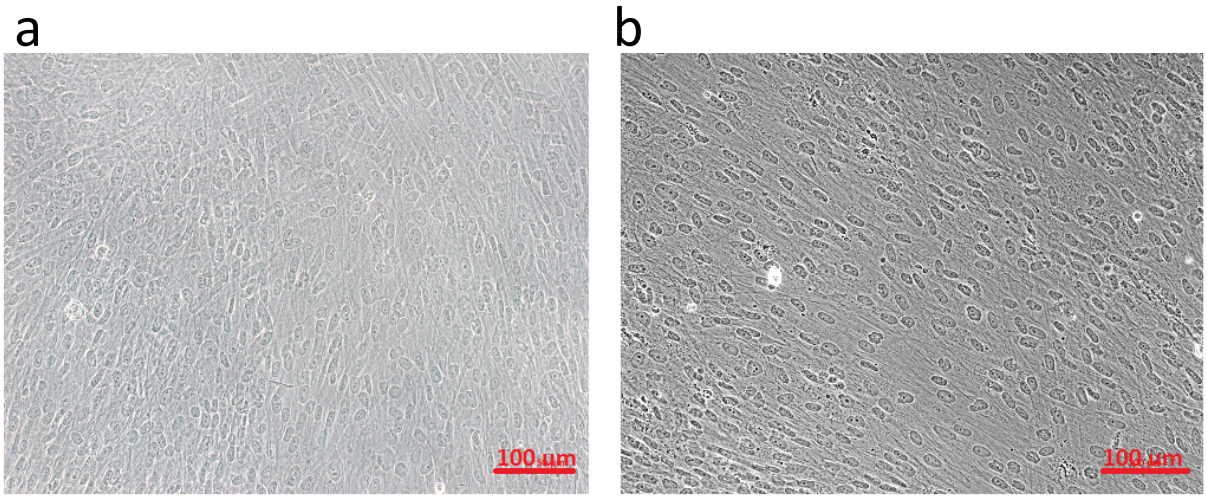

Fig. S2. Optical microscopy images for control cells after 96 h (a) and 14 d (b) incubation

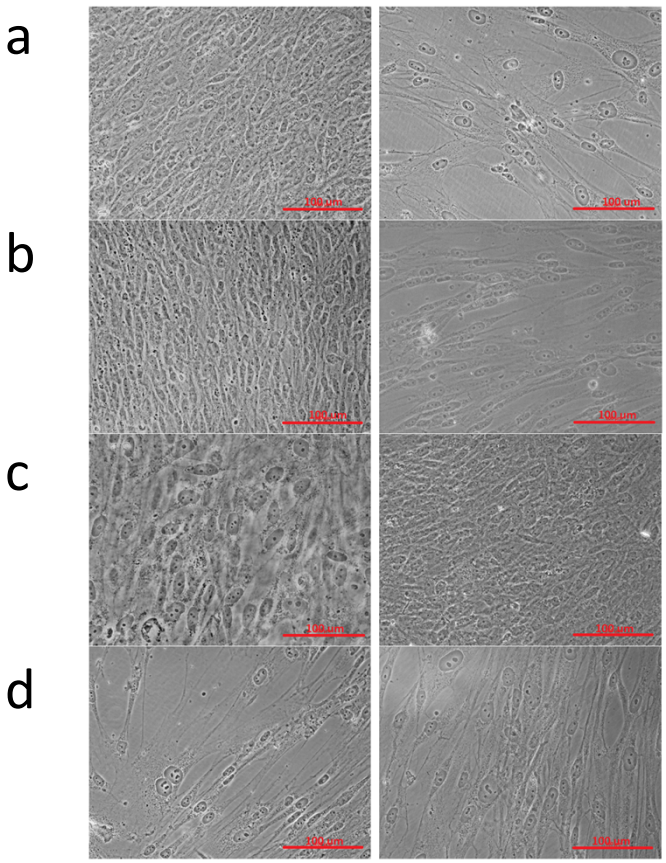

Fig. S3. Light microscopy images for the cells incubated with the reference organic semiconductors within 96 h (left column) or 14 d (right column): a) ADT, b) C_{60}, c) DNTT, d) pentacene.