Supplementary material

The effect of uncoated SPIONs on hiPSC-differentiated endothelial cells

Barbara Salingova¹, Pavel Simara¹, Pavel Matula¹, Lenka Zajickova², Petr Synek³, Ondrej Jasek³, Lenka Veverkova⁴, Miroslava Sedlackova⁵, Zuzana Nichtova⁶, Irena Koutna^{1*}

- ¹ Centre for Biomedical Image Analysis, Faculty of Informatics, Masaryk University, Kamenice 5, 625 00, Czech Republic
- ² RG Plasma Technologies, CEITEC Masaryk University, Purkynova 656/123, 612 00, Czech Republic
- ³ Department of Physical Electronics, Faculty of Science, Masaryk University, (Kotlarska 2, Brno 602 00, Czech Republic
- ⁴ 1st Surgical Department, Faculty of Medicine, Masaryk University and St. Ann's Hospital, Pekarska 53, 656 91 Brno, Czech Republic
- ⁵ Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic
- ⁶ Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Dubravska cesta 9, 840 05 Bratislava, Slovak Republic
- *Correspondence: Assoc. prof. Irena Koutna PhD., <u>qkoutna@gmail.com</u> (+420) 549 493 976

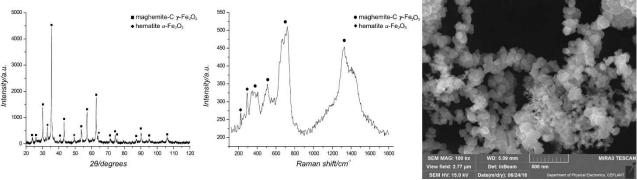


Figure S1 Characterisation of the prepared uSPIONs. Left, sample diffraction pattern. Middle, phase composition of the prepared sample analyzed by Raman spectroscopy. Right, SEM of the synthetized uSPIONs (size: 20-50 nm). Abbreviations: SEM, scanning electron microscopy.

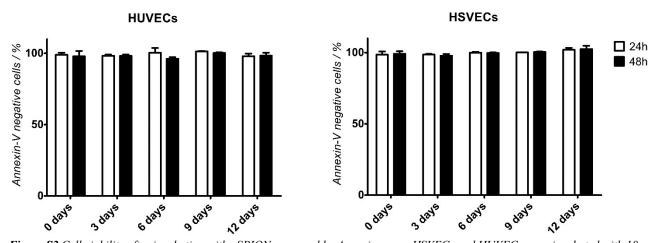


Figure S2 Cell viability after incubation with uSPIONs assessed by Annexin assay. HSVECs and HUVECs were incubated with 10 μ g/ml uSPIONs for 24 and 48 h and observed up to 12 days after incubation. Viability was assessed by annexin assay during passaging – every 3 days ($N=\pm 3$ SEM). Viability was standardized to the control. The statistical evaluation (t-test) showed no significant difference in cell survival among the cell lines (p>0.05). Abbreviations: HUVECs, human umbilical vein endothelial cells; HSVECs, human saphenous vein endothelial cells.

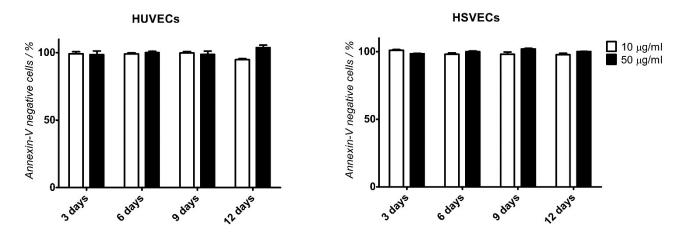


Figure S3 Cell viability during long-term incubation with uSPIONs assessed by Annexin assay. HUVECs and HSVECs were incubated with 10 and 50 μ g/ml uSPIONs for 3, 6, 9 and 12 days (N= ± 3 SEM). Viability was standardized to the control. The statistical evaluation (t-test) showed no significant difference in cell viability among the cell lines (p> 0.05). Abbreviations: HUVECs, human umbilical vein endothelial cells; HSVECs, human saphenous vein endothelial cells.

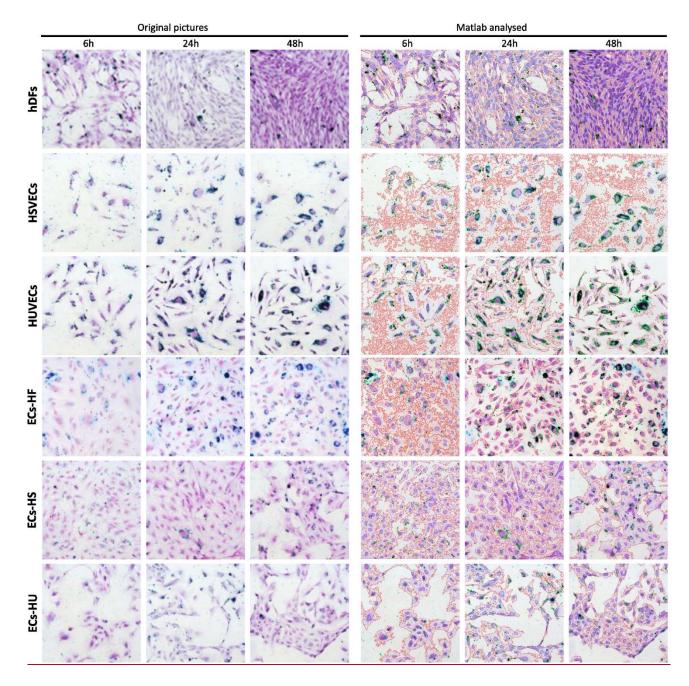


Figure S4 Internalization of uSPIONs in 6 cell types, Matlab analysis. Left - Representative pictures of internalization of uSPIONs visualized after Prussian blue staining. Cells were incubated with uSPIONs for 6 h, 24 h and 48 h, stained with Prussian blue for iron detection and observed under a light microscope. Right — Matlab analysis of internalization of uSPIONs visualized after Prussian blue staining. Analysis of number of uSPIONs inside the cell. Red lines — cell boarders, green — uSPIONs. Abbreviations: HUVECs, human umbilical vein endothelial cells; HSVECs, human saphenous vein endothelial cells; hDFs, adult human dermal fibroblasts; ECs-HUs, endothelial cells differentiated from hiPSCs-HU; ECs-HS, endothelial cells differentiated from hiPSCs-HF.