HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS IN THROMBOSIS-ON-A-CHIP DEVICES

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ABSTRACT

A microfluidic thrombosis-on-a-chip platform was developed to compare the pro-thrombotic response of healthy and inflamed monolayers of human umbilical vein endothelial cells (HUVECs) and human induced pluripotent stem cell-derived endothelial cells (hiPSC-ECs). Inflammation was induced by exposing the endothelial cells (ECs) to an inflammatory cytokine Tumor Necrosis Factor-α (TNF-α). After human whole blood perfusion at an arterial shear rate, the platelet coverage and average clot size were determined. Healthy endothelium showed a lower platelet coverage than inflamed endothelium. A minor difference was measured for both platelet coverage and average clot sizes on inflamed HUVECs versus hiPSC-ECs.

KEYWORDS: Thrombosis, Organ-on-a-Chip, Vasculature, Human Induced Pluripotent Stem Cells, Endothelial Cells, Human Umbilical Vein Endothelial Cells, Whole Blood, Microfluidics.

INTRODUCTION

We have used a microfluidic thrombosis-on-a-chip platform to perform human whole blood perfusion experiments to compare the pro-thrombotic response of inflamed monolayers of HUVECs and hiPSC-ECs with each other and their untreated counterparts. In these devices the interaction of ECs, platelets and blood-derived factors can be studied [1,2]. No thrombosis-on-a-chip devices have yet been reported that include hiPSC-ECs, and little is known about how platelets will react to both non-activated and inflamed hiPSC-ECs. In this study we will investigate the difference in thrombotic response of HUVECs and hiPSC-ECs when exposed to an inflammatory cytokine TNF-α by determining the platelet coverage and clot characteristics after whole blood perfusion.

EXPERIMENTAL

Polydimethylsiloxane devices were moulded using wafer-based soft lithography techniques and were bonded to glass slides using plasma treatment. The 300×50 μm (width×height) channels were treated with a 0.1 mg/ml collagen-I (Rat-tail collagen-I, Corning) solution. Human skin fibroblasts were reprogrammed and differentiated into hiPSC-ECs using previously reported protocols [4]. The channels were lined with HUVECs (Lonza) or hiPSC-ECs using 10×10^6 cells/ml. Inflammation was induced by exposure to a 10 ng/ml TNF-α (Sigma-Aldrich) solution for 15 hours. Re-calcified citrated whole blood was perfused for 20 minutes at an arterial shear rate of 1000 s^{-1} (Fig. 1 J). Afterwards the channels were washed and fixated. Platelets were labeled using CD-41 PE (Invitrogen), fibrin was stained using labeled fibrinogen (Invitrogen) and ECs were stained using a nuclear (NucBlue, Invitrogen) and VE-cadherin (abcam) staining. Platelet coverage was determined at four areas in the channel using the triangle threshold [5] script in MATLAB 2016b followed by a summation of thresholded areas. Only the middle 200 μm was analyzed to account for edge-effects. Surface area per clot was obtained using the regionprops script.

RESULTS AND DISCUSSION

Whole blood perfusion in microfluidic devices lined with healthy or inflamed monolayers of HUVECs or hiPSC-ECs were compared under arterial flow conditions. Both HUVECs and hiPSC-ECs show a confluent monolayer after 24 hours of culturing. The hiPSC-ECs show a more elongated morphology in comparison to the cobblestone-like HUVECs. No significant morphological changes of the monolayer were observed after exposure to TNF-α (Fig. 1 A-D). After whole blood perfusion platelet coverage was measured and fibrin formation observed in the microfluidic chips (n=2, healthy and n=3, TNF-α). The healthy condition resulted in 0.4% and 0.5% platelet coverage while the inflammatory condition resulted in 2.8% and 3.1% platelet coverage for hiPSC-ECs and

HUVECs respectively (Fig. 1 E-I). However, more experiments need to be conducted before statistical significance can be evaluated. Besides platelet coverage also the individual clot size per condition was investigated. The average clot size was determined to be 44 μm^2 for HUVECs and 38 μm^2 for hiPSC-ECs.

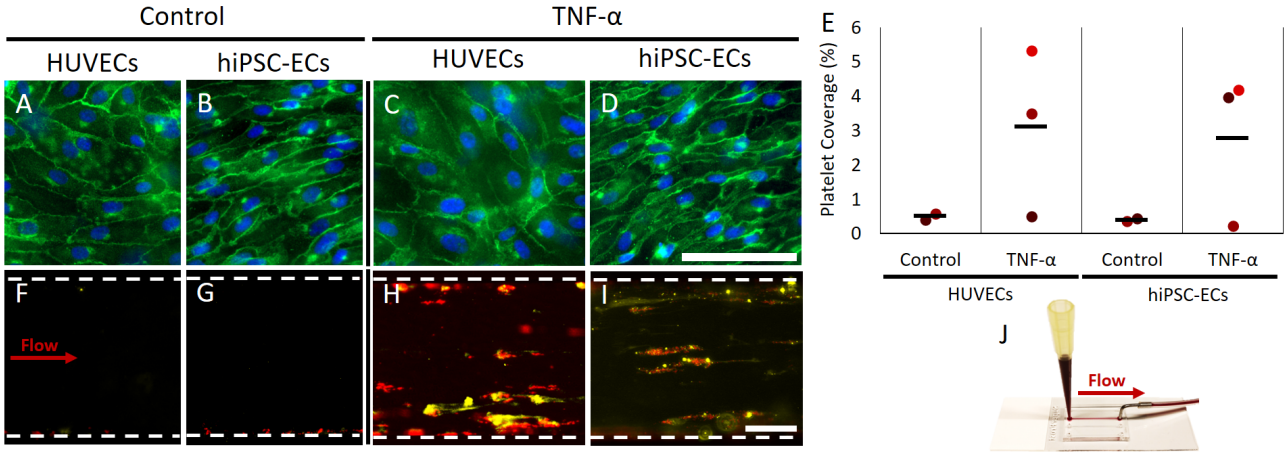

Figure 1: HUVECs (A,C) and hiPSC-ECs (B,D) form confluent monolayers when cultured in microfluidic channels. No morphological changes (nuclei, blue, VE-cadherin, green) are seen in inflamed monolayers (C,D) in comparison to untreated monolayers (A,B). Platelet coverages per condition are displayed as data points, the mean coverage is shown as a horizontal line (E). Fluorescence microscopy images of channels lined with HUVECs (F,H) and hiPSC-ECs (G,I) after perfusion with whole blood (platelets, red, fibrin, yellow). Healthy conditions (F,G) show almost no platelet coverage, while inflamed conditions (H,I) do show platelet aggregation and fibrin. A microfluidic chip perfused with whole blood (J). Scale bars, 100 µm.

CONCLUSION

A proof-of-concept of a thrombosis-on-a-chip device lined with hiPSC-ECs was presented here. The cells formed a confluent monolayer after 24 hours of culture. It was demonstrated that hiPSC-ECs responded to treatment with TNF-α and promoted platelet aggregation on inflamed monolayers. In addition, a comparison between healthy and inflamed HUVECs and hiPSC-ECs showed a lower platelet coverage and clot size on inflamed hiPSC-ECs. However, more experiments need to be done to prove a significant difference. In future experiments, using both whole blood and hiPSC-ECs from healthy and diseased subjects would open up possibility for a personalized approach in drug testing. Furthermore the mechanism behind the decreased coverage and average clot size for hiPSC-ECs will be investigated by improving the image analysis script to include clot height and composition.

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