# ATHEROSCLEROSIS-ON-A-CHIP: A TUNABLE 3D STENOTIC BLOOD VESSEL MICRODEVICE

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Atherosclerosis, the leading cause of cardiovascular diseases [1–3], is a chronic inflammatory disorder characterized by deposition of cholesterol-containing low-density lipoproteins (LDL) in arterial walls, resulting in blood vessel narrowing and impaired perfusion, and increased leukocyte recruitment [4,5]. Current atherosclerosis *in vitro* models are 2-dimensional (2D), and fail to reproduce important features of atherogenesis including blood flow-induced shear stress and leukocyte-endothelial interactions [6–7]. Herein, we report a novel biomimetic blood vessel model to study the hemodynamics and leukocyte-endothelial interactions using a tunable, 3-dimensional (3D) endothelial barrier to mimic stenotic plaque. We first characterized the effects of THP-1 monocyte adhesion on activated endothelial monolayer, followed by whole blood perfusion at different channel constrictions to study leukocyte rolling phenotype.

A schematic of the polydimethylsiloxane (PDMS) microfluidic device is shown in Figure 1A. It consists of 3 layers, with a top cell culture chamber (800×100 μm (H×W)) for endothelial (HUVECs) cell culture, and a bottom pneumatic channel (1000×100 μm (H×W)), separated by a thin PDMS membrane (10 μm thick). By varying air flow into the pneumatic channel, the membrane is deflected upwards, thereby creating tunable constrictions in the cell culture chamber (Figure 1B). Endothelial inflammation is mimicked by growing HUVECs to confluency and treating them with tumor necrosis factor-α (TNF-α) to study leukocyte-endothelial interactions in cell culture media (RPMI) and whole blood flow.

We first performed confocal imaging to visualize channel constriction using FITC dye and 3D endothelial "stenotic plaque" (Figure 1B, C). The laminar flow profile over the 3D stenosis was studied from the rolling velocities of 10 μm beads, which varied significantly in the stenosis region (Figure 1D). As observed from Figure 2A, HUVECs expressed higher ICAM-1 due to TNF-α-treatment which facilitated binding of THP-1 cells. Interestingly, distinct adherence patterns of THP-1 for 50% and 80% constrictions was observed under flow (1 dyne/cm$^2$) with increased adherence at the top of the constriction (Figure 2B, C). Upon increasing flow rate to 10 dyne/cm$^2$, THP-1 adhesion was reduced in 80% constriction while completely eliminated in 50% constriction. (Figure 2D, E). Finally, whole blood was perfused through the device to study blood flow under stenosis conditions (Figure 3). As expected, significant leukocyte (mostly neutrophils) rolling and adhesion were observed, and average leukocyte rolling velocity was lowest with an 80% constriction (Figure 3D), possibly due to low shear at the top of the stenotic "plaque". On-going studies are performed to gain better insights into the stenosis-induced hemodynamics effects on leukocyte-endothelial interactions.

In conclusion, the developed atherosclerosis-on-a-chip mimics a physiologically-relevant 3D stenotic blood vessel which enables long-term perfusion cell culture and on-chip visualization of leukocyte-endothelial interactions (rolling, adhesion). This model can also be further developed to study thrombus formation and other endothelial-related dysfunctions in cardiovascular diseases.

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## Figures

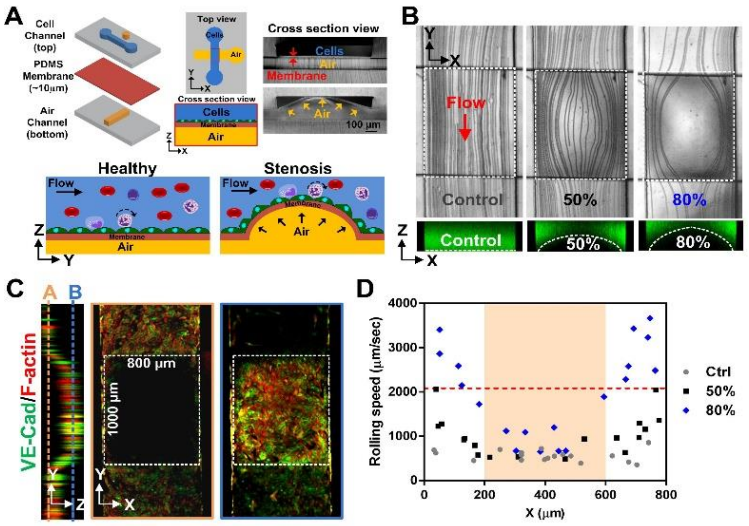


***Figure 1 (A)*** *Schematic illustration of the multilayered PDMS–based, atherosclerosis-on-a-chip. Membrane is deflated upwards by pumping air into the bottom channel, thereby creating a "stenotic plaque" in the cell culture chamber at the top.* ***(B)*** *(top) Overlaid high speed images illustrating 10 μm beads flow profile at different conditions. (Below) Confocal images of cell culture channel loaded with FITC dye to illustrate channel constriction.* ***(C)*** *Confocal images of HUVEC monolayer stained with VE-cadherin (green) and F-actin (red) at 80% channel constriction. White dotted box indicates membrane region.* ***(D)*** *Rolling velocity of 10 μm beads at different channel constrictions. Rolling velocities are lowest at the central region (shaded) and membrane and higher at the sides with increasing constriction.*

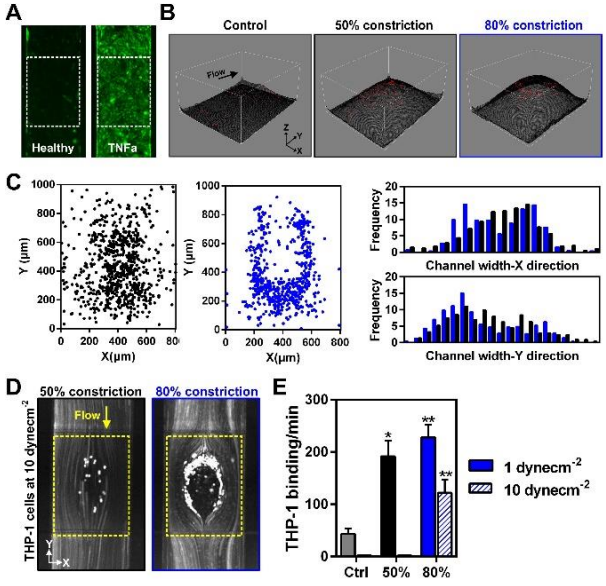


***Figure 2 (A)*** *ICAM-1 expression (green) of healthy and TNFa-treated HUVECs monolayer.* ***(B)*** *3D image reconstruction of the stenosis channel using FITC fluorescence distribution. A higher number of THP-1 cells (red) are adhered at the top of the constriction in the membrane region.* ***(C)*** *THP-1 cell adherence distribution for 50% and 80% constriction at 1 dyne/cm$^2$.* ***(D)*** *Overlaid fluorescence images indicating THP-1 cell adherence distribution for 50% and 80% constriction in the membrane region (yellow dotted box) at 10 dyne/cm$^2$.* ***(E)*** *THP-1 binding efficiencies to HUVECs at different channel constrictions and flow conditions. THP-1 adhesion is significantly reduced at 10 dyne/cm$^2$.*

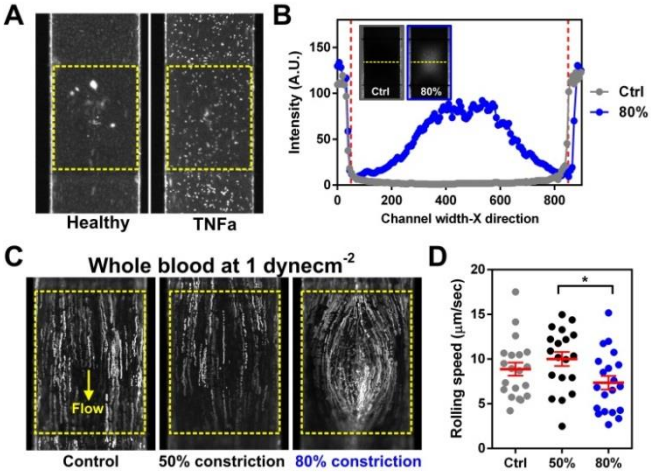


***Figure 3 (A)*** *Significant increase in leukocyte rolling and adhesion in TNFa-treated HUVECs under whole blood flow.* ***(B)*** *Intensity profile of cell culture chamber illustrating differences in blood flow over the stenosis region. (Inset brightfield images correspond to whole blood flow).* ***(C)*** *Overlaid fluorescence images indicating leukocyte (stained with R6G) rolling trajectories over the membrane region (yellow dotted box) at different channel constrictions.* ***(D)*** *Leukocyte rolling speed at the membrane region. The average rolling velocity is lowest for 80% channel constriction due to reduced flow through the center of the constriction.*

## References


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