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

Abstract #3347

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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. This was followed by perfusion through the microfluidic channel with whole blood (pre-incubated TNFα for 1 hour) at 5 dyn/cm^2 for 5 minutes or THP-1 cells (activated with TPA) at 1 dyn/cm^2 for 1 minute followed by 0.5 dyn/cm^2 for 10 minutes. 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 (<0.1 dyn/cm^2) 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/cm^2 for 5 mins and followed by a wash with HBSS containing 10 µg/mL Hoechst 33342 (H33342) for 1 min.

To activate THP-1 cells, 12-O-tetra-decanoylphorbol-13-acetate (TPA) in DMSO was added to cell samples (<0.1% v/v) at 1 ng/mL and transferred to low adhesive and adhesive flasks for 24 hours to determine successful activation. THP-1 cells were not pre-incubated with TNFα, but were labelled with 4 µM Calcein AM (0.5% v/v). For 5-hour experiments, THP-1 cells were perfused through channels at 1 dyn/cm^2 for 1 minute followed by 0.5 dyn/cm^2 for 10 min. Channels were washed with HBSS containing H33342.

Images were acquired using the Bioflux™ Montage software from multiple fields of view per channel under the brightfield, FITC (green; Calcien AM) and DAPI (blue; H33342) filters. Platelet/THP-1 adhesion was calculated using Montage software based on pixel area. One-way ANOVA with multiple comparisons *** p<0.001.

For 24-hour TNFα experimentation, un-labelled, activated THP-1 cells were perfused at 2 dyn/cm^2 for 30 seconds followed by 0.5 dyn/cm^2 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/cm^2 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).

Results

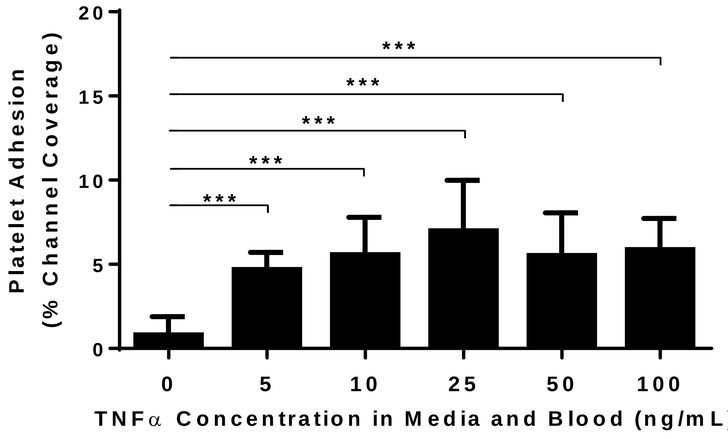


Figure 1A. Whole Blood adhesion to HAECs following TNFα stimulation (1-hour Blood + 5-hour HAECs) *** p<0.001

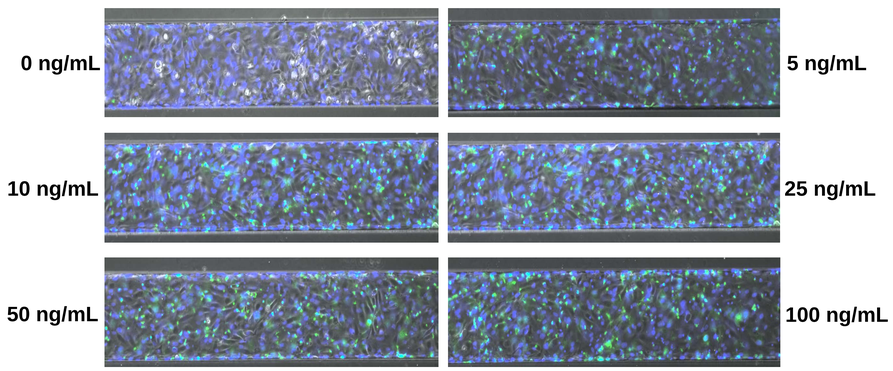
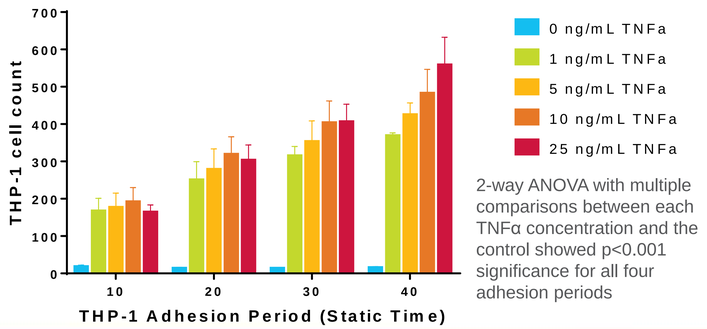


Figure 1B. Representative images of Whole Blood (FITC) adhesion to HAECs (DAPI) following 5-hour TNFα incubation



2-way ANOVA with multiple comparisons between each TNFα concentration and the control showed p<0.001 significance for all four adhesion periods

Figure 3. THP-1 Adhesion to HAECs following repeat THP-1 addition (24 hour TNFα)

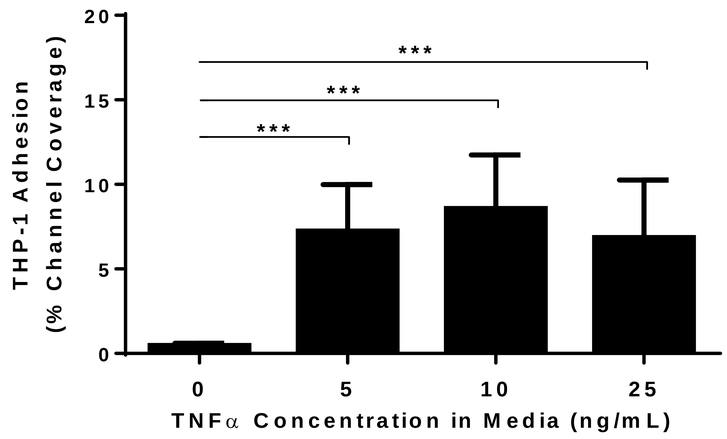


Figure 2A. THP-1 adhesion to HAECs following TNFα stimulation for 5-hours *** p<0.001

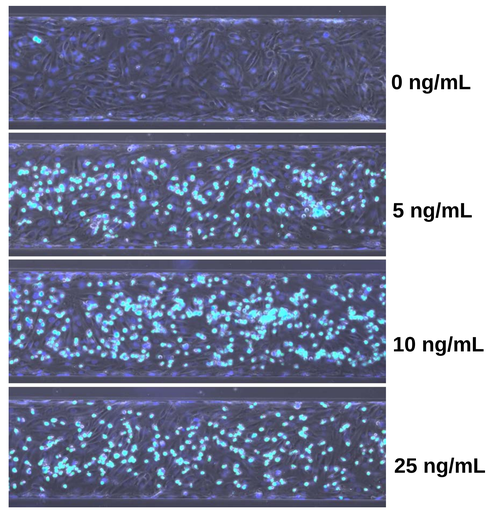


Figure 2B. Representative images of THP-1 (FITC) Adhesion to HAECs (DAPI) following 5-hour TNFα incubation

References

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