Platelet and Monocyte Binding to Human Aortic Endothelial Cells in well plate microfluidic devices after TNFα stimulation

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Abstract

Well plate microfluidic devices have been demonstrated to provide a physiologically relevant environment to model the human vasculature. Here we utilized the BioFlux™ microfluidic technology in conjunction with human aortic endothelial cells (HAECs) to observe the development of TNFα-induced platelet and monocyte adhesion to endothelial cells (i.e., thrombosis). HAEC monolayers were grown in microfluidic channels and were then exposed to a range of TNFα concentrations (5-100 ng/mL) for 5 hours under gravity flow. THP-1 monocytes were activated with TPA and exposed to a range of TNFα concentrations (5-100 ng/mL) for 5 hours under gravity flow. Whole blood and THP-1 cells were pre-labeled with Calcein AM to aid quantification of cell binding (percent channel area) with BioFlux™ Montage software. We demonstrate that both platelets and monocytes (THP-1) are able to bind to HAECs in microfluidic devices after exposure to TNFα and furthermore we have shown that this trend appears to increase in a dose-responsive manner before plateauing in both whole blood and THP-1 experimentation. We believe that these microfluidic devices have the potential to evaluate pro-inflammatory compounds and mixtures that may be relevant to cardiovascular disease such as atherosclerosis.

Material and Methods

HAECs were maintained in VascuLife® VEGF Endothelial Cell Culture Medium supplemented with VEGF LifeFactor Kit (LifeLine Cell Technology). Human THP-1 monocytic leukemia (THP-1) cells were supplied by ATCC and maintained in the recommended RPMI-1640 medium. Fresh whole blood samples were collected from fasting donors using 0.32% sodium citrate BD vacutainers™ and were used within 3 hours of collection. Methodology for BioFlux™ system and plate set up were adapted from Conant et al., 2009 and 2011. HAEC monolayers were exposed to TNFα under gravity flow (<1 dyn/cm²) for 5 or 24 hours as required. Whole blood was labelled with 4 μM Calcein AM (0.5% v/v). TNFα was incubated with whole blood (10% v/v) for 1 hour on a slow moving rocking platform. The whole blood was perfused through channels at 5 dyn/cm² for 5 mins and followed by a wash with HBSS containing 10 ng/mL, Hoechst 33342 (H33342) for 1 min.

Results

For 24-hour TNFα experimentation, un-labelled, activated THP-1 cells were perfused at 2 dyn/cm² for 30 seconds followed by 0.5 dyn/cm² for 1 min. THP-1 cells were left for a static adhesion period followed by a wash with HBSS containing H33342 at 2.5 and 6 dyn/cm² for 1 min. The perfusion, adhesion period and wash cycle was performed four times with increasing adhesion periods (10, 20, 30 and 40 mins). THP-1 numbers were quantified by manual counting of phase contrast images from three fields of view. Two-way ANOVA with multiple comparisons p<0.001.

Summary and Conclusions

Both platelets and monocytes are able to bind to HAECs in microfluidic devices after TNFα exposure for 5 hours (Figures 1 & 2).

Binding of monocytes to HAECs in microfluidic devices is increased after TNFα exposure for 24 hours (Figure 3).

References