INVESTIGATION OF THE EFFECTS OF ACUTE NICOTINE EXPOSURE AND LONG TERM SMOKING ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS FROM IDENTICAL TWINS WITH DM.

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The 20% of global mortality is caused by smoke-related diseases. Human induced pluripotent stem cells (hiPSC) provide a personalised link between clinical and in vitro cardiovascular models. hiPSC lines were generated from a pair of identical twins with diabetes mellitus to test long and short-term effects of nicotine.
Mononuclear cell fractions were reprogramed into hiPSC with Yamanaka factors (Cytotune 2.0) via Sendai viral transduction. Genotype and pluripotency of the iPSC were checked. hiPSC line from non-diabetic healthy control was used as control. The hiPSC lines were differentiated into endothelial cells (ECs). hiPSC-derived ECs were treated with e-cigarette liquid (vehicle: propylene glycol PG & vegetable glycerin VG 1:1, nicotine concentration: 18 mg/ml) in EC growth medium, 1:80 (ECL18) and the same vehicle without nicotine (ECL0) or nicotine in EC growth medium (0.225 mg/ml). The cells were analysed with high content microscopy (HCS, Opera), using Hoechst, apoptotic marker caspase3/7, mitochondrial TMRM and endothelial CD31 dyes.
On the clinical side, the smoking member of the twins (“C”) was diagnosed and treated with a severe coronary disease, whilst the other (“D”) was a non-smoker and does not suffer from any coronary artery disease. C cell line showed a higher endothelial-to-mesenchymal transition rate and non-endothelial drift as compared to D line at 3 passages after FACS sorting for CD31. The toxicity of ECL18 treatment resulted in higher cell death rate in the control and in D cell line compared to C. Caspase intensity histogram became bimodal as frequencies at higher caspase intensity values arise in response to nicotine treatments, especially in ECL18 treated wells.
Our developed patient-specific in vitro hiPSC model showed alteration in endothelial phenotype associated with nicotine exposure. A scalable platform can provide further cohort level information on toxic agent like nicotine to the cardiovascular system.

Keywords: hiPSC, identical twins, smoking, nicotine, endothelial cells